Bundesinstitut für Risikobewertung

Inter-laboratory comparison exercise on the determination of primary aromatic amines and amides from cold water extracts of paper FCM

Report on the inter-laboratory comparison exercise NRL-DE-FCM-01/2021 of the German National Reference Laboratory (NRL) for Food Contact Materials

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1 Summary

The inter-laboratory comparison (ILC) exercise NRL-DE-FCM-01/2021 was organized by the German National Reference Laboratory for Food Contact Materials (NRL-DE-FCM) established within the Unit Product Analytics of the Department of Chemical and Product Safety at the German Federal Institute for Risk Assessment (BfR).

In BfR recommendation XXXVI [1], the BfR restricts the release of primary aromatic amines (PAAs) from paper and board FCM. PAAs should not be released from these materials in a "detectable amount". For the sum of PAAs an analytical detection limit of 0.01 mg kg^{-1} food or food simulant should be applied. PAAs classified as carcinogens in class 1A and 1B of the regulation (EC) No. 1272/2008 [2] should not be detectable presuming an analytical detection limit of 0.002 mg kg^{-1} food or food simulant. Therefore, this ILC was primarily designed to assess the analytical capabilities of the participating laboratories to correctly identify and quantify PAAs in cold water extracts (CWEs) of colored napkins and in solution in these low concentration ranges.

In total, twenty laboratories enrolled in the ILC and seventeen laboratories from twelve Member States of the EU submitted results. The participating laboratories received a red-colored napkin sample to perform a CWE in triplicate according to DIN EN 645 [3] with slight adjustments [4] and three solutions which were prepared by the NRL-DE-FCM. Participants did not know which analytes to expect, so the scope of the ILC comprised the correct identification and quantification of the compounds in the test items.

Results reported by the laboratories were evaluated qualitatively, based on the correct identification of the compounds, and quantitatively, based on the reported concentrations for the identified compounds. Quantitative evaluation was conducted in accordance with ISO 13528 [5] by calculating either z or z' and zeta (ζ) scores for aniline in CWEs of Sample 1 as well as for 2,4-dimethylaniline and 4-aminoazobenzene in Solution No. 3. For aniline in Solution No. 2 as well as for 4-chloroaniline and *o*-anisidine in Solution No. 3 the results were assessed using estimates of deviation ($D\%$) in order to evaluate the individual laboratory's deviation from the calculated participants' robust mean. Moreover, relative standard measurement uncertainties were calculated and compared across the laboratories.

Based on expert judgment, the relative standard deviation for proficiency assessment (σ_{pt}) was set to 25 % for the extraction experiments (CWE of Sample 1) and to 20 % for the analysis of the provided solutions.

Four laboratories were able to identify all seven PAAs in the test items correctly and three laboratories did not identify any PAAs. The other laboratories were able to identify some of the analytes in the test items. Aniline as the representative substance in the class of PAAs was correctly identified in the CWE of the napkin sample (Sample 1) by more than 80 % of participants. More than half of the laboratories were able to identify selected PAAs at low concentrations of <2 µg L^{-1}.

Most of the participating laboratories (11 of 14) performed satisfactorily (according to z scores) for the analysis of aniline in the CWE of Sample 1 prepared under the terms of DIN EN 645 [3]. One laboratory received a questionable z score and two an unacceptable z score. The majority of the laboratories (8 of 11) obtained acceptable ζ scores for aniline in the CWE of Sample 1. A slightly better performance in terms of z' scores was demonstrated for the analysis of 4-aminoazobenzene and 2,4-dimethylaniline in Solution No. 3: 91 % (10 of 11) of the results were found to be acceptable for 4-aminoazobenzene and 89 % (8 of 9) for 2,4-dimethylaniline.

The results for aniline in Solution No. 2 as well as for 4-chloroaniline and *o*-anisidine in Solution No. 3 were assessed using estimates of deviation (*D%*). Most laboratories reported results with deviations from the robust mean value smaller than $2*\sigma_{pt}$. However, strong positive deviations were observed for three laboratories.

Most laboratories reported measurement uncertainties (MU) for the quantified compounds. Calculated relative standard uncertainties $u(x_i)_{\%}$ ranged from 2 to 22 % and were comparable for solutions and the CWE. Although expected because of the additional uncertainty due to the extraction step, only few participants reported a higher standard uncertainty for the CWE compared to the solutions. Taking the relative standard uncertainty calculated from the ILC results into account, the estimation of the reported measurement uncertainties for aniline in the CWE of Sample 1 was reasonable for 64 % (7 of 11) of the laboratories.

In general, this ILC demonstrates that most laboratories established analytical methods that perform well in terms of quantification of PAAs. Nevertheless, some laboratories showed distinct bias in the reported results. While a great majority of laboratories was able to identify at least one of the PAAs correctly, only four of them correctly identified all seven PAAs. Moreover, LC-DAD based methods showed to be not adequately suited for both, identification and quantification of PAAs in the low concentration range tested here. The findings of the ILC thus emphasize the need for further improvements and harmonization in the analysis of PAAs in the context of FCM monitoring.

2 List of abbreviations and symbols

BfR	German Federal Institute for Risk Assessment
CWE	Cold water extract
DAD	Diode array detector
FCM	Food contact material
ILC	Inter-laboratory comparison
LC	Liquid chromatography
LOD	Limit of detection
LOQ	Limit of quantification
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MU	Measurement uncertainty
NRL	National Reference Laboratory
OCL	Official Control Laboratory
PAA	Primary aromatic amine
c	$c=F1\sigma_{allow}^2+F2s_w^2$; is used to expand the criterion to allow for the actual sampling error and repeatability
$F1, F2$	Factors used in testing for sufficient homogeneity
k	Coverage factor
$s_{\bar{x}}$	Standard deviation of sample averages
s_w	Within-sample standard deviation
s_s	Estimate of between-sample standard deviation
$u(x_i)$	Calculated standard uncertainty of mean value from participant i
$u(x_i)_{\%}$	Calculated relative standard uncertainty of mean value from participant i
$U(x_i)$	Expanded uncertainty of reported result from participant i
$U(x_i)_{\%}$	Calculated relative expanded uncertainty from participant i
x_i	Mean value, calculated from single values reported by the participant i
x_{pt}	Assigned value
$\bar{\bar{x}}_{pt}$	Robust mean of participants' results
$u(x_{pt})$	Standard uncertainty of the assigned value
$U(x_{pt})$	Expanded uncertainty of the assigned value
$u(\bar{\bar{x}}_{pt})$	Standard uncertainty of the robust mean of participants' results
$U(\bar{\bar{x}}_{pt})$	Expanded uncertainty of the robust mean of participants' results
z	Score used for proficiency assessment
z'	Modified z score that includes the uncertainty of the assigned value
ζ	Modified z score that includes uncertainties for the participant result and the assigned value
$D\%$	Estimate of deviation
σ_{allow}	$\sigma_{allow} = 0.3\ \sigma_{pt}$; criterion of sufficient homogeneity
σ_{pt}	Standard deviation for proficiency assessment

3 Introduction

The inter-laboratory comparison (ILC) exercise NRL-DE-FCM-01/2021 on the determination of primary aromatic amines (PAAs) and amides from cold water extracts (CWEs) of paper food contact materials (FCM) was organized by the German National Reference Laboratory for Food Contact Materials (NRL-DE-FCM) established within the Unit Product Analytics of the Department of Chemical and Product Safety at the German Federal Institute for Risk Assessment (BfR). The primary aims of the exercise were the identification and quantification of PAAs in CWEs of a red-colored napkin sample prepared according to DIN EN 645 [3] and in solutions provided to the participants by the ILC organizer.

In BfR recommendation XXXVI [1], BfR restricts the release of PAAs from paper and board FCM. PAAs should not be released from these materials in a "detectable amount". For the sum of PAAs an analytical detection limit of 0.01 mg kg^{-1} food or food simulant should be applied. PAAs classified as carcinogens in class 1A and 1B of the EU regulation (EC) No. 1272/2008 [2] should not be detectable presuming an analytical detection limit of 0.002 mg kg^{-1} food or food simulant for each substance. This ILC was primarily designed to assess the analytical capabilities of the participating laboratories to correctly identify and quantify PAAs in solution and in CWEs of colored napkins in these low concentration ranges.

The following samples and solutions were provided to the participants:

- Sample 1: red-colored napkin sample
- Solution No. 1: blank matrix spiked with selected PAAs in low concentration (<2 µg L^{-1} for individual PAAs)
- Solution No. 2: blank matrix spiked with the same analytes as found in Sample 1
- Solution No. 3: blank matrix spiked with selected PAAs (>10 µg L^{-1} for the sum of PAAs)

The solutions were prepared in the labs of the NRL-DE-FCM. Solution No. 1 was blank matrix (CWE of white, i.e. non-colored napkins) spiked with *o*-toluidine and 4-aminobiphenyl. Solution No. 2 was blank matrix spiked with aniline, 3-hydroxy-2-naphthanilide, and 3-hydroxy-2-naphthoic acid to match the CWE of Sample. 1. Solution No. 3 was blank matrix spiked with 4-aminoazobenzene, *o*-anisidine, 4-chloroaniline and 2,4-dimethylaniline.
The cold water extraction of the red-colored napkin sample (Sample 1) had to be carried out in triplicate according to DIN EN 645 [3] with the adjustments specified in the instructions provided to the participants along with the samples (see chapter 13.1 and [4]). Participants could freely choose the analytical technique (e.g. LC-DAD or LC-MS/MS) for the identification and quantification of analytes in CWEs and in Solutions No.1–3.

This proficiency test was open to National Reference Laboratories (NRLs), Official Control Laboratories (OCLs) and the European Union Reference Laboratory for Food Contact Materials (EURL-FCM). This report summarizes the outcome of the ILC exercise.

Table 1: Participating laboratories.

Organization	Country
Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit (LGL) (OCL)	Germany
Bundesinstitut für Risikobewertung (BfR) (NRL)	Germany
Centro Nacional Alimentacion – Agencia Española de Seguridad Alimentaria y Nutrición (AESAN) (NRL)	Spain
Chemisches und Veterinäruntersuchungsamt (CVUA) Münsterland-Emscher-Lippe (OCL)	Germany
Chemisches und Veterinäruntersuchungsamt (CVUA) Stuttgart (OCL)	Germany
Croatian Institute of Public Health (NRL)	Croatia
Fera Science Ltd. (NRL)	United Kingdom
General Chemical State Laboratory (NRL)	Greece
Health Board (NRL)	Estonia
Kantonales Labor Zürich (KLZH) (NRL)	Switzerland
Landesuntersuchungsanstalt für das Gesundheits- und Veterinärwesen (LUA) Sachsen (OCL)	Germany
National Institute of Public Health (SZU) (NRL)	Czech Republic
National Laboratory of Health, Environment and Food (NRL)	Slovenia
Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES) (NRL)	Austria
Regional Public Health Authority (NRL)	Slovakia
Universidade Católica Portuguesa – Escola Superior de Biotecnologia (NRL)	Portugal
Zentrales Institut des Sanitätsdienstes der Bundeswehr München (UA Bundeswehr) (OCL)	Germany

The laboratory codes were allocated randomly to the participants and do not correspond to the alphabetical order shown here.

4 Scope

As stated in Regulation (EU) 2017/625 [6] one of the core duties of NRLs is to organize ILCs and proficiency tests between OCLs. The present ILC primarily aimed to assess the analytical capabilities of OCLs regarding the correct identification and quantification of PAAs in CWEs of a red-colored napkin sample and in solutions provided by the ILC organizer and was additionally open for NRLs and the EURL-FCM. The CWE should be prepared according to DIN EN 645 [3] with slight adjustments [4].

This ILC is identified as “NRL-DE-FCM-01/2021”.

5 Set up of the exercise

5.1 Time frame of the ILC

The invitation for the NRL-DE-FCM-01/2021 was sent on 20th of April 2021 and registration was open until 1st of May 2021. Samples were sent to the participants on 4th of May 2021 and the deadline for reporting of results was set to 30th of June 2021. Due to the pandemic situation, this deadline was extended until 16th of July 2021 for individual laboratories. For one laboratory, extension of the deadline was granted until 21st of July 2021.

5.2 Quality assurance

The NRL-DE-FCM has a quality management system according to DIN EN ISO/IEC 17025 [7]. The reported results were evaluated following the relevant administrative and logistic procedures.

5.3 Confidentiality

The procedures used for the organization of this ILC exercise guarantee that the identity of the participants and the information provided by them is treated as confidential. The participants in this ILC received a unique laboratory code used throughout this report.

5.4 Distribution

Each participant received:

- 1 red-colored napkin sample (Sample 1; >30 g)
- 3 solutions (Solutions No. 1, No. 2, and No. 3; 5 mL each)
- NRL_DE_FCM_01_2021_Confirmation of receipt_LC_0__.pdf
- NRL_DE_FCM_01_2021_Instructions.pdf
- NRL_DE_FCM_01_2021_Questionnaire_Results_LC_0__.xlsx

5.5 Instructions to participants

Participants were asked to check and report whether the test items were undamaged after transport using the “NRL_DE_FCM_01_2021_Confirmation of receipt_LC_0__.pdf” form.

Detailed instructions on the ILC were given to the participants in the document “NRL_DE_FCM_01_2021_Instructions.pdf”. In brief, participants were asked to prepare CWEs of the provided red-colored napkin sample (Sample 1) according to DIN EN 645 [3] with the adjustments stated in the provided instructions (see chapter 13.1) and to analyze the CWEs along with the Solutions No. 1–3.

Results and general information about the analytical procedure were inquired in the form “NRL_DE_FCM_01_2021_Questionnaire_Results_LC_0__.xlsx”. The questionnaire form was divided into three sheets: “General”, “CWE”, and “Results”. The sheet “General” contained questions about the laboratory and the analytical method used. Information about the experimental procedure was inquired in the sheet “CWE”. In the sheet “Results”, the identified compounds along with the CAS number should be named. Additionally, the results [in µg L^{-1}] for

the identified compounds along with the corresponding expanded measurement uncertainty (MU) and the coverage factor *k*, the limit of detection (LOD) and the limit of quantification (LOQ) were inquired.

To evaluate the data from the ILC more comprehensively, the ILC organizer inquired further information on the analyses dates and storage conditions of the provided test items from the participants after closure of the results submission period.

6 Test items

6.1 Preparation

6.1.1 Red-colored napkin sample

Commercially available red-colored napkins were cut into pieces (1–2 cm^2) according to DIN EN 645 [3], mixed by manual shaking and stored in a wide neck barrel at 22 °C. The aliquots of Sample 1 (>30 g) for the participants were provided in air tightly sealed clear plastic bags (which were free of PAAs).

6.1.2 Solutions

Solutions No. 1–3 were prepared in blank matrix. Blank matrix was a CWE of white, i.e. non-colored napkins, prepared according to DIN EN 645 [3] and autoclaved before use. Selected PAAs, 3-hydroxy-2-naphthanilide and 3-hydroxy-2-naphthoic acid were spiked to yield the following solutions, which were prepared shortly before shipment and stored light protected at 4 °C until dispatch.

Table 2: Overview of Solutions No. 1–3.

	Compound Name	CAS-No.	Target concentration
Solution No. 1	*o*-Toluidine 4-Aminobiphenyl	95-53-4 92-67-1	<2 µg L^{-1} for individual PAAs
Solution No. 2	Aniline 3-Hydroxy-2-naphthanilide* 3-Hydroxy-2-naphthoic acid*	62-53-3 92-77-3 92-70-6	Comparable to CWE of Sample 1
Solution No. 3	4-Aminoazobenzene *o*-Anisidine 4-Chloroaniline 2,4-Dimethylaniline	60-09-3 90-04-0 106-47-8 95-68-1	>10 µg L^{-1} for the sum of PAAs

* not evaluated within the scope of this report.

6.2 Homogeneity and stability

Homogeneity and stability studies as well as statistical data evaluation were performed by the NRL-DE-FCM. Homogeneity of Sample 1 and Solutions No. 1–3 was tested using statistical methods described in DIN ISO 13528 [5] and in IUPAC's harmonized protocol [8]. The test items were demonstrated to be adequately homogeneous (see chapter 13.2).

The stability of aniline in Sample 1 was confirmed according to ISO 13528 B.5.1 [5] over a period of about 10 months.

The stability of PAAs in the provided solutions was evaluated according to ISO 13528 B.5.2c [5] with a σ_{pt} of 20 %. This evaluation revealed that the expected stability of the PAAs spiked to Solutions No. 2 and 3 is as follows: 87 days for 4-chloroaniline, 74 days for 2,4-dimethylaniline, 65 days for 4-aminoazobenzene, 59 days for *o*-anisidine, and 42 days for aniline. Except for aniline, these periods are longer than the period initially planned for the ILC (57 days). Most of the laboratories reported the results before the initial deadline. However, due to the pandemic situation, the deadline was extended for 16 to 21 days for individual laboratories. In order to ensure that the results are not significantly influenced by the instability of the components, additional stability evaluations (see chapter 8.4.1) were conducted from the results submitted by the participants. For these evaluations, additional information inquired from the participants regarding analysis day and storage conditions prior to the analysis were considered.

The findings from these additional analyses support the statement that the stability of analyzed PAAs was sufficient for the period of this ILC.

Nevertheless, in the light that a significant variability was observed in the participants' results, a potential influence of PAA instability cannot be excluded and measures were taken for appropriate evaluation of the ILC data (e.g. *z'* score and *D%,* see paragraphs 0 and 0).

7 Assigned values and standard uncertainties

No reference values were available for the measurands in question. Thus, for the evaluation of aniline in the CWEs of Sample 1 the assigned value x_{pt} was calculated as a robust mean (using Hampel estimator [5, 9]) of the single results reported by the participants. For 4-amino-azobenzene and 2,4-dimethylaniline in Solution No. 3, x_{pt} was calculated as a robust mean of the results reported by the participants. Following the recommendations of ISO 13528 [5] and IUPAC's harmonized protocol [8], outliers were identified via Grubb's test [10] and eliminated before calculating x_{pt} and $u(x_{pt})$.

No reliable x_{pt} values could be assigned for aniline in Solution No. 2 as well as for 4-chloroaniline and *o*-anisidine in Solution No. 3, since the proportions $u(x_{pt})/\sigma_{pt}$ were found to be much higher than 0.3. For these PAAs the robust mean (using Hampel estimator [5, 9]) was calculated after outlier elimination (Grubb's test [10], according to recommendations in [5] and [8]) and defined as participants' robust mean $\bar{\bar{x}}_{pt}$.

The standard uncertainties of the assigned values $u(x_{pt})$ were estimated according to ISO 13528 [5]:

$$u(x_{pt}) = 1.25\frac{s^*}{\sqrt{p}} \qquad \text{Equation 1}$$

where s^* is the robust standard deviation of mean values (according to the Q/Hampel method [5, 9]) of the results reported by the participants and p is the number of participants. In this model, where the assigned value x_{pt} and the robust standard deviation s^* are determined from participants results, the uncertainty of the assigned value $u(x_{pt})$ can be assumed to include the effects of uncertainty due to inhomogeneity, transport, and instability [5].

The standard uncertainties of the participants' robust means $u(\bar{\bar{x}}_{pt})$ were calculated accordingly.

Based on expert judgment, relative standard deviations for proficiency assessment σ_{pt} were set to 25 % for the extraction experiments (Sample 1) and to 20 % for the test solutions (Solutions No. 2–3).

o-Toluidine and 4-aminobiphenyl were spiked to Solution No. 1 in concentrations below 2 µg L^{-1} to test the laboratories' analytical limits. The data were evaluated qualitatively only, based on correct identification of the analytes in question.

Table 3 summarizes the relevant parameters needed for scoring of PAAs in the test items.

Table 3: Relevant parameters related to the determination of PAAs in the CWE and in the solutions.

CWE of Sample 1						
	x_{pt} ± $U(x_{pt})$* [µg L^{-1}]		σ_{pt} [µg L^{-1}]	[% of x_{pt}]	$u(x_{pt})/\sigma_{pt}$	Score
Aniline	4.56 ± 0.73		1.14	25	0.32	*z*

Solution No. 1	
o-Toluidine	Qualitative evaluation only
4-Aminobiphenyl	

Solution No. 2					
	$\bar{\bar{x}}_{pt}$ ± $U(\bar{\bar{x}}_{pt})$* [µg L^{-1}]	σ_{pt} [µg L^{-1}]	[% of $\bar{\bar{x}}_{pt}$]	$u(\bar{\bar{x}}_{pt})/\sigma_{pt}$	Score
Aniline	2.57 ± 0.74	0.51	20	0.72	*D%*

Solution No. 3					
	x_{pt} ± $U(x_{pt})$* [µg L^{-1}]	σ_{pt} [µg L^{-1}]	[% of x_{pt}]	$u(x_{pt})/\sigma_{pt}$	Score
4-Aminoazobenzene	2.40 ± 0.55	0.48	20	0.57	*z´*
2,4-Dimethylaniline	4.28 ± 0.96	0.86	20	0.56	*z´*
	$\bar{\bar{x}}_{pt}$ ± $U(\bar{\bar{x}}_{pt})$* [µg L^{-1}]	σ_{pt} [µg L^{-1}]	[% of $\bar{\bar{x}}_{pt}$]	$u(\bar{\bar{x}}_{pt})/\sigma_{pt}$	Score
4-Chloroaniline	1.99 ± 0.53	0.40	20	0.67	*D%*
o-Anisidine	4.60 ± 1.43	0.92	20	0.78	*D%*

x_{pt} and $u(x_{pt})$ as well as $\bar{\bar{x}}_{pt}$ and $u(\bar{\bar{x}}_{pt})$ were estimated from the results reported by the participants using the Q/Hampel method (outlier eliminated following Grubb's test); *$U(x_{pt})$ and $U(\bar{\bar{x}}_{pt})$ are the expanded uncertainties at a coverage factor of *k*=2.

8 Evaluation

8.1 Scores and evaluation criteria

The individual laboratory's performance for aniline in the CWE of Sample 1 was expressed in terms of *z* and *ζ* scores according to ISO 13528 [5]. The *z* score describes the deviation between the participants' mean and the assigned value in terms of the standard deviation for proficiency assessment (σ_{pt}). The *ζ* score is a modified *z* score that includes uncertainties of the participants' results and the assigned value. It can be used in addition to the *z* score in order to evaluate whether the participants' results are close to the assigned value within their reported uncertainty. The *z* and *ζ* scores for the proficiency test results x_i were calculated as follows:

$$z_i = \frac{x_i - x_{pt}}{\sigma_{pt}} \qquad \text{Equation 2}$$

$$\zeta_i = \frac{x_i - x_{pt}}{\sqrt{u^2(x_i) + u^2(x_{pt})}} \qquad \text{Equation 3}$$

z' scores were used instead of *z* scores, when the proportion $u(x_{pt})/\sigma_{pt}$ was significantly higher than 0.3.

$$z_i' = \frac{x_i - x_{pt}}{\sqrt{\sigma_{pt}^2 + u^2(x_{pt})}} \qquad \text{Equation 4}$$

where:

x_i	is the mean value, calculated from single values reported by the participant *i,*
x_{pt}	is the assigned value,
σ_{pt}	is the standard deviation for proficiency test assessment,
$u(x_i)$	is the calculated standard uncertainty of mean value from participant *i,*
$u(x_{pt})$	is the standard uncertainty of the assigned value.

The interpretation of the *z* and *ζ* performance scores is done according to ISO 13528 [5]:

$\lvert z_i \rvert \leq 2.00$	acceptable performance	(green in chapter 13.4),
$2.00 < \lvert z_i \rvert < 3.00$	questionable performance	(yellow in chapter 13.4),
$\lvert z_i \rvert \geq 3.00$	unacceptable performance	(red in chapter 13.4).

No *z* or *z'* scores were calculated when the proportion $u(x_{pt})/\sigma_{pt}$ was higher than 0.6. In this case the results were assessed using estimates of deviation (*D%*, see ISO 13528 [4]). This parameter was not scored, however, it may allow participants to compare their results with each other.

$$D\% = 100\% \frac{x_i - \bar{\bar{x}}_{pt}}{\bar{\bar{x}}_{pt}} \qquad \text{Equation 5}$$

where:

x_i	is the value reported by the participant *i,*
$\bar{\bar{x}}_{pt}$	is the robust mean of participants' results.

The standard measurement uncertainty for the individual analytes in each laboratory $u(x_i)$ was calculated by dividing the reported expanded measurement uncertainty $U(x_i)$ by the reported coverage factor k.

In order to verify how reasonable these measurement uncertainties are, an additional assessment was performed for each $u(x_i)$ [5]. For this purpose, the relative standard uncertainty of the mean value from participant *"i"* was calculated:

$$u(x_i)_{\%} = 100\% \left(\frac{u(x_i)}{x_i} \right) \qquad \text{Equation 6}$$

The values of $u(x_i)_{\%}$ were divided into three groups:

a: $u_{min\,\%} \leq u(x_i)_{\%} \leq u_{max\,\%}$ reasonable estimation of $u(x_i)_{\%}$,

b: $u(x_i)_{\%} < u_{min\%}$ underestimation of $u(x_i)_{\%}$,

c: $u(x_i)_{\%} > u_{max\,\%}$ overestimation of $u(x_i)_{\%}$,

where:

$u_{min\,\%} = u(x_{pt})_{\%} = 100\% \left(\frac{u(x_{pt})}{x_{pt}} \right)$ is the minimum of the accepted relative standard uncertainty

$u_{max\,\%} = \sigma_{pt\,\%} = 100\% \left(\frac{\sigma_{pt}}{x_{pt}} \right)$ is the maximum of the accepted relative standard uncertainty

If $u(x_i)_{\%}$ is in the range between the minimum and maximum of the allowed uncertainty (case "a") the laboratory's standard uncertainty may have been reasonably estimated.
If $u(x_i)_{\%}$ is smaller than $u_{min\%} = u(x_{pt})_{\%}$ (case "b") the laboratory's standard uncertainty may have been underestimated. However, the following should be taken into account. Because the values of $u(x_{pt})$ were derived from the robust standard deviation of the single results reported by the participants, these values include contributions from inhomogeneity, transport, and instability. Therefore, a relative standard uncertainty $u(x_i)_{\%}$ smaller than $u(x_{pt})_{\%}$ is possible and plausible if these contributions are significant [5].

If $u(x_i)_{\%}$ is larger than $u_{max\%} = \sigma_{pt\%}$ (case "c") the laboratory's standard uncertainty may have been overestimated. However, if $u(x_i)_{\%} > \sigma_{pt\%}$ but x_i agrees with x_{pt} within their respective expanded measurement uncertainties, then the measurement uncertainty is properly assessed. In this case, however, the usefulness of the corresponding z score for the performance evaluation may be questionable.

8.2 General observations

Originally, twenty laboratories registered for the ILC, of which seventeen from twelve EU Member States submitted results. Of the latter, two reported lacks in sensitivity due to high LOQs and were not able to quantify the analytes in the provided test items.

While most laboratories used liquid chromatography in combination with tandem quadrupole or quadrupole/time-of-flight mass spectrometry (LC-MS) for the quantification of analytes in this ILC, four laboratories used LC in combination with diode array detectors (LC-DAD) (see Table 4).

Table 4: Analytical techniques used in this ILC for the analysis of PAAs.

Technique	No. of Labs
LC-MS	13
LC-DAD	4
Total	17

Since the primary aim of the ILC was the identification and quantification of PAAs in CWEs of a colored napkin sample and in solutions, results were evaluated qualitatively (based on correctly identified compounds) and quantitatively (based on reported concentrations).

8.3 Qualitative Evaluation

Solution No. 1 was spiked with PAAs in concentrations <2 µg L^{-1} in order to assess the capability of laboratories to identify the compounds based on BfR Recommendation XXXVI [1]. This recommendation stipulates that PAAs classified as carcinogens in class 1A and 1B of EU regulation (EC) No. 1272/2008 should not be released from paper and board FCM presuming an analytical detection limit of 0.002 mg kg^{-1} food or food simulant. Solution No. 1 was spiked with *o*-toluidine (Carc. 1B) and 4-aminobiphenyl (Carc. 1A). Figure 1 presents an overview of the compounds identified by the participating laboratories.

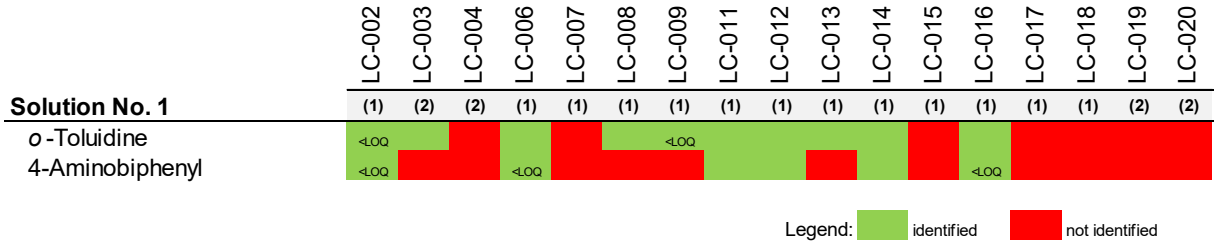

Figure 1: Qualitative evaluation of the PAAs spiked to Solution No. 1. *o*-Toluidine and 4-aminobiphenyl were spiked to blank matrix (CWE of non-colored napkins) at a concentration of ≈ 1 µg L^{-1}. The analytical method used is indicated as follows: (1) LC-MS and (2) LC-DAD; <LOQ: found concentration was below the laboratory's limit of quantification.

Solution No. 3 was spiked with 4-aminoazobenzene, *o*-anisidine, 4-chloroaniline and 2,4-dimethylaniline with a sum concentration >10 µg L^{-1}, which relates to the current limit defined in BfR Recommendation XXXVI (sum of PAAs ≤10 µg L^{-1}) [1]. The individual concentrations of the analytes in Solution No. 3 were about 2–5 fold higher compared to those of the compounds in Solution No. 1. Figure 2 summarizes the performance of laboratories in identifying compounds in Solution No. 3.

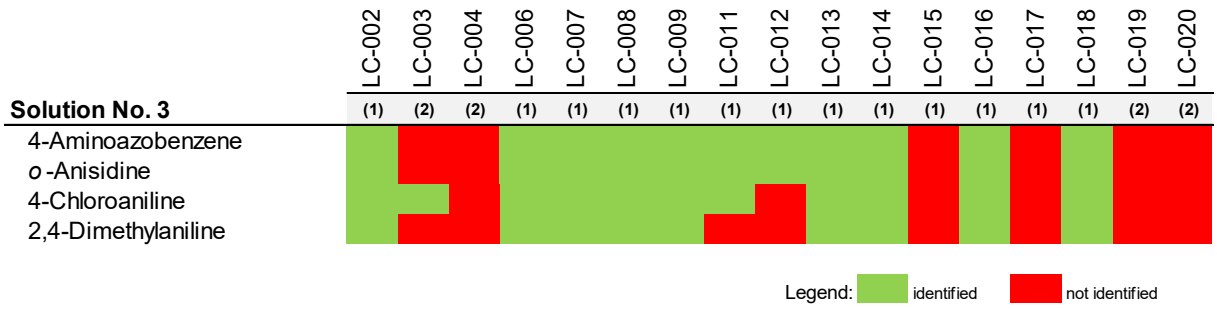

Figure 2: Qualitative evaluation of the PAAs spiked to Solution No. 3. 4-Aminoazobenzene, *o*-anisidine, 4-chloroaniline and 2,4-dimethylaniline were spiked to blank matrix (CWE of non-colored napkins) at a sum concentration of >10 µg L^{-1}. The analytical method used is indicated as follows: (1) LC-MS and (2) LC-DAD.

It is noticeable that mostly LC-DAD based methods were not suitable for the identification of PAAs. Five laboratories were not able to identify any compound in Solutions No. 1 or 3, three of which used LC-DAD based methods. Only one laboratory using LC-DAD was able to identify at least one analyte. A reason might be lacking sensitivity, which has been reported by one lab. Moreover, the number of not identified or falsely identified analytes was higher for LC-DAD compared to LC-MS. It should be noted that some laboratories using LC-MS apparently had difficulties in the identification of PAAs, too, and one laboratory also reported lacking sensitivity. Nevertheless, from the present data it can be concluded that the DAD detector is not suitable for correct identification and subsequent quantification of PAAs in concentrations below 10 µg L^{-1}.

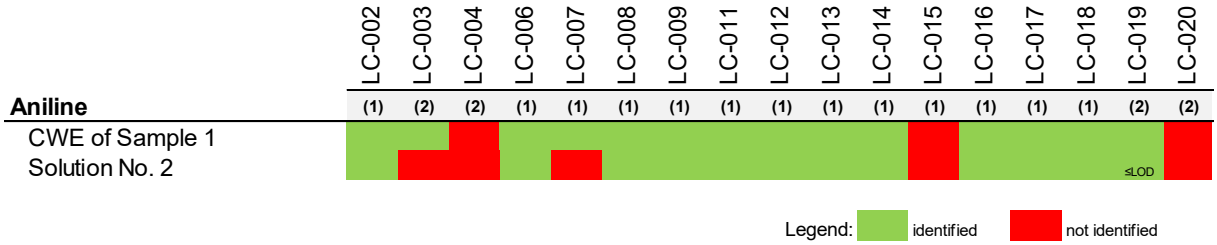

Figure 3: Qualitative evaluation of aniline in the CWE of Sample 1 and in Solution No. 2. The analytical method used is indicated as follows: (1) LC-MS and (2) LC-DAD. ≤LOD: found concentration was below or equal to the laboratory's limit of detection.

Regarding the CWE of Sample 1, the majority of laboratories was able to correctly identify aniline as a representative PAA (Figure 3). Most of these laboratories also correctly identified aniline in Solution No. 2. Again, mainly labs running LC-DAD based methods had problems identifying aniline correctly.

8.4 Quantitative evaluation – Laboratory results and scorings

Detailed evaluation of the quantitative data for the individual laboratories can be found in the Annex (see chapter 13).

8.4.1 Stability of analytes in provided solutions

Although limited stability of PAAs in solution is a known issue, only little experimental data is available on this topic. Unpublished data indicates that stability of PAAs is highly dependent on the experimental conditions during extraction or migration experiments (e.g. temperature, pH and time) as well as on the matrix and simulant used. These are crucial parameters, in particular if PAAs are stored in the extract or migrate prior to the analysis. Experiments performed by the NRL-DE-FCM prior to the ILC revealed that all PAAs investigated in this ILC are stable for the whole ILC-period (57 days as initially planned; 78 days with extension) in the CWE matrix prepared from red-colored napkins. However, in the CWE blank matrix prepared from non-colored napkins, the stability of the selected spiked PAAs was found to be limited. According to the performed experiments, the expected stability of the selected PAAs in the blank matrix (calculated according to ISO 13528, B.5.2c [5] with a σ_{pt} of 20 %) is as follows: 87 days for 4-chloroaniline, 74 days for 2,4-dimethylaniline, 65 days for 4-aminoazobenzene, 59 days for *o*-anisidine, and 42 days for aniline. For all selected PAAs, except for aniline, these periods are longer than the period initially planned for the ILC (57 days). Despite the limited stability of aniline, the non-colored napkin matrix has a number of advantages. This matrix was free of PAAs of interest (<LOD) allowing the preparation of solutions at low concentrations, it has a lower optical density, and was thus assumed to be better suited for UV-VIS detection

(e.g. DAD). Therefore, the non-colored napkin matrix was selected for the preparation of the solutions for this ILC round.

Most of the laboratories reported the results before the initial deadline. However, due to the pandemic situation, the deadline was extended for 16–21 days for individual laboratories.

In order to ensure that the results were not significantly impaired by the instability of the components, the participating laboratories were asked to provide information about the analysis day and storage conditions prior to the analysis. All laboratories reported that the samples were stored at temperatures ≤ 9 °C and that the samples were analyzed in a period of 8–73 days after dispatch. The reported number of days prior to the analysis can be found in Figure 4.

Despite the large interval between the first and the last analysis (65 days), 8 of 11 laboratories (submitting results for Solutions No. 2 and 3) performed the analysis within 28 days and 6 of 11 laboratories within 7 days (Figure 4). It is of note that for all analyzed PAAs the robust mean calculated for all submitted results (65 days) differs only insignificantly from those calculated for much narrower periods of analysis (28 and 7 days) (calculated using Student's t-test). The differences between the calculated mean values for all analyzed PAAs are within the range of 3–12 %, which is within the respective standard uncertainties (see Figure 5). Moreover, no clear correlation between the reported results and the period prior to the analysis was observed ($R^2 < 0.48$, data not shown). These findings support the statement that the stability of analyzed PAAs was sufficient in the context of this ILC.

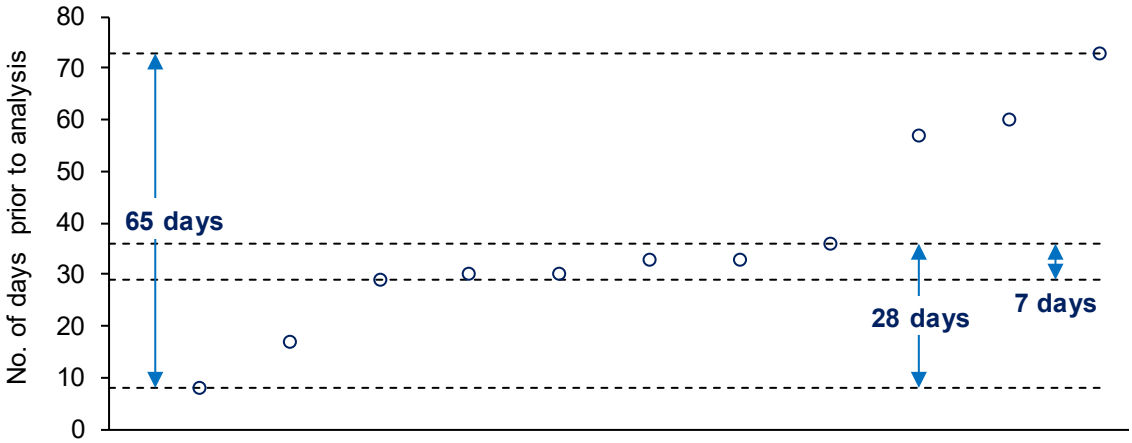

Figure 4: Number of days between samples' dispatch and the reported analysis date. For this evaluation, only the 11 laboratories submitting results for Solutions No. 2 and 3 were considered. The dotted lines and arrows indicate the periods used for stability assessment (see Figure 5).

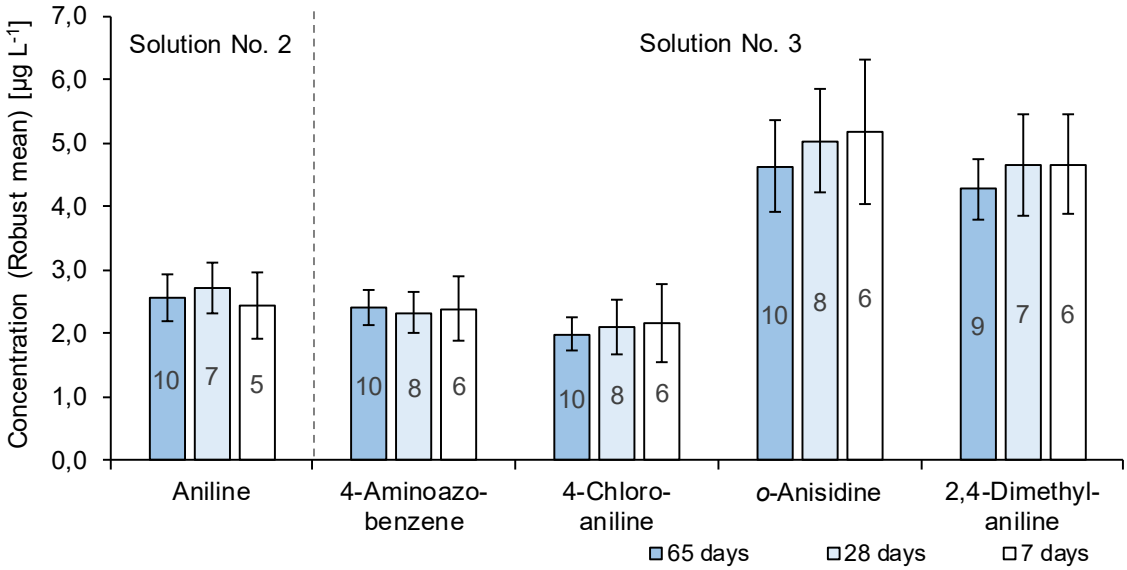

Figure 5: Participants' robust mean values calculated for all submitted results (65 days analysis interval) in comparison to those calculated for 28 and 7 days analysis interval. Bars indicate corresponding standard uncertainties and numbers inside the bars reflect the considered number of results. Prior to calculations, outliers were identified (Grubb's test, 2-site, 99 %) and eliminated.

Nevertheless, in the view of a potential influence of the instability and because the proportions $u(x_{pt})/\sigma_{pt}$ were found to be higher than 0.6, no *z* or *z'* scores were calculated for aniline in Solution No. 2 and for 4-chloroaniline and *o*-anisidine in Solution No. 3. Instead, results were assessed using estimates of deviation (*D%*). This parameter was not scored; however, it may allow participants to compare their results with each other.

8.4.2 Performance

z scores were calculated for the results of aniline in CWEs of Sample 1. For the results of 2,4-dimethylaniline and 4-aminoazobenzene in Solution No. 3 *z'* scores instead of *z* scores were calculated since the proportions $u(x_{pt})/\sigma_{pt}$ were significantly higher than 0.3. For aniline in Solution No. 2 as well as for 4-chloroaniline and *o*-anisidine in Solution No. 3 the results were assessed using *D%* because the proportions $u(x_{pt})/\sigma_{pt}$ were found to be higher than 0.6. ζ scores were calculated for all laboratories reporting MUs. A graphical overview of the laboratories' performance for aniline in the CWE of Sample 1 as well as for 2,4-dimethylaniline and 4-aminoazobenzene in Solution No. 3 expressed by *z, z'* and ζ scores is given in Figure 6.

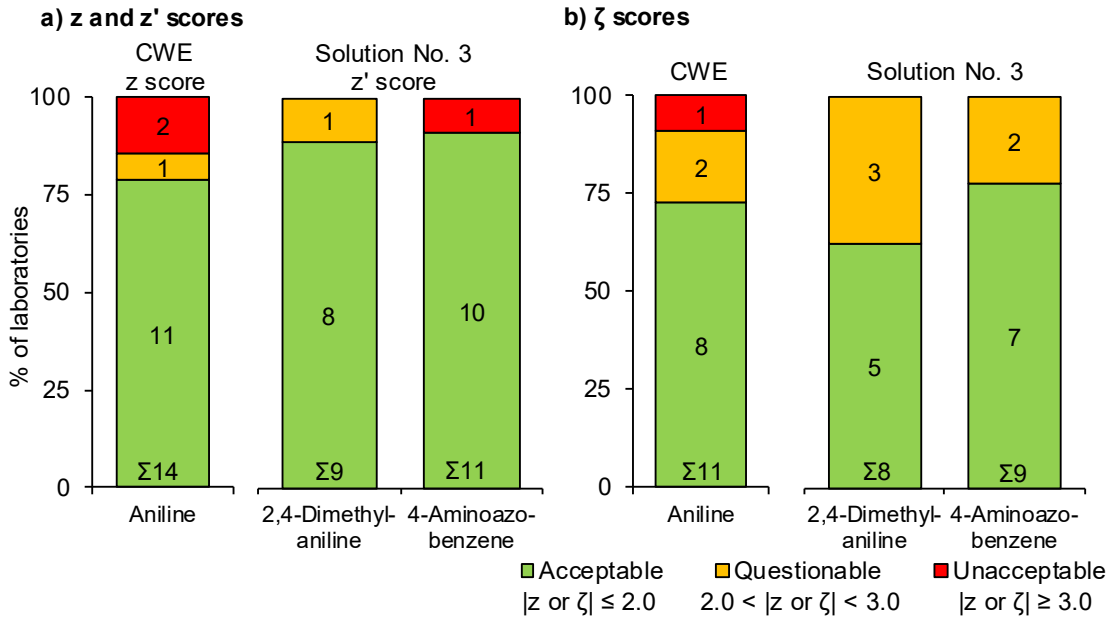

Figure 6: Overview of the laboratories' performance according to a) *z* or *z'* and b) *ζ* scores for the analysis of aniline in CWEs of Sample 1 as well as for 2,4-dimethylaniline and 4-aminoazobenzene in Solution No. 3. σ_{pt} was defined as 25 % of x_{pt} for CWEs and 20 % of x_{pt} for Solution No. 3. *z, z'* and *ζ* scores were determined using x_{pt} and $u(x_{pt})$ calculated from the participants' results. The numbers in the bars correspond to the number of laboratories assigned with the respective scoring. Three laboratories did not report MUs, therefore the number of laboratories differs between *z/z'* and *ζ* scores.

The majority of laboratories received acceptable *z* scores for the analysis of aniline in the CWE (79 %) and *z'* scores for the analysis of PAAs in Solution No. 3 (89 % for 2,4-dimethylaniline and 91 % for 4-aminoazobenzene). For each compound, *z* or *z'* scores for only one to three laboratories were either questionable or unacceptable. Most of the questionable or unacceptable results for the different compounds could be assigned to the same three laboratories (see chapter 13.4). Comparison of the results for the CWEs (extraction step included) to the results obtained for the solutions (no extraction step included) revealed that two of these laboratories received unacceptable results for both types of test items.

ζ scores are a measure to evaluate the closeness of the reported value to the assigned value taking into account the MUs reported by the laboratories. Three laboratories did not submit MUs for the results; hence, no *ζ* scores could be calculated. From the remaining laboratories, the majority reached acceptable *ζ* scores for aniline (73 %) in CWEs of Sample 1 as well as for 2,4-dimethylaniline (63 %) and 4-aminoazobenzene (78 %) in Solution No. 3.

The results for aniline in Solution No. 2 as well as for 4-chloroaniline and *o*-anisidine in Solution No. 3 were assessed using *D%* and are depicted in Table 5.

Table 5: *D%* (estimate of deviation) for the PAAs in Solutions No. 2 and 3.

LC-	002	003	006	007	008	009	011	012	013	014	016	017	018
Solution No. 2													
Aniline	-30	-	32	-	-41	-7	43	379	-39	2	31	-14	23
Solution No. 3													
4-Chloroaniline	-40	6449	5	76	-26	-13	-16	-	-5	-16	39	-	21
o-Anisidine	-20	-	6	80	-37	-34	5	631	-30	3	25	-	23

"-" no result was submitted.

Similarly to the observed for *z/z'* scores, three laboratories demonstrated strong positive deviations (76 to 6449 %). However, most laboratories showed deviations from the calculated participants' robust mean within the range of 2 to 43 %, which is in the range of about $2*\sigma_{pt}$ (σ_{pt} was set to 20 % for solutions).

Overall, questionable (positive) *z/z'* scores and strong positive deviations according to *D%* were observed for the same laboratories. Therefore, it can be presumed that these laboratories have a positive bias due to their analytical methods.

8.4.3 Measurement uncertainties (MU)

According to the questionnaire, the majority of laboratories (12 out of 16) reported that they usually provide measurement uncertainties. Three laboratories did not submit MUs for their results, although they usually provide MUs for their customers. The questionnaire revealed that most laboratories estimated the measurement uncertainty based on in-house validation (11 out of 16) and without including the extraction step (11 out of 16). Other methods used were NORDTEST, GUM or the Horwitz equation.

Relative standard uncertainties $u(x_i)_{\%}$ were calculated from the submitted results and are shown in Table 6. Calculated relative $u(x_i)_{\%}$ ranged from 5–22 % for CWEs of Sample 1 and from 2–15 % for Solutions No. 2 and 3. Due to the additional uncertainty of the extraction procedure, the expected $u(x_i)_{\%}$ for CWEs should be higher compared to the solutions. However, only 3 of 9 laboratories submitted a higher $u(x_i)_{\%}$ for aniline in CWEs compared to Solution No. 2. In most cases (5 out of 9) the similar $u(x_i)_{\%}$ for CWEs and solutions were reported and one laboratory even submitted a higher $u(x_i)_{\%}$ for the solutions.

Table 6: Calculated relative standard uncertainties ($u(x_i)_{\%}$). The values of $u(x_i)_{\%}$ are rounded to the nearest hundredths.

LC-	002	006	007	008	009	011	014	016	017	018	019*
CWE											
Aniline	5	8	15	14	7	9	13	8	10	15	22
Solution No. 2											
Aniline	14	8	-	14	7	9	7	5	2	15	-
Solution No. 3											
4-Aminoazobenzene	3	8	15	12	5	8	8	8	-	15	-
o-Anisidine	12	8	15	12	13	10	7	8	-	15	-
4-Chloroaniline	8	7	15	13	6	10	7	8	-	15	-
2,4-Dimethylaniline	11	7	15	12	7	-	8	5	-	15	-

"-" no results were submitted for this analyte; * *k* was set to 2 by the ILC coordinator because no coverage factor was reported.

The calculated relative standard uncertainties were compared to the maximum and minimum of accepted relative standard uncertainty values (see and assigned to one of three cases: a) reasonable estimation of $u(x_i)_{\%}$, b) underestimation of $u(x_i)_{\%}$, and c) overestimation of $u(x_i)_{\%}$. The results are depicted in Figure 7. Most laboratories (7 out of 11) estimated $u(x_i)_{\%}$ reasonably for aniline in CWEs of Sample 1. Due to the high value of $u(x_{pt})/\sigma_{pt}$, the reported measurement uncertainties for the analysis of PAAs in Solutions No. 2 and No. 3 were not evaluated.

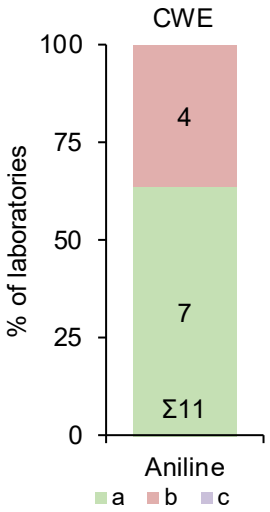

Figure 7: Evaluation of the reported relative measurement uncertainties $u(x_i)_{\%}$ for the analysis of aniline in CWEs of Sample 1. The numbers in the bars correspond to the number of laboratories assigned to the respective cases: "a": $u_{min\,\%} \leq u(x_i)_{\%} \leq u_{max\,\%}$; "b": $u(x_i)_{\%} < u_{min\,\%}$; "c": $u(x_i)_{\%} > u_{max\,\%}$.

8.5 Additional information extracted from the questionnaire

Additional information on general aspects of the ILC, the preparation of CWEs and the analytical method was extracted from the questionnaire and is summarized below. All questions and answers are listed in the Annex (see chapter 13.5).

General information

All participating laboratories have a quality management system according to ISO 17025 [7].

Six laboratories reported to use accredited methods and four laboratories used validated methods, while six laboratories used methods which were neither accredited nor validated. However, one of the latter reported to have validated the method for selected PAAs in other matrices than CWEs and two laboratories reported that the validation is in process.

Most laboratories test blank samples (15 out of 16); however, the type of blank differs. Examples of reported blank samples include water, CWEs of white napkins and PAA-free paper-matrix spiked with internal standards. Only four laboratories used certified reference materials for quality control.

Experience of the laboratories with the analytical methods used to determine PAAs is diverse. Six laboratories have been using the method for less than a year of which four reported that they never use the method. One laboratory uses the method 51–250 times a year. Six laboratories reported to have been using the method for more than five years. Out of these laboratories, four use the method 1–50 times a year and two use the method 51–250 times a year. No laboratory reported to use the method more than 250 times a year.

Most laboratories neither did encounter difficulties with the sample analysis nor applied any special treatment to the samples provided.

Cold water extracts

Most laboratories used glass fiber filters for CWEs (14 out of 17) and only three laboratories used different kinds of filters. Of these, two laboratories used filter papers and one laboratory used glass frits for filtration of the cold water extraction solution. Twelve laboratories used 11–50 mL to fill the volumetric flask after the extraction steps. Only one laboratory needed less than 10 mL and four laboratories needed more than 50 mL of water to fill the volumetric flask.

9 Conclusions

This ILC had two distinct aims. First, PAAs that were present in the provided samples (red-colored napkin sample and spiked solutions prepared at the NRL-DE-FCM) should be identified correctly. Second, the identified PAAs should be quantified.

The laboratories' performances in their capability to identify PAAs differed significantly. Most laboratories were able to identify some PAAs in the provided solutions and in CWEs while three did not identify any PAA. Only four labs identified all seven PAAs correctly. Of note, mainly participants using LC-DAD based methods had difficulties in identifying the correct PAAs in the test items. Therefore, LC-DAD seems not to be suited for the analysis of PAAs at the given (rather low) concentration ranges.

Quantitative results submitted by the participating laboratories were highly variable for selected PAAs. Experimental data suggests that the stability of analytes (except for aniline) in the provided solutions was sufficient for the time period initially planned for the ILC. However, to exclude influence on the results by potential instability of the analytes, additional data evaluation was conducted to investigate the effect of storage time in the laboratories prior to the analysis on the ILC results. No clear correlation was found. Therefore, it can be concluded that limited analyte stability is not a major issue contributing to the observed variability. Nevertheless, a systematic investigation of the stability of PAAs under various conditions (temperature, pH, time) is needed in order to further improve PAA analysis.

Despite these limitations, most laboratories scored well for the analysis of PAAs in CWEs and in solutions (according to z, z' and ζ scores). This emphasizes that many laboratories have established well performing analytical methods for quantification of PAAs. Notably, most of the questionable or unacceptable results observed were assigned to the same three laboratories, which showed a strong positive bias for both, CWEs and solutions. Moreover, two laboratories reported high LOQs and were not able to identify or quantify PAAs at concentrations relevant in the legal context.

Overall, the findings of this ILC highlight the need for further improvement and harmonization of PAA analysis in order to improve detection and quantification with regard to the legal limits set by national and international regulations.

10 References

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10. *DIN ISO 5725-2:2002-12; Accuracy (trueness and precision) of measurement methods and results - Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method.* 2002.

11 List of figures

12 List of tables

13 Annex

13.1 Instructions

Please perform cold water extracts (DIN EN 645) as specified below. Analyze the three additionally provided solutions together with the extracts. Please determine primary aromatic amines for Solutions No. 1 and 3 as well as primary aromatic amines and optionally amides for Solution No. 2 provided in the glass vials. All solutions are aqueous.

For added value of this entire study, we would appreciate if you could send us an aliquot (~15 mL) of each of the respective extracts. In consequence, we will examine all incoming solutions in one sequence with our LC-MS/MS. With a growing dataset we expect to improve the data basis for the estimation of the measurement uncertainty for the estimation of primary aromatic amines and amides from cold water extracts.

Before starting the experiments, please read the Questionnaire carefully so that you can answer all questions.

1. Cold water extract of the paper sample No. 1 (napkin) according to DIN EN 645
Please perform the cold water extracts according to DIN EN 645 in triplicate (use ultrapure water of at least type I), shaking is not necessary. Do **not** only decant the extract, instead perform a vacuum filtration of the extract with a glass fiber filter (e.g. Whatman; GF / C; 1.2 µm). The solution has to be transferred as completely as possible. The Erlenmeyer flask has to be rinsed twice with ultrapure water (2 x 20 mL) and the rinsing solution is poured over the filter cake. If necessary, carefully squeeze the remaining water out of the filter cake. The filtrate has to be transferred to a 250 mL volumetric flask and made up to the mark with ultrapure water. Please estimate (see questionnaire) the added volume (mL water) to finally fill the volumetric flask. Identify the primary aromatic amines and optionally the amides and determine their mass fractions in all extracts. Please send an aliquot (~15 mL) of each of the three extracts to the German NRL-FCM for further examination.

13.2 Homogeneity and stability of the samples and solutions

13.2.1 Homogeneity assessment of Sample 1

Table 7: Results for the homogeneity assessment of Sample 1 (red-colored napkin sample). Twelve test items were prepared and analyzed in duplicate. Results were evaluated according to ISO 13528 [5] and IUPAC's harmonized protocol [8] using the expanded criterion ($\sqrt{c}$) to consider the actual sampling error and repeatability. All results are reported in [µg L^{-1}].

	Aniline	
	1st	2nd
1	5.301	5.426
2	5.157	5.744
3	5.541	5.592
4	5.006	5.087
5	5.096	5.315
6	5.021	5.322
7	5.217	5.108
8	5.071	5.272
9	4.758	4.886
10	5.147	5.355
11	5.105	5.048
12	4.276	4.262
Mean*	5.130	
$s_{\bar{x}}$	0.333	
s_w	0.161	
s_s	0.313	
σ_{pt} (25% of Mean)	1.282	
σ_{allow}	0.385	
F1	1.790	
F2	0.860	
σ^2_{allow}	0.148	
c	0.287	
$\sqrt{c}$	0.536	
$s_s \leq \sqrt{c}$	passed	
Homogenous	YES	

* The mean concentration of aniline in the homogeneity assessment does not correspond to the robust mean calculated from participants' results because of the between-laboratory bias.

Where: $s_{\bar{x}}$ is the standard deviation of sample averages,
s_w is the within-sample standard deviation,
s_s is the estimate of between-sample standard deviation,

$$s_s = \sqrt{\max\left(0; s_x^2 - \frac{s_w^2}{m}\right)} \quad \text{Equation 7}$$

σ_{pt} is the standard deviation for proficiency assessment,
σ_{allow} = 0.3 σ_{pt}; criterion of sufficient homogeneity,
F1, F2 are factors for use in testing for sufficient homogeneity, can be calculated or be taken from standard statistical tables
c = $F1\ \sigma^2_{allow} + F2\ s_w^2$; is used to expand the criterion to allow for the actual sampling error and repeatability.

13.2.2 Homogeneity assessment of Solution No. 1

Table 8: Results for the homogeneity assessment for Solution No. 1. The analysis was performed in duplicate. Results were evaluated according to ISO 13528 [5]. All results are reported in [µg L^{-1}].

	4-Aminobiphenyl		*o*-Toluidine	
	1st	2nd	1st	2nd
1	1.057	0.948	1.071	1.005
2	0.993	0.953	1.049	0.987
3	1.049	1.029	1.053	0.971
4	1.090	0.946	0.992	1.039
5	1.013	1.095	1.057	1.052
6	0.918	1.011	1.160	0.991
7	0.976	0.974	1.084	1.065
8	1.044	0.975	1.048	1.152
9	1.019	0.929	0.997	1.032
10	1.062	1.017	1.035	1.090
11	1.044	0.969	1.072	1.077
12	1.090	0.932	1.073	1.059
Mean	1.005		1.050	
$s_{\bar{x}}$	0.029		0.030	
s_w	0.063		0.051	
s_s	0.000		0.000	
σ_{pt} (20% of Mean)	0.201		0.210	
σ_{allow}	0.060		0.063	
$s_s \leq \sigma_{allow}$	passed		passed	
Homogenous	YES		YES	

Where:
- $s_{\bar{x}}$ is the standard deviation of sample averages,
- s_w is the within-sample standard deviation,
- s_s is the estimate of between-sample standard deviation, see Equation 7
- σ_{pt} is the standard deviation for proficiency assessment,
- σ_{allow} = 0.3 σ_{pt}; criterion of sufficient homogeneity.

13.2.3 Homogeneity assessment of Solution No. 2

Table 9: Results for the homogeneity assessment for Solution No. 2. The analysis was performed in duplicate. Results were evaluated according to ISO 13528 [5]. All results are reported in [µg L^{-1}].

	Aniline	
	1st	2nd
1	4.150	3.395
2	3.909	3.530
3	3.722	3.487
4	3.698	3.719
5	3.730	3.539
6	3.619	4.838
7	2.812	4.050
8	3.837	3.551
9	4.003	3.810
10	4.122	3.641
11	4.132	3.758
12	4.388	4.103
Mean*	3.814	
$s_{\bar{x}}$	0.242	
s_w	0.428	
s_s	0.000	
σ_{pt} (20% of Mean)	0.763	
σ_{allow}	0.229	
$s_s \leq \sigma_{allow}$	passed	
Homogenous	YES	

*The mean concentration of aniline in the homogeneity assessment does not correspond to the robust mean calculated from participants' results because of the combination of between laboratory bias and stability issues (see chapter 8.4.1).

Where:
- $s_{\bar{x}}$ is the standard deviation of sample averages,
- s_w is the within-sample standard deviation,
- s_s is the estimate of between-sample standard deviation, see Equation 7
- σ_{pt} is the standard deviation for proficiency assessment,
- σ_{allow} = 0.3 σ_{pt}; criterion of sufficient homogeneity.

13.2.4 Homogeneity assessment of Solution No. 3

Table 10: Results for the homogeneity assessment for Solution No. 3. The analysis was performed in duplicate. Results were evaluated according to ISO 13528 [5]. All results are reported in [µg L^{-1}].

	4-Aminoazobenzene		2,4-Dimethylaniline		*o*-Anisidine		4-Chloroaniline	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
1	3.603	3.589	4.928	4.798	6.182	6.337	2.712	2.622
2	3.405	3.450	5.016	4.329	6.019	6.104	2.825	2.387
3	3.521	3.543	5.399	4.607	6.167	6.348	3.021	2.730
4	3.625	3.574	4.765	4.639	5.847	6.173	2.932	2.525
5	3.379	3.278	5.065	4.547	6.213	6.205	2.683	2.602
6	3.412	3.301	5.209	5.158	6.425	6.051	2.839	2.736
7	3.385	3.420	4.982	5.146	6.245	6.177	2.696	2.737
8	3.113	3.447	5.355	5.085	5.933	6.060	2.926	2.640
9	3.305	3.336	5.409	5.410	6.298	6.241	2.912	2.922
10	3.576	3.449	5.459	5.300	6.325	6.474	3.251	2.912
11	3.480	3.538	5.042	5.153	6.405	5.992	2.908	2.902
12	3.757	3.339	5.315	5.156	6.318	6.263	3.174	2.957
Mean*	3.451		5.053		6.200		2.815	
$s_{\bar{x}}$	0.113		0.249		0.120		0.157	
s_w	0.118		0.255		0.149		0.172	
s_s	0.075		0.171		0.058		0.099	
σ_{pt} (20% of Mean)	0.690		1.011		1.240		0.563	
σ_{allow}	0.207		0.303		0.372		0.169	
$s_s \leq \sigma_{allow}$	passed		passed		passed		passed	
Homogenous	YES		YES		YES		YES	

*The mean concentrations of PAAs in the homogeneity assessment do not correspond to the corresponding robust mean values calculated from participants' results because of the combination of between-laboratory bias and stability issues (see chapter 8.4.1).

Where:
- $s_{\bar{x}}$ is the standard deviation of sample averages,
- s_w is the within-sample standard deviation,
- s_s is the estimate of between-sample standard deviation, see Equation 7
- σ_{pt} is the standard deviation for proficiency assessment,
- σ_{allow} = 0.3 σ_{pt}; criterion of sufficient homogeneity.

13.3 Stability assessment of Sample 1

Table 11: Results of the stability assessment of Sample 1. Stability was tested over a period of about 10 months. All values are reported in [µg L^{-1}].

	Aniline
m_0	5.130
m_{10}	4.962
$\lvert m_0-m_{10}\rvert$	0.167
σ_{pt}	1.282
0.3 σ_{pt}	0.385
$\lvert m_0-m_{10}\rvert \leq 0.3\ \sigma_{pt}$	Passed
Assessment	Stable

Where: m_0 is the analysis in the beginning of the stability study,
m_{10} is the analysis in the end of the stability study,
σ_{pt} is the standard deviation for proficiency assessment, 25 %.

13.4 Results

13.4.1 Results for the determination of aniline in CWEs of Sample 1

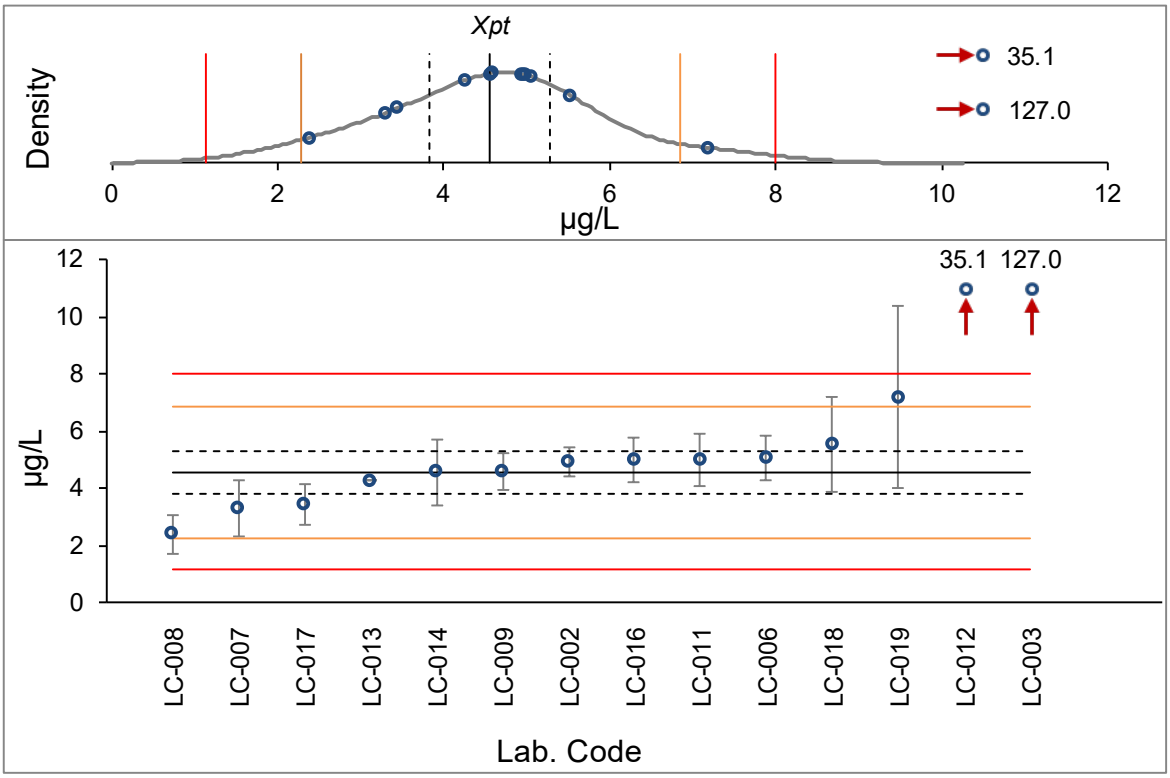

Figure 8: Measurement result range reported by the participants for the determination of aniline in CWEs of Sample 1. Circles and bars represent the reported results [x_i] with the corresponding expanded uncertainties [$U(x_i)$]; orange and red lines represent *z* scores = 2 and 3, respectively; solid and dotted black lines represent the assigned value [x_{pt}] and its expanded uncertainty [$U(x_{pt})$].

Table 12: Results for the determination of aniline in CWE of Sample 1. Assigned range: x_{pt} = 4.561 ± 0.726 µg L^{-1}; σ_{pt} = 1.140 µg L^{-1}; x_i and $U(x_i)$ values are reported in µg L^{-1}.

Lab. Code	x_i	$U(x_i)$	k	*z* score*	ζ score*	$u(x_i)_{\%}$ est.§
LC-002	4.95	0.50	2	0.34	0.87	b
LC-003	127.00	-	-	107.38	-	-
LC-006	5.07	0.76	2	0.44	0.96	b
LC-007	3.30	0.99	2	-1.11	-2.05	a
LC-008	2.39	0.67	2	-1.91	-4.41	a
LC-009	4.58	0.66	2	0.01	0.03	b
LC-011	4.98	0.90	2	0.37	0.72	a
LC-012	35.08	-	-	26.76	-	-
LC-013	4.26	-	-	-0.27	-	-
LC-014	4.56	1.14	2	0.00	-0.01	a
LC-016	4.96	0.78	2	0.35	0.76	b
LC-017	3.45	0.69	2	-0.98	-2.22	a
LC-018	5.52	1.66	2	0.84	1.06	a
LC-019	7.19	3.18	2**	2.31	1.61	a

* color code for z and ζ scores: green for |z or ζ| ≤ 2.00, orange for 2.00 < |z or ζ| < 3.00 and red for |z or ζ| ≥ 3.00.
§ (a) Reasonable estimation of $u(x_i)$; (b) underestimation of $u(x_i)$; (c) overestimation of $u(x_i)$.
** k = 2 was set by the ILC coordinator because no coverage factor was reported.

13.4.2 Results for the determination of aniline in Solution No. 2

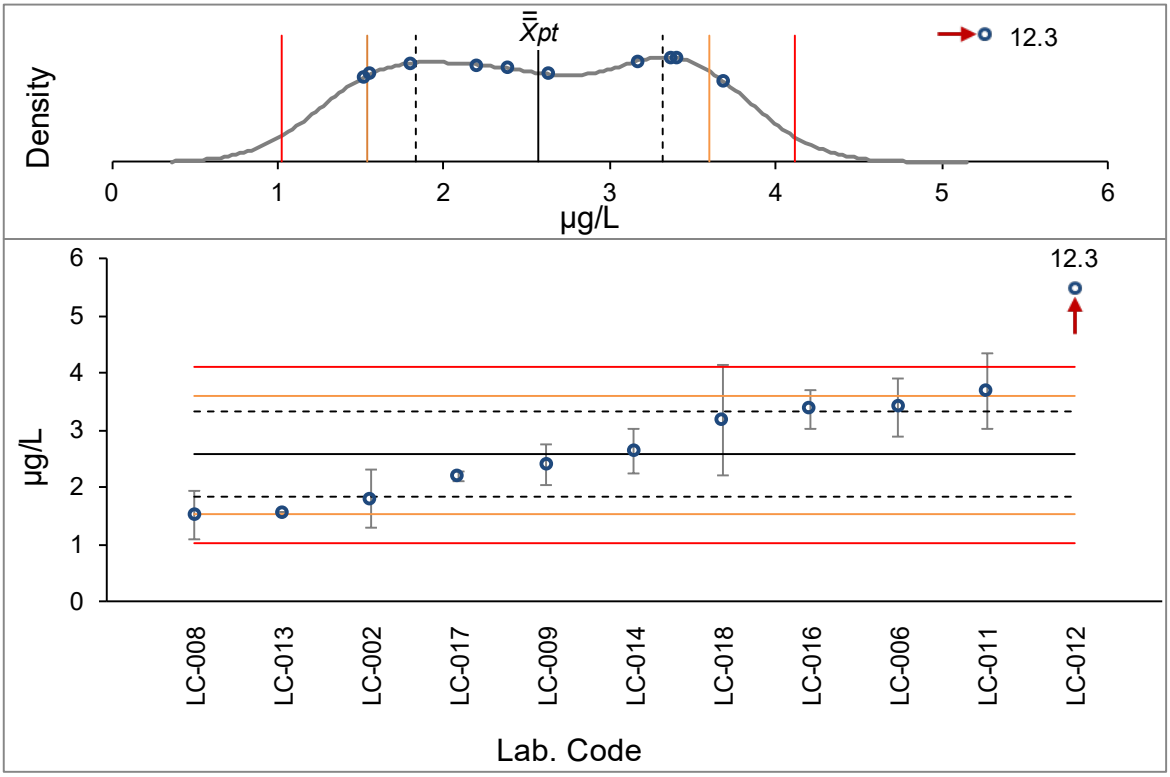

Figure 9: Measurement result range reported by the participants for the determination of aniline in Solution No. 2. Circles and bars represent the reported results [x_i] with the corresponding expanded uncertainties [$U(x_i)$]; orange and red lines represent 2*σ_{pt} and 3*σ_{pt}, respectively; solid and dotted black lines represent the robust mean $\bar{\bar{x}}_{pt}$ and its expanded uncertainty [$U(\bar{\bar{x}}_{pt})$].

Table 13: Results for the determination of aniline in Solution No. 2. Robust mean $\bar{\bar{x}}_{pt}$ = 2.572 ± 0.741 µg L^{-1}; σ_{pt} = 0.514 µg L^{-1}; x_i and $U(x_i)$ values are reported in µg L^{-1}.

Lab. Code	x_i	$U(x_i)$	k	D%*
LC-002	1.80	0.50	2	-30
LC-006	3.40	0.51	2	32
LC-008	1.52	0.43	2	-41
LC-009	2.39	0.35	2	-7
LC-011	3.68	0.66	2	43
LC-012	12.31	-	-	379
LC-013	1.56	-	-	-39
LC-014	2.63	0.39	2	2
LC-016	3.37	0.34	2	31
LC-017	2.20	0.08	2	-14
LC-018	3.17	0.95	2	23

* *D%* was used instead of *z* or *z'* scores, because the proportion $u(x_{pt})/\sigma_{pt}$ was found to be higher than 0.6.

13.4.3 Results for the determination of 4-aminoazobenzene in Solution No. 3

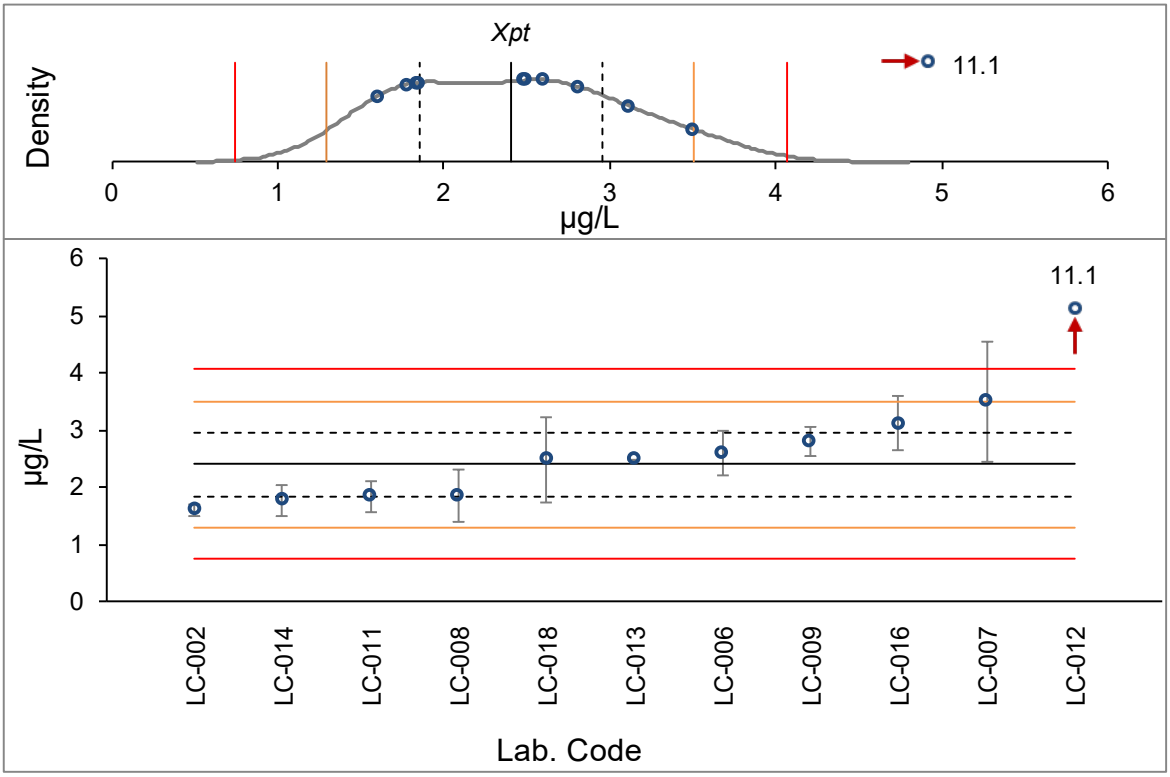

Figure 10: Measurement result range reported by the participants for the determination of 4-aminoazobenzene in Solution No. 3. Circles and bars represent the reported results [x_i] with the corresponding expanded uncertainties [$U(x_i)$]; orange and red lines represent *z'* scores = 2 and 3, respectively; solid and dotted black lines represent the assigned value [x_{pt}] and its expanded uncertainty [$U(x_{pt})$].

Table 14: Results for the determination of 4-aminoazobenzene in Solution No. 3. Assigned range: x_{pt} = 2.401 ± 0.551 µg L^{-1}; σ_{pt} = 0.480 µg L^{-1}; x_i and $U(x_i)$ values are reported in µg L^{-1}.

Lab. Code	x_i	U_i	k	*z'* score#, *	ζ score*	$u(x_i)_{\%}$ est.§
LC-002	1.60	0.10	2	-1.45	-2.86	**
LC-006	2.60	0.39	2	0.36	0.59	**
LC-007	3.50	1.05	2	1.99	1.85	a
LC-008	1.85	0.46	2	-0.99	-1.53	a
LC-009	2.81	0.26	2	0.73	1.34	**
LC-011	1.84	0.28	2	-1.01	-1.82	**
LC-012	11.09	-	-	15.70	-	-
LC-013	2.49	-	-	0.16	-	-
LC-014	1.78	0.27	2	-1.12	-2.02	**
LC-016	3.11	0.47	2	1.29	1.97	**
LC-018	2.48	0.74	2	0.14	0.17	a

* color code for z and ζ scores: green for |z or ζ| ≤ 2.00, orange for 2.00 < |z or ζ| < 3.00 and red for |z or ζ| ≥ 3.00.
# *z'* score was used instead of *z* score, because the proportion $u(x_{pt})/\sigma_{pt}$ was found to be significantly higher than 0.3.
§ (a) Reasonable estimation of $u(x_i)$; (b) underestimation of $u(x_i)$; (c) overestimation of $u(x_i)$.
** Case (b) for $u(x_i)_{\%}$ est. is not reasonable due to the high value of $u(x_{pt})$.

13.4.4 Results for the determination of 2,4-dimethylaniline in Solution No. 3

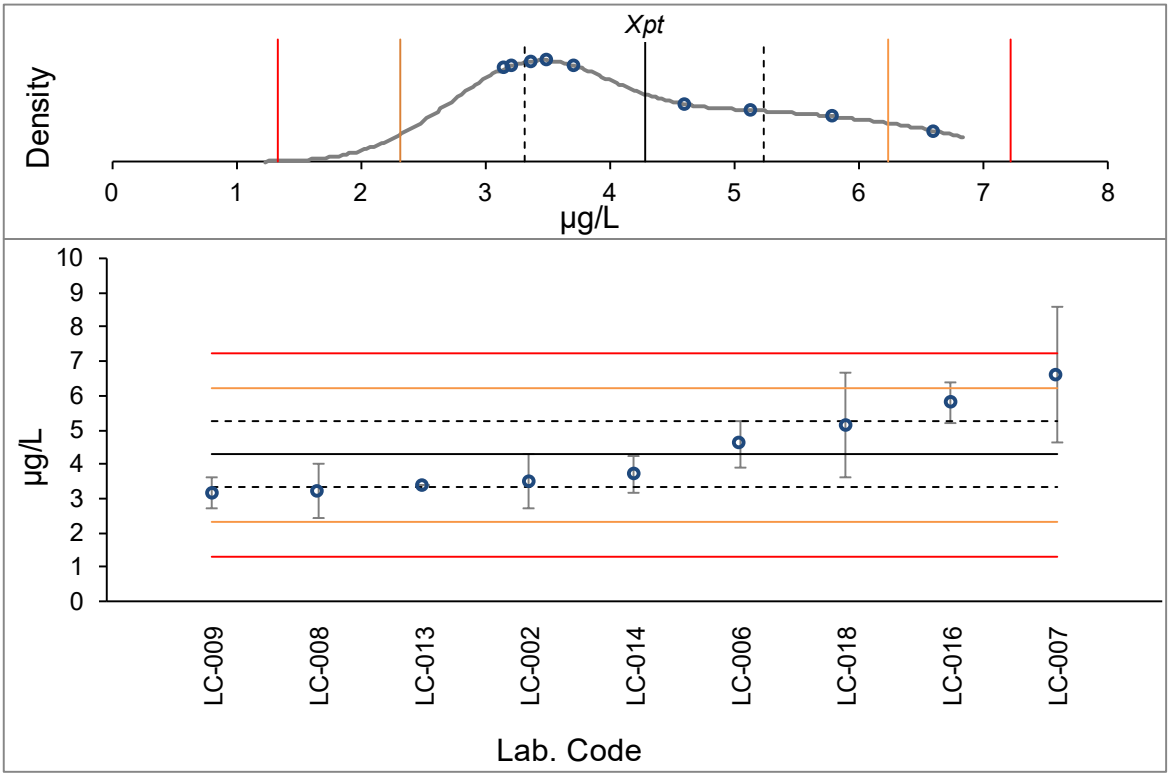

Figure 11: Measurement result range reported by the participants for the determination of 2,4-dimethylaniline in Solution No. 3. Circles and bars represent the reported results [x_i] with the corresponding expanded uncertainties [$U(x_i)$]; orange and red lines represent z´ scores = 2 and 3, respectively; solid and dotted black lines represent the assigned value [x_{pt}] and its expanded uncertainty [$U(x_{pt})$].

Table 15: Results for the determination of 2,4-dimethylaniline in Solution No. 3. Assigned range: x_{pt} = 4.276 ± 0.962 µg L^{-1}; σ_{pt} = 0.855 µg L^{-1}; x_i and $U(x_i)$ values are reported in µg L^{-1}.

Lab. Code	x_i	U_i	k	z' score#, *	ζ score*	$u(x_i)_{\%}$ est.§
LC-002	3.50	0.80	2	-0.79	-1.24	a
LC-006	4.60	0.68	2	0.33	0.55	**
LC-007	6.60	1.98	2	2.37	2.11	a
LC-008	3.21	0.80	2	-1.09	-1.70	a
LC-009	3.16	0.46	2	-1.14	-2.10	**
LC-013	3.37	-	-	-0.92	-	-
LC-014	3.71	0.56	2	-0.58	-1.02	**
LC-016	5.79	0.58	2	1.54	2.70	**
LC-018	5.14	1.54	2	0.88	0.95	a

* color code for z and ζ scores: green for |z or ζ| ≤ 2.00, orange for 2.00 < |z or ζ| < 3.00 and red for |z or ζ| ≥ 3.00.
# *z'* score was used instead of *z* score, because the proportion $u(x_{pt})/\sigma_{pt}$ was found to be significantly higher than 0.3.
§ (a) Reasonable estimation of $u(x_i)$; (b) underestimation of $u(x_i)$; (c) overestimation of $u(x_i)$.
** Case (b) for $u(x_i)_{\%}$ *est.* is not reasonable due to the high value of $u(x_{pt})$.

13.4.5 Results for the determination of 4-chloroaniline in Solution No. 3

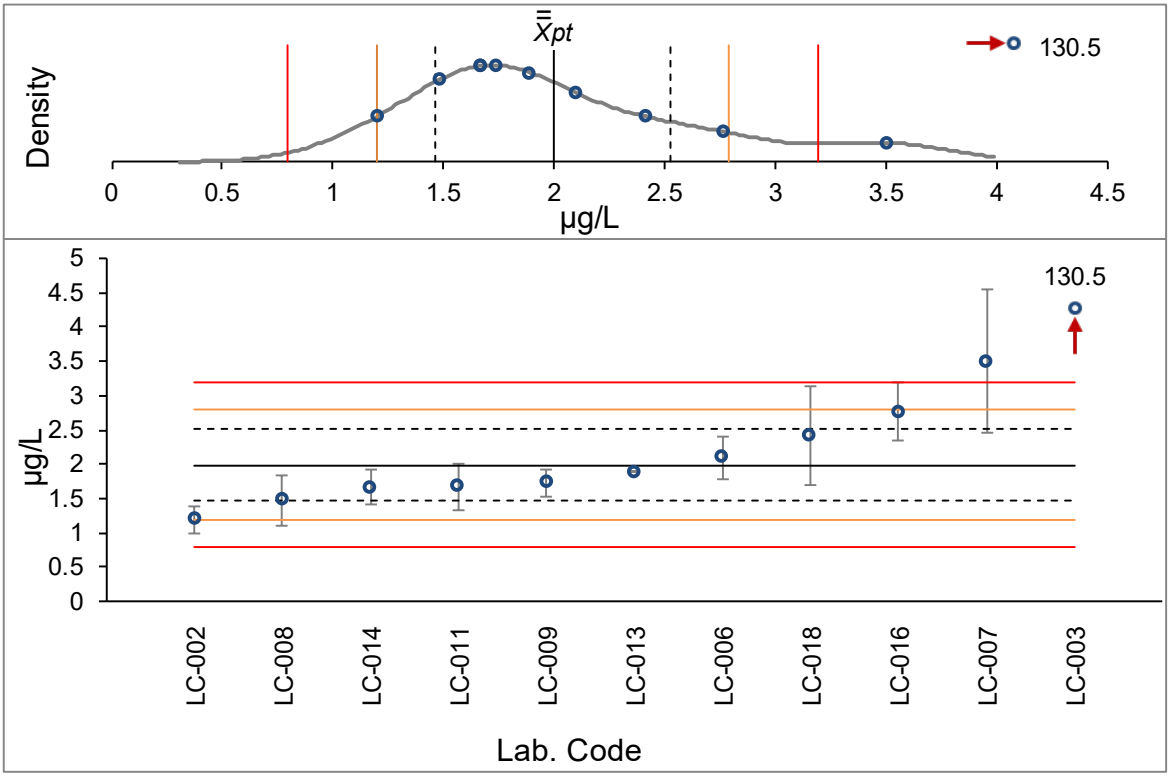

Figure 12: Measurement result range reported by the participants for the determination of 4-chloroaniline in Solution No. 3. Circles and bars represent the reported results [x_i] with the corresponding expanded uncertainties [$U(x_i)$]; orange and red lines represent 2*σ_{pt} and 3*σ_{pt}, respectively; solid and dotted black lines represent the robust mean $\bar{\bar{x}}_{pt}$ and its expanded uncertainty [$U(\bar{\bar{x}}_{pt})$].

Table 16: Results for the determination of 4-chloroaniline in Solution No. 3. Robust mean $\bar{\bar{x}}_{pt}$ = 1.993 ± 0.531 µg L^{-1}; σ_{pt} = 0.399 µg L^{-1}; x_i and $U(x_i)$ values are reported in µg L^{-1}.

Lab. Code	x_i	$U(x_i)$	k	$D\%$*
LC-002	1.20	0.20	2	-40
LC-003	130.50	-	-	6449
LC-006	2.10	0.31	2	5
LC-007	3.50	1.05	2	76
LC-008	1.48	0.37	2	-26
LC-009	1.74	0.20	2	-13
LC-011	1.67	0.33	2	-16
LC-013	1.89	-	-	-5
LC-014	1.67	0.25	2	-16
LC-016	2.77	0.42	2	39
LC-018	2.41	0.72	2	21

* $D\%$ was used instead of z or z' scores, because the proportion $u(x_{pt})/\sigma_{pt}$ was found to be higher than 0.6.

13.4.6 Results for the determination of *o*-anisidine in Solution No. 3

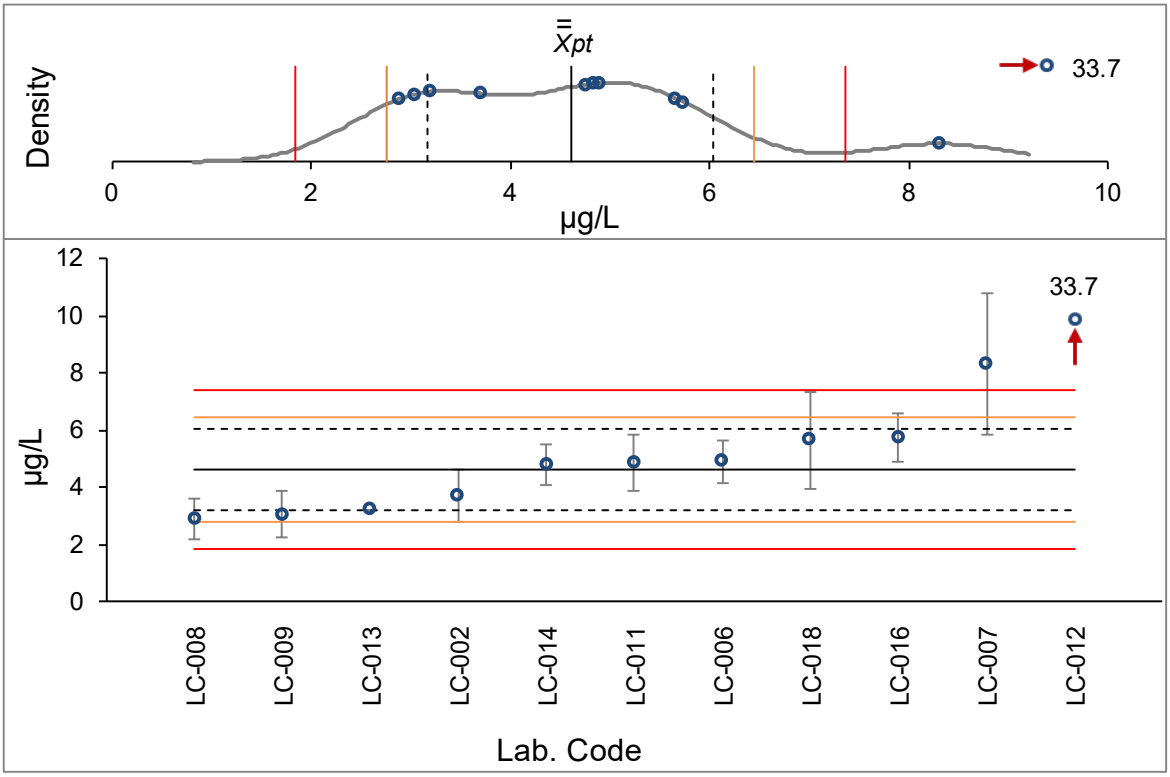

Figure 13: Measurement result range reported by the participants for the determination of *o*-anisidine in Solution No. 3. Circles and bars represent the reported results [x_i] with the corresponding expanded uncertainties [$U(x_i)$]; orange and red lines represent 2*σ_{pt} and 3*σ_{pt}, respectively; solid and dotted black lines represent the robust mean $\bar{\bar{x}}_{pt}$ and its expanded uncertainty [$U(\bar{\bar{x}}_{pt})$].

Table 17: Results for the determination of *o*-anisidine in Solution No. 3. Robust mean $\bar{\bar{x}}_{pt}$ = 4.603 ± 1.426 µg L^{-1}; σ_{pt} = 0.921 µg L^{-1}; x_i and $U(x_i)$ values are reported in µg L^{-1}.

Lab. Code	x_i	$U(x_i)$	k	$D\%$*
LC-002	3.70	0.90	2	-20
LC-006	4.90	0.74	2	6
LC-007	8.30	2.49	2	80
LC-008	2.89	0.72	2	-37
LC-009	3.04	0.82	2	-34
LC-011	4.84	0.97	2	5
LC-012	33.66	-	-	631
LC-013	3.20	-	-	-30
LC-014	4.76	0.71	2	3
LC-016	5.74	0.86	2	25
LC-018	5.65	1.70	2	23

* *D%* was used instead of *z* or *z'* scores, because the proportion $u(x_{pt})/\sigma_{pt}$ was found to be higher than 0.6.

13.5 Results of the questionnaire

13.5.1 General information

Table 18: General information

Lab. Code	1. Please identify yourself. You are …	2. Does your laboratory have a quality management system?	if YES, based on which standard?	3. Do you usually provide an uncertainty statement to your customer?
LC-002	OCL	Yes	ISO 17025	Yes
LC-003	NRL	Yes	ISO 17025	Yes
LC-004	NRL	Yes	ISO 17025	No
LC-006	NRL	Yes	ISO 17025	Yes
LC-007	OCL	Yes	ISO 17025	Yes
LC-008	NRL	Yes	ISO 17025	No
LC-009	NRL	Yes	ISO 17025	Yes
LC-011	NRL	Yes	ISO 17025	Yes
LC-012	Other	Yes	ISO 17025	Yes
LC-013	OCL	Yes	ISO 17025	Yes
LC-014	OCL	Yes	ISO 17025	Yes
LC-015	NRL	Yes	ISO 17025	No
LC-016	NRL	Yes	ISO 17025	Yes
LC-017	NRL	Yes	ISO 17025	No
LC-018	NRL	Yes	ISO 17025	Yes
LC-019	NRL	Yes	ISO 17025	Yes
LC-020*	NRL	Yes	ISO 17025	n.a.

* The laboratory provided information on the preparation of CWEs only.
n. a. – no information available

13.5.2 Analytical method (primary aromatic amines and amides)

Table 19: Information on the used analytical methods (Part 1)

Lab. Code	1. Which analytical technique was used for the analysis of primary aromatic amines and amides?	if other specify here	2. Is this method validated/accredited for the following conditions?	Describe shortly the way of the method validation
LC-002	LC-MS	-	Validated Method	Recovery experiments with spiked samples, participation in PTs
LC-003	LC-DAD	-	Not validated/accredited	-
LC-004	LC-DAD	-	Not validated/accredited	Accredited and validated only for 14 PAAs for plastics FCM and for food simulants 3 % acetic acid, 50 % ethanol, isooctane.
LC-006	LC-MS	-	Accredited method	Determination of recovery and intermediate precision, determination of calibration curve linearity, determination of LOQ

Continuation Table 19: Information on the used analytical methods (Part 1)

Lab. Code	1. Which analytical technique was used for the analysis of primary aromatic amines and amides?	if other specify here	2. Is this method validated/accredited for the following conditions?	Describe shortly the way of the method validation
LC-007	LC-MS	-	Validated Method	We used 5 samples of blank serviette matrix spiked with PAA (concentration: 8 µg/L) and blank samples to derive the standard deviation, repeatability and recovery. The quantitative evaluations were carried out under application of external matrix calibration in a range from 2–20 µg/L.
LC-008	LC-MS	-	Not validated/accredited	(method currently under validation)
LC-009	LC-MS	-	Accredited method	We follow FCM EURL Guidelines for performance criteria and validation procedures of analytical methods used in controls of food contact materials. (EUR 24105 EN) for the initial validation (12 PAAs) in aqueous extract of P&B; then if a new analyte of the same group is to be included in the method, a complementary reduced validation of linearity, precision and trueness is done. LOQ is the lowest validated level.
LC-011	LC-MS	-	Accredited method	Linearity, LOQ, repeatability, uncertainty according to Bratinova S, Raffael B, Simoneau C (2009)
LC-012	LC-MS	-	Not validated/accredited	-
LC-013	LC-MS	-	Not validated/accredited	(Validation is in progress)
LC-014	LC-MS	-	Accredited method	According to descriptions and methods DIN ISO 5725-1 to -6 and EURACHEN/CITAC
LC-015	LC-MS	-	Validated Method	According to our house-internal SOP based on ISO 17025
LC-016	LC-MS	-	Accredited method	Selectivity, specificity, recovery, precision, linearity, measurement uncertainty, LOD and LOQ
LC-017	LC-MS	-	Accredited method	-
LC-018	LC-MS	-	Validated Method	Validation of linearity of calibration function, LOQ, reproducibility, trueness
LC-019	LC-DAD		Not validated/accredited	-
LC-020*	n. a.	n. a.	n. a.	n. a.

* The laboratory provided information on the preparation of CWEs only.
n. a. – no information available

Table 20: Information on the used analytical methods (Part 2)

Lab. Code	3. How long and frequently is this method used in your laboratory?		4. Do you use certified reference materials for quality control?	5. Please enter the method for the estimation of the measurement uncertainty	if other specify here:	Is the uncertainty of the extraction-step included in the estimation of measurement uncertainty?
	year(s)	/year				
LC-002	>5	51–250	Yes	In-house validation	-	No
LC-003	<1	Never	No	In-house validation	-	No
LC-004	<1	Never	Yes	In-house validation	-	No
LC-006	>5	1–50	No	In-house validation	-	No
LC-007	2–5	1–50	No	In-house validation	-	Yes
LC-008	<1	Never	No	Other	estimation based on replicate analysis of samples	Yes
LC-009	1–2	1–50	No	In-house validation	-	No
LC-011	>5	51–250	No	In-house validation	-	No
LC-012	<1	1–50	Yes	In-house validation	-	Yes
LC-013	<1	Never	No	In-house validation	in progress; our LOD and LOQ are provisionally estimated	Yes
LC-014	2-5	1–50	No	In-house validation	-	Yes
LC-015	>5	1–50	No	In-house validation	-	No
LC-016	>5	1–50	No	NORDTEST	-	No
LC-017	>5	1–50	No	GUM	-	No
LC-018	<1	51–250	No	Other	-	No
LC-019	1-2	1–50	Yes	Other	Horwitz equation	No
LC-020*	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.

* The laboratory provided information on the preparation of CWEs only.
n. a. – no information available

Table 21: Information on the used analytical methods (Part 3)

Lab. Code	6. Did you test a blank sample?	if YES specify here	7. Did you substract these blank values?	8. Did you apply any special treatment to the samples provided?	if YES specify here
LC-002	Yes	millipore water	Yes	No	-
LC-003	No	-	No	No	-
LC-004	Yes	-	Yes	No	-
LC-006	Yes	extraction solution	No	No	-
LC-007	Yes	we used the unprinted serviette as a blank sample	No	No	-
LC-008	Yes	(a) Ultrapure water (b) CWE of a white paper napkin	No	No	-
LC-009	Yes	White kitchen paper	No	No	-
LC-011	Yes	Reagent blank	Yes	No	-
LC-012	Yes	PAA free paper-matrix spiked with internal standard	Yes	No	-

Continuation Table 21: Information on the used analytical methods (Part 3)

Lab. Code	6. Did you test a blank sample?	if YES specify here	7. Did you substract these blank values?	8. Did you apply any special treatment to the samples provided?	if YES specify here
LC-013	Yes	blanks were performed with white napkins	No	No	-
LC-014	Yes	Cold water extract, but without napkin sample.	No	Yes	The cold water extract was prepared with 4 g sample and 100 mL of water. Rinsing was performed with 2 x 8 mL.
LC-015	Yes	Ultrapure water which was also used for the CWE	No	No	-
LC-016	Yes	CWE of white paper napkin	Yes	No	-
LC-017	Yes	Blank without internal standards and a blank with internal standards	No	No	-
LC-018	Yes	We have prepared a blank sample, deionised water, which was treated according to the same procedure as samples. There was no response for analytes in the blank sample.	No	No	-
LC-019	Yes	Food simulant – water	No	No	-
LC-020*	n. a.	n. a.	n. a.	n. a.	n. a.

* The laboratory provided information on the preparation of CWEs only.
n. a. – no information available

Table 22: Information on the used analytical methods (Part 4)

Lab. Code	9. Did you encounter any problems with the sample analysis?	if YES specify here
LC-002	No	-
LC-003	No	-
LC-004	Yes	With HPLC-DAD it was very difficult to determine the PAAs. We had to buy many standards to identify and find out which substance is what and the list on possible PAAs is quite large. For qualitative analysis LC-MS would be much more suitable, but unfortunately, we don't have this possibility in our laboratory yet.
LC-006	No	-
LC-007	No	-
LC-008	No	-
LC-009	No	-
LC-011	No	-
LC-012	Yes	Solutions 1 to 3 had to be diluted so that the internal standard could be added. After dilution, the amount of *o*-anisidine is below the limit of quantification.
LC-013	Yes	some analytes are very unstable, partial high loss during extraction
LC-014	No	-
LC-015	Yes	Limit of quantification of our method is insufficient for these samples
LC-016	No	-
LC-017	No	-

Continuation Table 22: Information on the used analytical methods (Part 4)

Lab. Code	9. Did you encounter any problems with the sample analysis?	if YES specify here
LC-018	No	-
LC-019	No	-
LC-020*	n. a.	-

* The laboratory provided information on the preparation of CWEs only.
n. a. – no information available

13.5.3 Cold water extract

Table 23: Information on the cold water extract

Lab code	1. Did you use a glass-fiber filter for the filtration of the extract-solution?	if NO specify here	2. How much water did you add to fill the volumetric flask up to the mark?
LC-002	Yes	-	11–50 mL
LC-003	No	we did use it, but because of inappropriate equipment and our improvisation, first 2 extracts were destroyed by tap water reflux. 3rd extract we filtered with Schleicher&Schuell black ribbon ashless filter paper.	more than 50 mL
LC-004	Yes, glass-fiber filter (size C)	0.45 µm	11–50 mL
LC-006	-	Filter funnel – glass filter Por. 4	11–50 mL
LC-007	No	We used paper filter and checked the absence of PAA before.	0–10 mL
LC-008	Yes, glass-fiber filter (size C)	-	11–50 mL
LC-009	Yes, glass-fiber filter (size C)	-	11–50 mL
LC-011	No	Glass frit was used for filtration	more than 50 mL
LC-012	Yes, glass-fiber filter (other size)	-	11–50 mL
LC-013	Yes, glass-fiber filter (other size)	-	11–50 mL
LC-014	Yes, glass-fiber filter (size C)	-	11–50 mL
LC-015	Yes, glass-fiber filter (size C)	-	more than 50 mL
LC-016	Yes, glass-fiber filter (size C)	-	11–50 mL
LC-017	Yes	Fritted glass, filter porosity 4 (nominal size 90), as quoted in DIN EN 645	11–50 mL
LC-018	Yes, glass-fiber filter (other size)	-	more than 50 mL
LC-019	Yes, glass-fiber filter (size C)	-	11–50 mL
LC-020*	Yes, glass-fiber filter (other size)	GF/C 1.2 µm	11–50 mL

* The laboratory provided information on the preparation of CWEs only.