Bundesinstitut für Risikobewertung

Interlaboratory comparison exercise on the determination of 3-MCPD, 2-MCPD and 1,3-DCP in cold water extracts of paper FCM

Report on the interlaboratory comparison exercise NRL-DE-FCM-01/2022 of the German National Reference Laboratory (NRL) for Food Contact Materials

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1 Summary

The interlaboratory comparison (ILC) exercise NRL-DE-FCM-01/2022 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 participating laboratories received two paper straw samples (Sample 1 and 2) to perform cold water extracts (CWEs) in triplicate according to DIN EN 645 [1] with specifications described in the method collection for paper and board of the BfR [2]. In addition, they received a solution (Solution 1) which was a CWE of a white kitchen roll spiked with selected analytes of interest. Solution 1 was prepared by the NRL-DE-FCM. The scope of the ILC comprised the quantification of 3-monochloropropane-1,2-diol (3-MCPD), 2-monochloropropane-1,3-diol (2-MCPD) and 1,3-dichloro-2-propanol (1,3-DCP) in the provided test items.

In total, eleven laboratories enrolled in the ILC. Ten laboratories were from three member states of the EU and one participant was from a non-EU state. All laboratories reported results for 3-MCPD in the CWEs of paper straw samples. For Solution 1 five laboratories reported results for 2-MCPD and all laboratories reported results for 1,3-DCP. One laboratory reported two results, since they used two derivatization reagents.

Results reported by the laboratories were evaluated quantitatively based on the reported concentrations for the identified compounds in accordance with ISO 13528 [3] by calculating either *z* and *zeta* (*ζ*) scores or the estimate of deviation *D%*. *z* and *ζ* scores were calculated for 3-MCPD in CWEs of Sample 1 and 2 and for 1,3-DCP in Solution 1. *D%* was calculated for 2-MCPD in Solution 1. Moreover, relative measurement uncertainties were calculated and compared across the laboratories. The relative standard deviation for proficiency assessment (σ_{pt}) was set to 20 % for the extraction experiment of Sample 1, to 27 % for the extraction experiment of Sample 2 and to 15 % for the analysis of the provided Solution 1.

All participating laboratories obtained acceptable *z* scores for 3-MCPD in the CWE of Sample 1. The majority of the laboratories (8 of 9) received acceptable *z* scores for 3-MCPD in the CWE of Sample 2. The corresponding *ζ* scores were also acceptable for the majority of the laboratories (7 of 11 (Sample 1) and 6 of 9 (Sample 2)). The results of 2-MCPD in Solution 1 were assessed using the estimate of deviation (*D%*). Only five laboratories reported results; for four laboratories the *D%* values were smaller than $2*\sigma_{pt}$. The results of 1,3-DCP were also evaluated using *z* and *ζ* scores. Most laboratories (9 of 12) received acceptable *z* scores. *ζ* scores were acceptable for 7 of 10 laboratories.

Reported expanded measurement uncertainties ranged from 13 to 45 %. Taking the relative standard uncertainties calculated from the ILC results into account, the measurement uncertainties (MUs) reported for 3-MCPD in the CWEs of Sample 1 and 2 were estimated reasonably for 64 % and 100 % of the laboratories, whereas they were only reasonable for 40 % of the laboratories for 1,3-DCP in Solution 1.

In general, it could be clearly demonstrated that the CWE according to DIN EN 645 [1] as well as the analytical methods of all participating laboratories perform well for the determination of 3-MCPD and 1,3-DCP. 2-MCPD is not yet included in the analytical methods of most of the participating laboratories because of a missing classification and the associated lack of limit values.

2 List of abbreviations and symbols

BfR	German Federal Institute for Risk Assessment
CWE	Cold water extract
FCM	Food contact material
ILC	Interlaboratory comparison
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
3-MCPD	3-Monochloropropane-1,2-diol
2-MCPD	2-Monochloropropane-1,3-diol
1,3-DCP	1,3-Dichloro-2-propanol
c	$c=F1\ \sigma_{allow}^2+ F2\ s_w^2$; 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
ζ	Modified z score that includes uncertainties for the participants' 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 interlaboratory comparison (ILC) exercise NRL-DE-FCM-01/2022 on the determination of 3-monochloropropane-1,2-diol (3-MCPD), 2-monochloropropane-1,3-diol (2-MCPD) and 1,3-dichloro-2-propanol (1,3-DCP) 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 aim of the exercise was the quantification of chloropropanols in CWEs of paper straw samples prepared according to DIN EN 645 [1] with specifications described in the method collection for paper and board of the BfR [2] and in a solution provided to the participants by the ILC organizer.

1,3-DCP is classified as carcinogenic in category 1B (presumed to have carcinogenic potential for humans) by Regulation (EC) No. 1272/2008 [4]. 3-MCPD is classified in category 2B (possibly carcinogenic to humans) by the International Agency for Research on Cancer [5]. Consequently, the BfR recommendation XXXVI for paper and board for food contact stipulates limit values [6]. According to the BfR recommendation 1,3-DCP must not be detected in the water extract (detection limit 2 µg L^{-1}) and the detectable amount of 3-MCPD in the water extract must be as low as technically achievable, whereby a limit of 12 µg L^{-1} must not be exceeded [6].

The following samples and solutions were provided to the participants:

- Sample 1: paper straws for CWE
- Sample 2: paper straws for CWE
- Solution 1: CWE of a kitchen roll spiked with 2-MCPD and 1,3-DCP

Solution 1 was a CWE of a kitchen roll spiked with 2-MCPD as well as 1,3-DCP and was prepared in the labs of the NRL-DE-FCM. According to the analyses of the NRL-DE-FCM, interfering signals were found at the retention time of 3-MCPD and it was decided by the ILC organizer to exclude its evaluation in Solution 1 from the ILC. The cold water extraction of the paper straw samples (Sample 1 and 2) had to be carried out in triplicate according to DIN EN 645 [1] with the adjustments specified in the instructions provided to the participants along with the samples (see chapter 13 and [2]). Participants could freely choose the analytical technique (e.g. GC-MS or GC-MS/MS) for quantification of the analytes in CWEs and in Solution 1.

This proficiency test was open to Reference Laboratories and Official Control Laboratories (OCLs). This report summarizes the outcome of the ILC exercise.

Table 1: Participating laboratories.

Organization	Country
Amt für Verbraucherschutz und Veterinärwesen Schweiz (NRL)	Switzerland
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
Landeslabor Schleswig-Holstein (OCL)	Germany
Landesuntersuchungsamt Rheinland-Pfalz (OCL)	Germany
Landesuntersuchungsanstalt für das Gesundheits- und Veterinärwesen (LUA) Sachsen (OCL)	Germany
Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit (LAVES) (OCL)	Germany
Thüringer Landesamt für Verbraucherschutz (OCL)	Germany
Service Commun des Laboratoires (SCL) (Joint Control Laboratories) (NRL)	France

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 [7] 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 the German OCLs regarding the quantification of chloropropanols in CWEs of paper samples prepared according to DIN EN 645 [1] with slight specifications (see chapter 13 and [2]) and in a solution provided by the ILC organizer. The ILC was open for other NRLs and OCLs.

This ILC is identified as “NRL-DE-FCM-01/2022”.

5 Set up of the exercise

5.1 Time frame of the ILC

The invitation for the NRL-DE-FCM-01/2022 was sent on October 10, 2022 and registration was open until October 14, 2022. Samples were sent to the participants on October 19, 2022 and the deadline for reporting of results was set to November 11, 2022. This deadline was extended until November 18, 2022 for individual laboratories.

5.2 Quality assurance

The NRL-DE-FCM has a quality management system according to DIN EN ISO/IEC 17025 [8]. 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:

- 2 paper straw samples (Sample 1 and 2; ~5 g)
- 1 solution (Solution 1; 19 mL)
- NRL_DE_FCM_01_2022_Confirmation of receipt_LC_0__.pdf
- NRL_DE_FCM_01_2022_Instructions.pdf
- NRL_DE_FCM_01_2022_Questionnaire_Results_LC_0__.xlsx

5.5 Instructions to participants

Participants were sent the participation letter containing the lab code and were asked to check and report whether the test items were undamaged after transport using the “NRL_DE_FCM_01_2022_Confirmation of receipt_LC_0__.pdf” form.

Detailed instructions on the ILC were given to the participants in the document “NRL_DE_FCM_01_2022_Instructions.pdf”. In brief, participants were asked to prepare CWEs of the provided paper straw samples (Sample 1 and 2) according to DIN EN 645 [1] with the specifications [2] stated in the provided instructions and to analyze the CWEs along with Solution 1. Moreover, participants were asked to send an aliquot (~15 mL) of the prepared CWEs to the NRL-DE-FCM.

Results and general information about the analytical procedure were inquired in the form “NRL_DE_FCM_01_2022_Questionnaire_Results_LC_0__.xlsx”. The questionnaire form was divided into four sheets: “General”, “CWE”, “Analysis” and “Results”. The sheet “General” contained questions about the laboratory and the analytical methods used. Information about the experimental procedure was inquired in the sheet “CWE”. The sheet “Analysis” contained

questions about sample preparation and analysis. In the sheet “Results”, the results [in µg L^{-1}] for the identified compounds along with the corresponding measurement uncertainty (MU) and the coverage factor *k*, the limit of detection (LOD) and the limit of quantification (LOQ) were inquired.

6 Test items

6.1 Preparation

6.1.1 Paper straw samples

Commercially available paper straws were cut into pieces (1 cm^2) according to DIN EN 645 [1], mixed by automated shaking and stored in a glass bottle at room temperature. The aliquots of Sample 1 and 2 (~5 g) for the participants were provided in aluminum foil.

6.1.2 Solutions

Solution 1 was prepared in matrix. Matrix was a CWE of a white kitchen roll prepared according to DIN EN 645 [1]. The CWE was autoclaved before spiking with 2-MCPD and 1,3-DCP to yield the following solution, which was prepared shortly before shipment and stored light-protected at 4°C until dispatch.

Table 2: Overview of Solution 1.

	Compound Name	CAS-No.	Target concentration
Solution 1	2-MCPD 1,3-DCP	497-04-1 96-23-1	12.0 µg L^{-1}* 2.0 µg L^{-1}

*see discussion for 2-MCPD in chapter 7

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 2 was tested using statistical methods described in DIN ISO 13528 [3] and in IUPAC's harmonized protocol [9]. The test items of Sample 1 were demonstrated to be adequately homogeneous (see chapter 13.2.1) with respect to 3-MCPD using a σ_{pt} of 20 % while Sample 2 was adequately homogenous using a σ_{pt} of 27 % (see chapter 13.2.2). The higher σ_{pt} for Sample 2 resulted from the low concentration of 3-MCPD in the sample which was near the LOQ of the analytical method used for homogeneity testing.

The stability of 2-MCPD and 1,3-DCP in Solution 1 was confirmed prior to the ILC according to ISO 13528 B.5.1 [3] over a period of three weeks (see chapter 13.2.3). The findings from these additional analyses support the statement that the stability of the analyzed chloropropanols was sufficient for the period of this ILC.

7 Assigned values and standard uncertainties

No reference values were available for the measurands of concern. Thus, for the evaluation of 3-MCPD in Sample 1 and 2 the assigned value x_{pt} was derived from the homogeneity tests by the ILC organizer.

The standard uncertainties of the assigned values $u(x_{pt})$ were estimated according to ISO 13528 [3]:

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

where s^* is the robust standard deviation of mean values (according to the Q/Hampel method [3, 10]) calculated from the results of the homogeneity investigations and p is the number of samples.

3-MCPD in Solution 1 was not evaluable: According to the GC-MS/MS analyses of the NRL-DE-FCM, a peak was present at the correct retention time but with a quantifier/qualifier ratio not matching the neat analytical standard. Hence, it could not be unambiguously determined whether 3-MCPD was present in the matrix material used and it was therefore not quantified. Nevertheless, in the results sheet of the questionnaire information on the concentration of 3-MCPD was inquired in order to check how the participating laboratories handled this issue (see also chapter 8.3.2).

The assigned value x_{pt} for the evaluation of 1,3-DCP in Solution 1 was defined as the concentration spiked into the solution. The standard uncertainty of the assigned value $u(x_{pt})$ was calculated from the individual uncertainties of the equipment (balance, glassware, pipettes) used to prepare the spiked solution and from the uncertainty of the purity of the analytical standard which was retrieved from the batch-specific certificate of the manufacturer.

For 2-MCPD, problems with the analytical standard occurred in the laboratory of the ILC organizer. The 2-MCPD standard was only available in an ampule in a very low amount and the exact amount weighed for the spiking solution had to be determined by weighting out the ampule. This procedure leads to strong deviations in the determination of the exact concentration. Furthermore, it could not be excluded that the purity reported by the manufacturer was incorrect. The 2-MCPD concentration measured by the participants was about eight times lower compared to the concentration measured in the laboratory of the ILC organizer. The assigned value for the evaluation of 2-MCPD in Solution 1 was calculated as a robust mean $\bar{\bar{x}}_{pt}$ of the results reported by the participants (using Hampel estimator [4, 10], following elimination of outliers via Grubb's test [11]). The standard uncertainty of the participants' robust mean $u(\bar{\bar{x}}_{pt})$ was calculated similarly to equation 1 using the reported results and the number of participants. Since only four participants (plus one result from the ILC organizer) reported results for 2-MCPD in Solution 1 above their limits of quantification the statistical statement for this result is low.

Relative standard deviations for proficiency assessment σ_{pt} were set to 20 % for the extraction experiment of Sample 1 and to 27 % for the extraction experiment of Sample 2 taking into account the results of the homogeneity tests (see chapter 6.2). Based on expert judgment σ_{pt} was set to 15 % for the test solution (Solution 1).

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

Table 3: Relevant parameters related to the determination of chloropropanols in the CWEs and in the solution.

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
3-MCPD	16.827#	±	1.194	3.365	20	0.177	*z*

CWE of Sample 2							
	x_{pt}	±	$U(x_{pt})$* [µg L^{-1}]	σ_{pt} [µg L^{-1}]	[% of x_{pt}]	$u(x_{pt})/\sigma_{pt}$	Score
3-MCPD	2.764§	±	0.257	0.746	27	0.172	*z*

Solution 1							
	$\bar{\bar{x}}_{pt}$	±	$U(\bar{\bar{x}}_{pt})$* [µg L^{-1}]	σ_{pt} [µg L^{-1}]	[% of x_{pt}]	$u(x_{pt})/\sigma_{pt}$	Score
2-MCPD	1.578‡	±	0.314	0.237	15	0.664	*D%*
	x_{pt}	±	$U(x_{pt})$* [µg L^{-1}]	σ_{pt} [µg L^{-1}]	[% of x_{pt}]	$u(x_{pt})/\sigma_{pt}$	Score
1,3-DCP	2.000$	±	0.042	0.300	15	0.071	*z*

* $U(x_{pt})$ and $U(\bar{\bar{x}}_{pt})$ are the expanded uncertainty at a given coverage factor (k=2).
# This value was calculated from 13 homogeneity test samples.
§ This value was calculated from 17 homogeneity test samples.
‡ This value was calculated from 4 participants' results (the result of the ILC organizer was eliminated as outlier).
$ Spiked concentration.

8 Evaluation

8.1 Scores and evaluation criteria

The individual laboratory's performance for 3-MCPD in the CWEs of Sample 1 and 2 was expressed in terms of *z* and *ζ* scores according to ISO 13528 [3]. 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:

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

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

The interpretation of the *z* and *ζ* performance scores is done according to ISO 13528 [3]:

$\lvert z_i \rvert \leq 2.00$	acceptable performance	(green in chapter 13.3),
$2.00 < \lvert z_i \rvert < 3.00$	questionable performance	(yellow in chapter 13.3),
$\lvert z_i \rvert \geq 3.00$	unacceptable performance	(red in chapter 13.3).

When the proportion $u(x_{pt})/\sigma_{pt}$ was higher than 0.6 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.

$$D\%_i = 100\% \ \frac{x_i - \bar{\bar{x}}_{pt}}{\bar{\bar{x}}_{pt}} \qquad \text{Equation 4}$$

where:

x_i is the mean, calculated from single values 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 $u(x_i)$ [3]. For this purpose, the relative standard uncertainty of the mean value from participant *"i"* was calculated:

$$u(x_i)_{\%} = 100\% \left(\frac{u(x_i)}{x_i} \right) \qquad \text{Equation 5}$$

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 range,

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

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. 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

While most laboratories used gas chromatography coupled with mass spectrometry (GC-MS) for the quantification of analytes, three laboratories used GC coupled to tandem mass spectrometry (MS/MS) (Table 4).

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

Technique	No. of Labs
GC-MS	8
GC-MS/MS	3
Total	11

8.3 Laboratory results and scorings

8.3.1 Performance

The results for 3-MCPD in CWEs of Sample 1 and 2 and for 1,3-DCP in Solution 1 were evaluated in terms of z scores. For 2-MCPD in Solution 1, 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 the results of all laboratories reporting MUs.

A graphical overview of the laboratories’ performance for 3-MCPD in the CWEs of Sample 1 and 2 expressed as z and ζ scores is given in Figure 1.

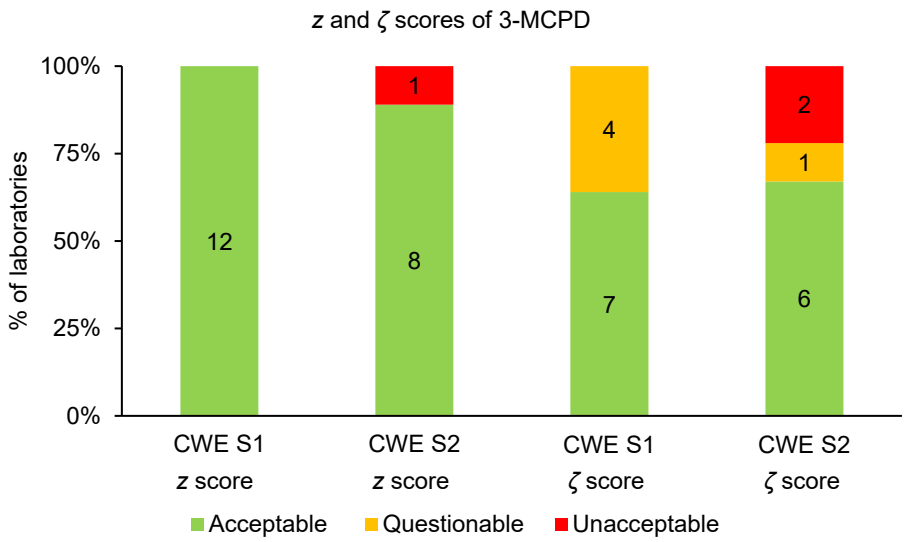

Figure 1: Overview of the laboratories' performance according to *z* and *ζ* scores for the analysis of 3-MCPD in CWEs of Sample 1 and 2 (CWE S1 and CWE S2). σ_{pt} was defined as 20 % of x_{pt} for the CWE of Sample 1 and as 27 % for the CWE of Sample 2. *z* and *ζ* scores were determined using x_{pt} and $u(x_{pt})$ calculated from the homogeneity measurements. The numbers in the bars correspond to the number of laboratories assigned with the respective scoring. One laboratory did not report MUs for the analysis of the CWE of Sample 1; therefore, the sum of laboratories may differ between *z* and *ζ* scores.

All laboratories received acceptable *z* scores for the CWE of Sample 1. Most laboratories (89 %) obtained acceptable *z* scores for the CWE of Sample 2, only one laboratory obtained an unacceptable *z* score.

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. One laboratory did not submit MUs for the results; hence, no *ζ* scores could be calculated for this laboratory. The majority (64 to 67 %) of the laboratories received acceptable *ζ* scores for their submitted results for the CWEs. Only 33 to 36 % of the laboratories obtained either questionable or unacceptable *ζ* scores.

The results for 2-MCPD in Solution 1 were evaluated using the estimate of deviation *D%* and are shown in Table 5. The deviations were positive for two laboratories and negative for two laboratories. The absolute deviations (4–12 %) were within the range of σ_{pt} (σ_{pt} was set to 15 % for Solution 1). *D%* of the ILC organizer was evaluated as outlier.

Table 5: *D%* (estimate of deviation) for 2-MCPD in Solution 1.

Lab. Code	002	003	006	008	ILC Org.*
2-MCPD	-7	4	-9	12	739

* Laboratory of the ILC organizer.

A graphical overview of the laboratories' performance for 1,3-DCP in Solution 1 expressed as *z* and *ζ* scores is given in Figure 2.

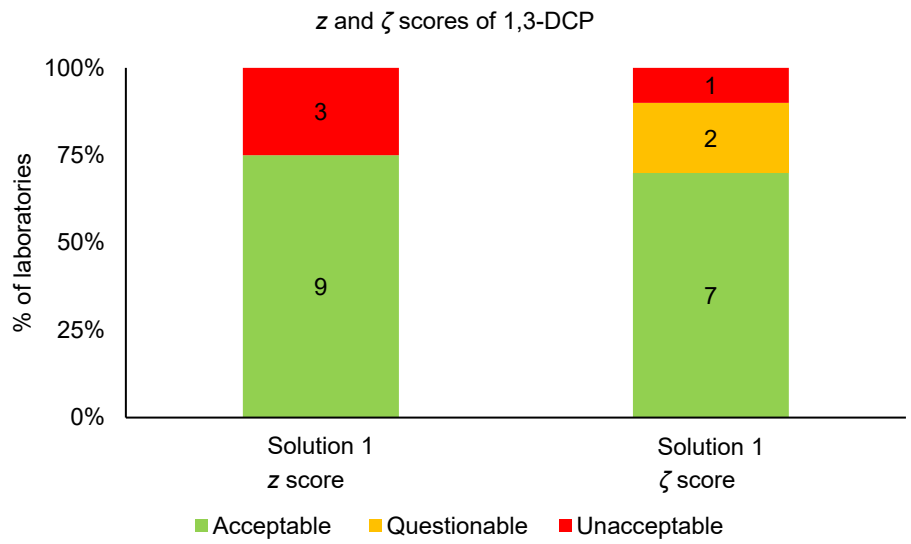

Figure 2: Overview of the laboratories' performance according to *z* and *ζ* scores for the analysis of 1,3-DCP in Solution 1. σ_{pt} was defined as 15 % of x_{pt} for the Solution 1. *z* and *ζ* scores were determined using x_{pt} and $u(x_{pt})$ calculated from the spiked concentration. The numbers in the bars correspond to the number of laboratories assigned with the respective scoring. Two laboratories did not report MUs for the analysis of Solution 1; therefore, the number of laboratories differs between *z* and *ζ* scores.

Z and *ζ* scores were calculated for 1,3-DCP in Solution 1. Most laboratories (75 %) obtained acceptable *z* scores and 25 % of the laboratories (3 out of 12 laboratories) received an unacceptable *z* score. Two laboratories did not report MUs for their results. The majority (70 %) of the laboratories achieved acceptable *ζ* scores; only 30 % of the laboratories obtained questionable or unacceptable *ζ* scores.

Notably, most of the questionable or unacceptable *z* and *ζ* scores observed could be assigned to the same three laboratories.

8.3.2 Discussion on 3-MCPD in Solution 1

Before spiking the matrix used for Solution 1, it was analyzed repeatedly at the NRL-DE-FCM. During these analyses a signal was observed at the retention time of 3-MCPD which quantifier/qualifier ratio differed more than 20 % from that of the neat analytical standard. Therefore, 3-MCPD could not be unambiguously identified and quantified and it was decided neither to spike 3-MCPD to the kitchen role matrix nor to evaluate this compound in the provided Solution 1. However, results for 3-MCPD were inquired in the questionnaire to evaluate how the participating laboratories would deal with this finding.

Three laboratories reported results above their respective LOQs in the range of 1.1 to 5.7 µg L^{-1}. The other laboratories reported that the results were either below their LOQ (four laboratories) or below LOD (one laboratory). Moreover, three laboratories stated that 3-MCPD was not detectable and one laboratory did not report any result.

In the comments of the questionnaire (worksheet "Analytical Method"), two laboratories (LC-004 and LC-005) mentioned problems with the quantifiers and qualifiers of 3-MCPD in the GC-MS chromatograms (for the exact answers see Table 18 in chapter 13.4.2). One of these laboratories observed similar problems with 3-MCPD in Sample 1 and the other laboratory increased the LOD in Solution 1 as a consequence to the findings.

Quantifier/qualifier ratios in samples that do not match those of the neat analytical standards may be a result of matrix interferences. All three laboratories reporting results above their respective LOQ used GC-MS. Matrix interferences due to lower selectivity of the MS method (compared to MS/MS) may have led to false positive results. Overall, the observations in this ILC show the importance of well-characterized and selective analytical methods for the correct identification and quantification of chloropropanols.

8.3.3 Measurement uncertainties (MU)

Within the questionnaire, the majority of laboratories (10 out of 11) reported that they usually provide measurement uncertainties. For the CWE, one laboratory did not submit MUs for their results, and one laboratory did not report its *k* value. For 1,3-DCP in Solution 1, two laboratories did not report measurement uncertainties. The questionnaire revealed that most laboratories estimated the measurement uncertainty based on in-house validation or NORDTEST (4 each). Other methods used were Horwitz' equation or interlaboratory comparisons.

Relative expanded measurement uncertainties (expressed as $U(x_i)_{\%}$) calculated from the submitted results ranged from 13 to 45 % and are shown in Table 6.

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

Lab. Code	001	002	003	004	005	006	007	008	009	010	011	012	ILC Org.
CWE Sample 1													
3-MCPD	40	42	30	20	26	40	40	45	13	-*	30	40	-
CWE Sample 2													
3-MCPD	40	43	30	-	27	40	40	44	-	-	30	40	-
Solution 1													
2-MCPD	-	45	30	-	-	-	-	44	-	-	-	-	30
1,3-DCP	41	43	30	30	39	20	40	-*	18	-*	20	40	-

"-" no results were submitted for this analyte.
"-*" no values for the uncertainty were submitted for this analyte.

The calculated relative standard uncertainties $u(x_i)_{\%}$ were compared to the accepted relative standard uncertainty range (see chapter 8.1) 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 3. Due to the high value of $u(x_{pt})/\sigma_{pt}$, the reported MUs for the analysis of 2-MCPD in Solution 1 were not evaluated in this context.

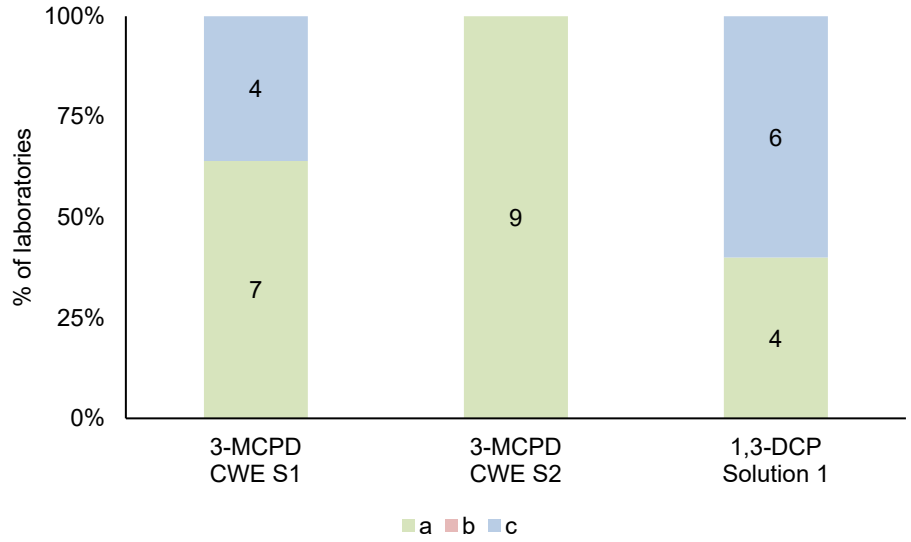

Figure 3: Evaluation of the laboratories' relative standard uncertainties $u(x_i)_{\%}$ for the analysis of 3-MCPD in CWEs of Sample 1 and 2 (CWE S1 and CWE S2) and 1,3-DCP in Solution 1. The numbers in the bars correspond to the number of laboratories *assigned to the respective cases:* "a": $u_{min\,\%} \leq u(x_i)_{\%} \leq u_{max\,\%}$; "b": $u(x_i)_{\%} < u_{min\,\%}$; "c": $u(x_i)_{\%} > u_{max\,\%}$.

8.4 Additional information extracted from the questionnaire

Additional information on general aspects of the ILC, the analytical method, the preparation of CWEs and the analysis was extracted from the questionnaire and is summarized below. All questions and answers are listed in the annex (see chapter 13.4).

General information

All participating laboratories have a quality management system according to ISO 17025 [8].

Five laboratories reported to use accredited methods and six laboratories used validated methods, while one laboratory used a method, which was not accredited for the matrix; information about a validation was not given.

All laboratories tested blank samples. Most laboratories performed either a CWE with just water or the whole sample preparation process with water.

Experience of the laboratories with the analytical methods used to determine chloropropanols is diverse. Four laboratories have been using the method for less than a year, two of which reported that they had no experience with the method. Three laboratories have used the method for more than five years, one of these laboratories is using the method 251–1000 times per year. Most laboratories have been using the method for 1 to 5 years and up to 250 times per year.

Cold water extracts

Six laboratories used glass fiber filters size C for CWEs, four laboratories used glass fiber filters in other sizes and one laboratory used fritted glass.

Six laboratories used 11–50 mL to fill the volumetric flask after the filtration. Four laboratories needed less than 10 mL and one laboratory needed more than 50 mL of water to fill the volumetric flask.

Sample preparation and analysis

All laboratories used a derivatization agent. Eight laboratories used *N*-methyl-*N*-(trimethylsilyl)-trifluoracetamid (MSTFA), two laboratories used *N*-heptafluorobutyrylimidazole (HFBI) and one laboratory used *p*-toluenesulfonic acid monohydrate.

All laboratories used internal standards. Eleven laboratories used 3-MCPD-d5 for 3-MCPD and one laboratory used 3-methoxy-1,2-propanediol. For 2-MCPD all nine laboratories, which submitted results, used 3-MCPD-d5. For 1,3-DCP six laboratories used 3-chloro-1-methoxy-propanol, three laboratories used 1,3-DCP-d5, one laboratory used 3-MCPD-d5 and one laboratory used 3-methoxy-1,2-propanediol.

9 Conclusions

The primary aim of this ILC was the quantification of 3-MCPD in CWEs of paper straw samples and the quantification of 3-MCPD, 2-MCPD and 1,3-DCP in a solution provided to the participants by the ILC organizer.

Most laboratories scored well for the analysis of chloropropanols according to z and ζ scores. This emphasizes that many laboratories have established well-performing analytical methods for the quantification of chloropropanols. This is especially important for the quantification of 1,3-DCP since it is classified as carcinogenic in category 1B according to Regulation (EC) No. 1272/2008 [4]. Notably, most of the questionable or unacceptable results observed could be assigned to the same three laboratories. 2-MCPD is not classified and no limit values in paper or board exist, therefore it is not yet included in the analytical methods of every participating laboratory.

Most laboratories reported reasonable measurement uncertainties for the analysis of 3-MCPD in the CWEs. However, the estimation of the measurement uncertainties for 1,3-DCP in the provided solution was not reasonable for the majority of the laboratories.

In general, it is concluded that the preparation of the CWEs and the implemented analytical methods work well for the analysis of chloropropanols. Nevertheless, few laboratories need to improve their current methods, e.g. regarding specific detection of 3-MCPD. It might be advisable to use GC-MS/MS analysis for an unambiguous quantification of 3-MCPD in low concentration ranges (<2 ppb) or to slightly increase the LOQ of the GC-MS method as already done by one of the participating labs.

10 References

1. *DIN EN 645:1994, Paper and board intended to come into contact with foodstuffs; Preparation of a cold water extract*, 1994.
2. German Federal Institute for Risk Assessment (BfR), *Methodensammlung Papier, Karton und Pappe,1.2 Kaltwasserextrakt,* https://www.bfr.bund.de/de/methodensammlung_papier__karton_und_pappe-32620.html, [24.01.2023].
3. *ISO 13528:2015, Statistical methods for use in proficiency testing by interlaboratory comparison*, 2015.
4. *Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006.* Official Journal of the European Union, 2008. **51**.
5. International Agency for Research on Cancer, https://www.iarc.who.int/, [11.01.2023].
6. German Federal Institute for Risk Assessment (BfR), *BfR Recommendation: XXXVI. Paper and board for food contact*. 2022.
7. *Regulation (EU) 2017/625 of the European Parliament and of the Council of 15 March 2017 on official controls and other official activities performed to ensure the application of food and feed law, rules on animal health and welfare, plant health and plant protection products.* Official Journal of the European Union, 2017. **60**.
8. *DIN EN ISO/IEC 17025:2018 , General requirements for the competence of testing and calibration laboratories (ISO/IEC 17025:2017); German and English version*, 2018.
9. Michael Thompson, Stephen L. R. Ellison, and Roger Wood, *The international harmonized protocol for the proficiency testing of (chemical) analytical laboratories.* Pure & Appl. Chem. , 2006. **78**(1): p. 145-196.
10. *DIN 38402-45:2014, German standard methods for the examination of water, waste water and sludge - General information (group A) - Part 45: Interlaboratory comparisons for proficiency testing of laboratories (A 45)*, 2014.
11. *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 tables

12 List of figures

13 Annex

13.1 Instructions

Please perform the cold water extraction of Sample 1 and 2 in triplicate according to DIN EN 645 with the adjustments specified below. Analyze the additionally provided aqueous Solution 1, if possible, in triplicate (spiked cold water extract, individual sample treatment for each analysis is necessary) together with the extracts. Determine mass fractions for 3-MCPD in Samples 1 and 2 as well as 3-MCPD, 2-MCPD and 1,3-DCP in Solution 1.

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 GC-MS/MS. With this dataset we expect to improve the data basis for the estimation of the measurement uncertainty for the estimation of 3-MCPD from cold water extracts.

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

Instructions for the preparation of the cold water extract of Samples 1 and 2 (paper straws) according to DIN EN 645 with the modifications laid out in the BfR method compilation for paper and cardboard as well as in BfR recommendation XXXVI.

Please perform the cold water extracts according to DIN EN 645 in triplicate from each sample (use ultra pure water of at least type II) with 1 g of sample, 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 filled to the mark with ultrapure water. Please estimate (see questionnaire) the added volume (mL water) for completely filling the volumetric flask. Determine the mass fractions of 3-MCPD in all extracts. Please send an aliquot (~15 mL) of each of the three extracts of each sample 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 (paper straw sample). Thirteen test items were prepared and analyzed in duplicate. Results were evaluated according to ISO 13528 [3] and IUPAC's harmonized protocol [9] using the expanded criterion ($\sqrt{c}$) to consider the actual sampling error and repeatability. All results are reported in [µg L^{-1}].

	CWE	
	3-MCPD	
	1st	2nd
1	18.521	17.593
2	15.025	14.771
3	17.642	17.327
4	17.293	16.125
5	16.703	17.344
6	16.875	19.657
7	16.944	19.750
8	19.391	18.397
9	18.103	17.986
10	13.500	14.246
11	13.867	14.386
12	16.877	16.182
13	16.262	16.267
Mean	16.809	
$s_{\bar{x}}$	1.640	
s_w	0.893	
s_s	1.514	
σ_{pt} (20 % of Mean)	3.362	
σ_{allow}	1.009	
F1	1.75	
F2	0.80	
σ^2_{allow}	1.017	
c	2.421	
$\sqrt{c}$	1.556	
$s_s \leq \sqrt{c}$	passed	
Homogenous	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,
- 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,
- *C* = $F1\ \sigma^2_{allow} + F2\ s^2_w$; is used to expand the criterion to allow for the actual sampling error and repeatability.

13.2.2 Homogeneity assessment of Sample 2

Table 8: Results for the homogeneity assessment of Sample 2 (paper straw sample). Seventeen test items were prepared and analyzed in duplicate. Results were evaluated according to ISO 13528 [3] and IUPAC's harmonized protocol [9] using the expanded criterion ($\sqrt{c}$) to consider the actual sampling error and repeatability. All results are reported in [µg L^{-1}].

	CWE	
	3-MCPD	
	1st	2nd
1	3.392	3.421
2	2.783	3.241
3	2.130	2.557
4	2.774	3.000
5	2.381	2.633
6	2.649	3.109
7	2.655	2.856
8	3.286	3.440
9	2.502	2.177
10	2.444	2.699
11	3.048	3.258
12	3.070	3.240
13	2.311	2.622
14	2.891	2.533
15	2.907	2.818
16	2.310	2.249
17	2.618	2.200
Mean	2.771	
$s_{\bar{x}}$	0.361	
s_w	0.206	
s_s	0.331	
σ_{pt} (27 % of Mean)	0.748	
σ_{allow}	0.224	
F1	1.64	
F2	0.64	
σ^2_{allow}	0.050	
c	0.110	
$\sqrt{c}$	0.332	
$s_s \leq \sqrt{c}$	passed	
Homogenous	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,
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,
C	= $F1\ \sigma^2_{allow} + F2\ s^2_w$; is used to expand the criterion to allow for the actual sampling error and repeatability.

13.2.3 Stability assessment of Solution 1

Table 9: Results of the stability assessment of Solution 1. Stability was tested over a period of three weeks. All values are reported in [µg L^{-1}].

	2-MCPD	1,3-DCP
d_0	12.246	2.184
d_{21}	12.031	2.249
$\|d_0-d_{21}\|$	0.215	0.065
σ_{pt}	1.837	0.328
0.3 σ_{pt}	0.551	0.098
$\|d_0-d_{21}\| \leq 0.3\ \sigma_{pt}$	passed	passed
Assessment	Stable	Stable

Where: d_0 is the analysis in the beginning of the stability study,
d_{21} is the analysis in the end of the stability study,
σ_{pt} is the standard deviation for proficiency assessment, 15 %.

13.3 Results

13.3.1 Results for the determination of 3-MCPD in CWE of Sample 1

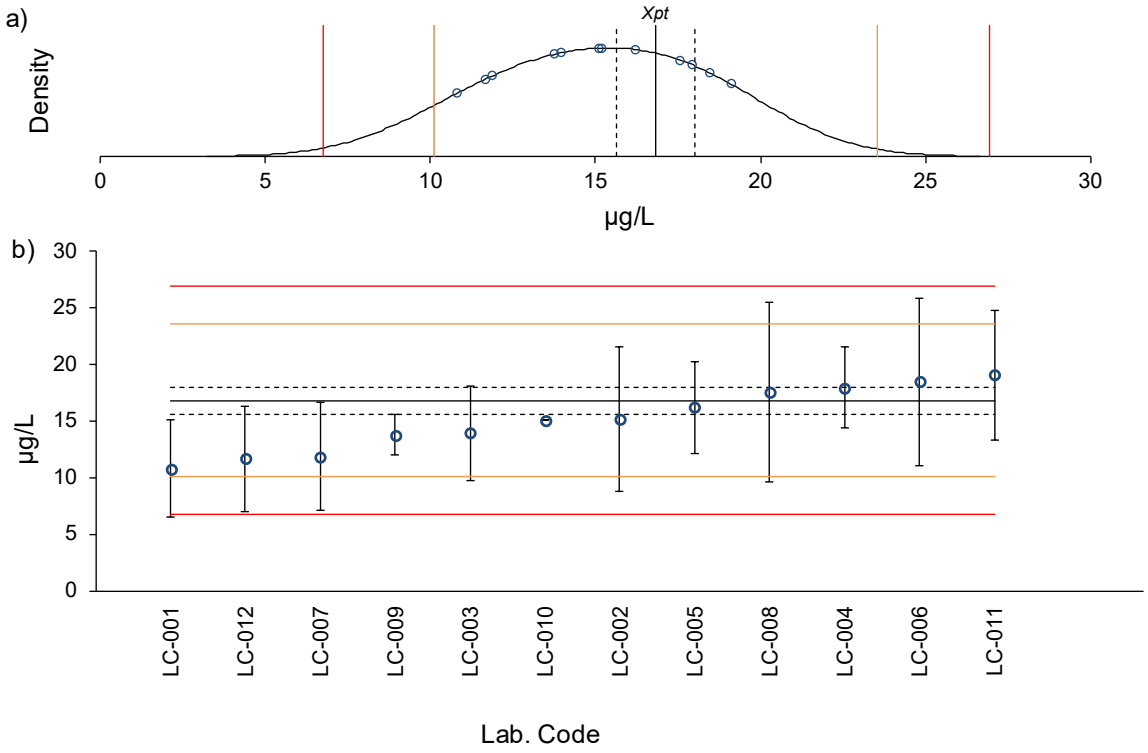

Figure 4: Measurement result range reported by the participants for the determination of 3-MCPD in CWE of Sample 1. Points and bars represent the reported results x_i with the corresponding expanded uncertainties $U(x_i)$; orange and red lines represent *z* scores = 2 and 3, respectively; solid and dotted black lines represent the assigned value x_{pt} and its expanded uncertainty $U(x_{pt})$.

Table 10: Results for the determination of 3-MCPD in CWE of Sample 1. Assigned range: x_{pt} = 16.827 ± 1.194 µg L^{-1}, $u(x_{pt})_{\%}$ = 3.5 %, σ_{pt} = 3.365 µg L^{-1} (20 %); x_i and $U(x_i)$ values are in µg L^{-1}.

Lab. Code	x_i	$U(x_i)$	k	$u(x_i)_{\%}$	*z* score	ζ score	$u(x_i)_{\%}$ est.§
LC-001	10.800	4.334	2	20	-1.79	-2.68	c
LC-002	15.200	6.400	2	21	-0.48	-0.50	c
LC-003	13.963	4.190	1.96	15	-0.85	-1.29	a
LC-004	17.936	3.587	2.576	8	0.33	0.73	a
LC-005	16.200	4.100	2	13	-0.19	-0.29	a
LC-006	18.467	7.387	2	20	0.49	0.44	a
LC-007	11.883	4.757	2	20	-1.47	-2.02	c
LC-008	17.580	7.966	2*	23	0.22	0.19	c
LC-009	13.767	1.800	2	7	-0.91	-2.83	a
LC-010	15.100	-	-	-	-0.51	-	-
LC-011	19.099	5.730	2	15	0.67	0.78	a
LC-012	11.667	4.653	2	20	-1.53	-2.15	a

* k=2 is set by the ILC organizer when no coverage factor k is reported.
§ (a) Reasonable estimation of $u(x_i)_{\%}$: $u(x_{pt})_{\%} \leq u(x_i)_{\%} \leq \sigma_{pt,\%}$; (b) underestimation of $u(x_i)_{\%}$; (c) overestimation of $u(x_i)_{\%}$.

13.3.2 Results for the determination of 3-MCPD in CWE of Sample 2

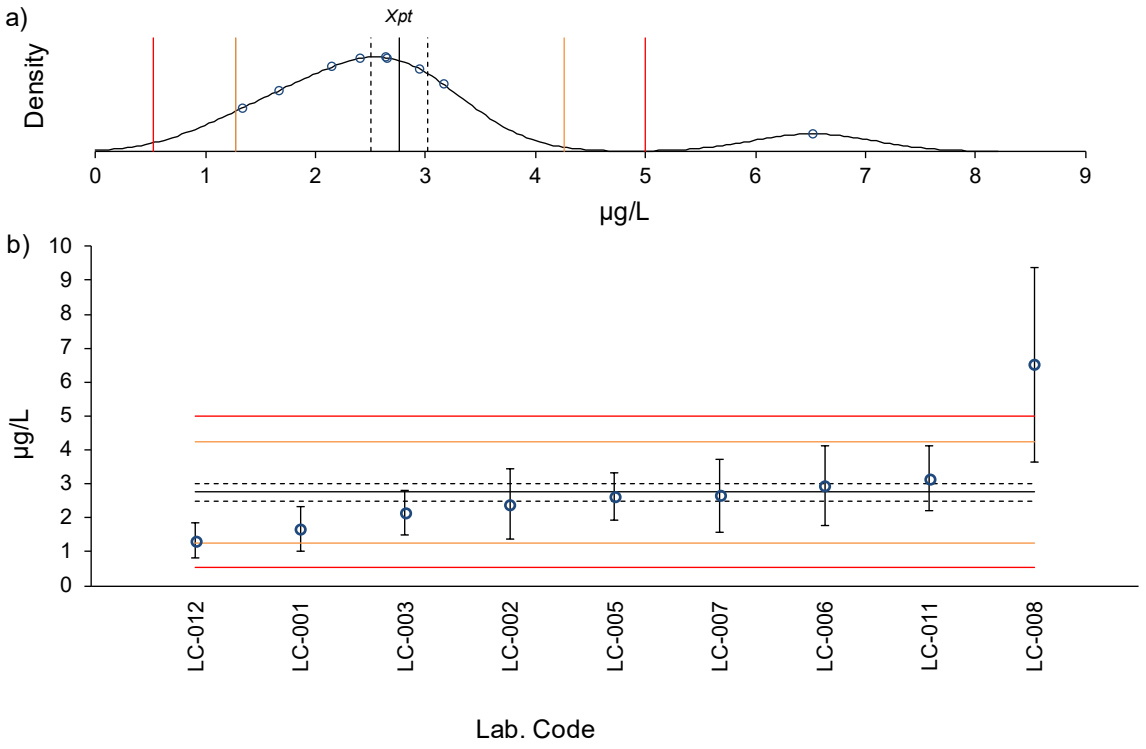

Figure 5: Measurement result range reported by the participants for the determination of 3-MCPD in CWE of Sample 2. Points and bars represent the reported results x_i with the corresponding expanded uncertainties $U(x_i)$; orange and red lines represent *z* scores = 2 and 3, respectively; solid and dotted black lines represent the assigned value x_{pt} and its expanded uncertainty $U(x_{pt})$.

Table 11: Results for the determination of 3-MCPD in CWE of Sample 2. Assigned range: x_{pt} = 2.764 ± 0.257 µg L^{-1}, $u(x_{pt})_{\%}$ = 4.6 %, σ_{pt} = 0.746 µg L^{-1} (27 %); x_i and $U(x_i)$ values are in µg L^{-1}.

Lab. Code	x_i	$U(x_i)$	k	$u(x_i)_{\%}$	*z* score	ζ score	$u(x_i)_{\%}$ est.§
LC-001	1.667	0.667	2	20	-1.47	-3.07	a
LC-002	2.400	1.033	2	22	-0.49	-0.68	a
LC-003	2.150	0.647	1.96	15	-0.82	-1.73	a
LC-005	2.633	0.700	2	13	-0.17	-0.35	a
LC-006	2.950	1.180	2	20	0.25	0.31	a
LC-007	2.650	1.063	2	20	-0.15	-0.21	a
LC-008	6.520	2.866	2*	22	5.03	2.61	a
LC-011	3.163	0.949	2	15	0.54	0.81	a
LC-012	1.333	0.533	2	20	-1.92	-4.83	a

* k=2 is set by the ILC organizer when no coverage factor k is reported.
§ (a) Reasonable estimation *of* $u(x_i)_{\%}$: $u(x_{pt})_{\%} \leq u(x_i)_{\%} \leq \sigma_{pt,\%}$; (b) underestimation of $u(x_i)_{\%}$; (c) overestimation of $u(x_i)_{\%}$.

13.3.3 Results for the determination of 2-MCPD in Solution 1

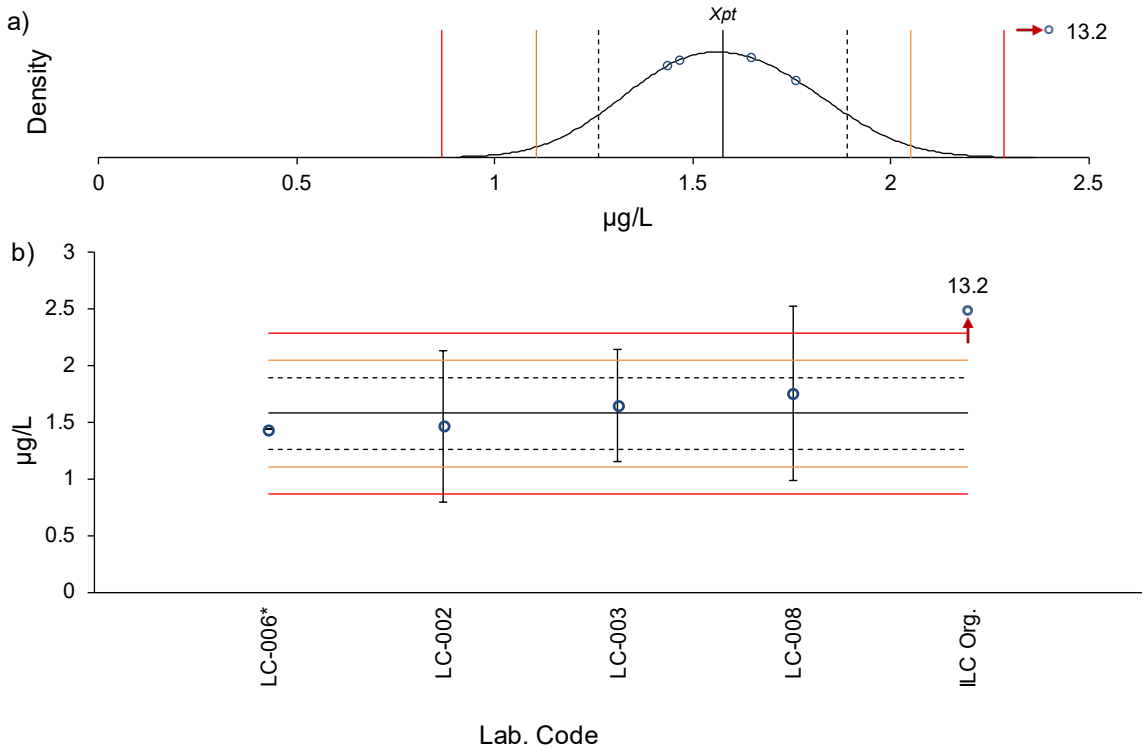

Figure 6: Measurement result range reported by the participants for the determination of 2-MCPD in Solution 1. Points and bars represent the reported results x_i with the corresponding expanded uncertainties $U(x_i)$; orange and red lines represent $2^*\sigma_{pt}$ and $3^*\sigma_{pt}$, respectively; solid and dotted black lines represent the robust mean $\overline{\overline{x}}_{pt}$ and its expanded uncertainty $U(\overline{\overline{x}}_{pt})$.

Table 12: Results for the determination of 2-MCPD in Solution 1. Robust mean: $\overline{\overline{x}}_{pt}$ = 1.578 ± 0.314 µg L^{-1}, σ_{pt} = 0.237 µg L^{-1}; x_i and $U(x_i)$ values are in µg L^{-1}.

Lab. Code	x_i	$U(x_i)$	k	$D\%^{\#}$
LC-002	1.467	0.668	2	-7
LC-003	1.647	0.493	1.96	4
LC-006	1.437	-	2*	-9
LC-008	1.760	0.770	2*	12
ILC Org.	13.238	3.972	2	739

* k=2 is set by the ILC organizer when no coverage factor k is reported.
\# $D\%$ was used instead of z scores, because the proportion $u(x_{pt})/\sigma_{pt}$ was found to be higher than 0.6.

13.3.4 Results for the determination of 1,3-DCP in Solution 1

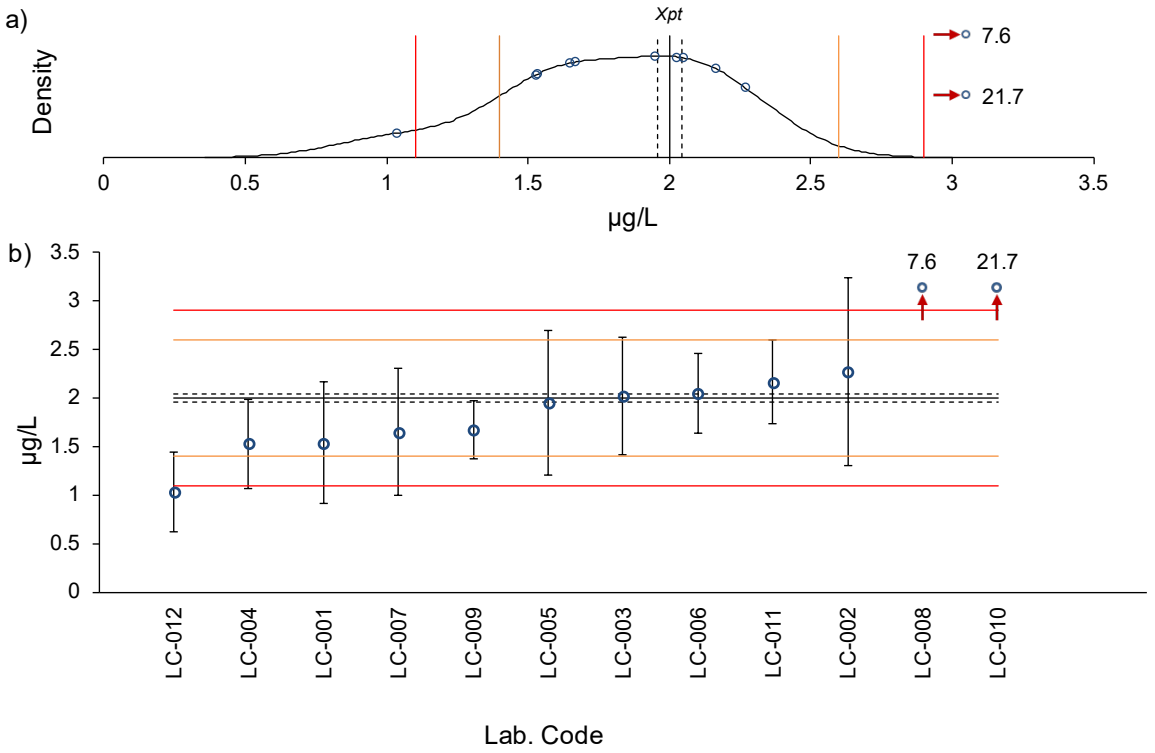

Figure 7: Measurement result range reported by the participants for the determination of 1,3-DCP in Solution 1. Points and bars represent the reported results x_i with the corresponding expanded uncertainties $U(x_i)$; orange and red lines represent z scores = 2 and 3, respectively; solid and dotted black lines represent the assigned value x_{pt} and its expanded uncertainty $U(x_{pt})$.

Table 13: Results for the determination of 1,3-DCP in Solution 1. Assigned range: x_{pt} = 2.000 ± 0.042 µg L^{-1}, $u(x_{pt})_{\%}$ = 1.1 %, σ_{pt} = 0.3 µg L^{-1} (15 %); x_i and $U(x_i)$ values are in µg L^{-1}.

Lab. Code	x_i	$U(x_i)$	k	$u(x_i)_{\%}$	z score	ζ score	$u(x_i)_{\%}$ est.§
LC-001	1.533	0.627	2	20	-1.56	-1.49	c
LC-002	2.267	0.970	2	21	0.89	0.55	c
LC-003	2.025	0.607	1.96	15	0.08	0.08	c
LC-004	1.530	0.457	2.576	12	-1.57	-2.63	a
LC-005	1.950	0.751	2	19	-0.17	-0.13	c
LC-006	2.047	0.409	2	10	0.15	0.23	a
LC-007	1.647	0.657	2	20	-1.18	-1.07	c
LC-008	7.600	-	-	-	18.66	-	-
LC-009	1.667	0.300	2	9	-1.11	-2.20	a
LC-010	21.733	-	-	-	65.77	-	-
LC-011	2.163	0.433	2	10	0.54	0.75	a
LC-012	1.033	0.413	2	20	-3.22	-4.65	c

§ (a) Reasonable estimation of $u(x_i)_{\%}$: $u(x_{pt})_{\%} \leq u(x_i)_{\%} \leq \sigma_{pt,\%}$; (b) underestimation of $u(x_i)_{\%}$; (c) overestimation of $u(x_i)_{\%}$.

13.4 Results of the questionnaire

13.4.1 General Information

Table 14: 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-001	Official Control Laboratory (OCL)	Yes	ISO 17025	Yes
LC-002	Official Control Laboratory (OCL)	Yes	ISO 17025	Yes
LC-003	Official Control Laboratory (OCL)	Yes	ISO 17025	Yes
LC-004	Official Control Laboratory (OCL)	Yes	ISO 17025	Yes
LC-005	Official Control Laboratory (OCL)	Yes	ISO 17025	Yes
LC-006	Official Control Laboratory (OCL)	Yes	ISO 17025	Yes
LC-007	Official Control Laboratory (OCL)	Yes	ISO 17025	No
LC-008	Official Control Laboratory (OCL)	Yes	ISO 17025	Yes
LC-009	Official Control Laboratory (OCL)	Yes	ISO 17025	Yes
LC-010	National Reference Laboratory (NRL)	Yes	ISO 17025	Yes
LC-011	National Reference Laboratory (NRL)	Yes	ISO 17025	Yes

13.4.2 Analytical Method (3-MCPD, 2-MCPD, 1,3-DCP)

Table 15: Information on the used analytical methods (Part I).

Lab. Code	1. Which analytical technique was used for the analysis of 3-MCPD, 2-MCPD and 1,3-DCP?	if other specify here	2. Is this method validated/accredited?	Describe shortly the way of the method validation
LC-001	GC-MS	-	Accredited method	a sample with added 3-MCPD and DCP concentration was treated according to the analysis instruction (5-fold determination). Evaluation against external calibration
LC-002	GC-MS	-	Accredited method	The following parameters were tested: specificity, variability between instruments, linearity, accuracy of GC/MS measurement, accuracy of sample preparation (including real samples and spiked samples), LOD and LOQ, accuracy tested with spiked samples, implementation of control charts
LC-003	GC-MS/MS	-	Validated Method	Linearity, repeatability, intermediate precision, recovery rate, LOD, LOQ, selectivity, uncertainty, spiked cold water extract

Continuation Table 15: Information on the used analytical methods (Part I).

Lab. Code	1. Which analytical technique was used for the analysis of 3-MCPD, 2-MCPD and 1,3-DCP?	if other specify here	2. Is this method validated/accredited?	Describe shortly the way of the method validation
LC-004	GC-MS	SPE with kieselgur and ethylacetate. Online Derivatization	Validated Method	According to Guidelines for performance criteria and validation procedures of analytical methods used in controls of food contact materials – 7 "BASIC LEVEL" VALIDATION SCHEMES FOR FCM METHODS
LC-005	GC-MS	-	Accredited method	Determination of LOD, LOQ, linearity range of calibration, recovery after standard addition to CWEs of paper samples, intra-/inter-day precision, expanded measurement uncertainty. Regular participation in inter-laboratory tests.
LC-006	GC-MS	-	Accredited method	LOD, LOQ, recovery studies, proficiency tests, stability, measurement uncertainty
LC-007	GC-MS	-	Validated Method	Precision and recovery determinated from spiked cold water extracts (blank matrix), LOD defined as the lowest concentration with the correct qualifier ratios and having a S/N-ratio of more than 3. LOQ = 2 x LOD (S/N-ratio more than 10), LOQ correspond to the lowest calibration point.
LC-008	GC-MS	-	Validated Method	Validated for 3- and 2-MCPD but not validated for 1,3-DCP
LC-009	GC-MS/MS	-	Accredited method	spiked cold water extract
LC-010	GC-MS	-	Please Select	Not accredited for this matrix, but for foods. Uncertainty is therefore not reported for this intercomparison
LC-011	GC-MS/MS	-	Validated Method	Selectivity – RT, ratio qualifier/quantifier; linearity – Mandeltest, Residualtest; LOD+LOQ – DIN 32645:2008; precision – EUR 24105 EN; trueness – 2002/657/EC; measurement uncertainty – EUR 24105 EN (chapter 5.2.8.3), NT TR 537 (Nordtest)

Table 16: Information on the used analytical methods (Part II).

Lab. Code	3. How long and frequently is this method used in your laboratory?		4. 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-001	>5	1–50	In-house validation	-	Yes
LC-002	2–5	1–50	In-house validation	-	Yes
LC-003	<1	1–50	NORDTEST	-	No
LC-004	<1	51–250	NORDTEST	-	No
LC-005	>5	251–1000	In-house validation	-	Yes
LC-006	1–2	51–250	NORDTEST	-	Yes
LC-007	1–2	1–50	Inter-laboratory comparison	-	Yes
LC-008	Please Select	51–250	Other	Horwitz	No
LC-009	1–2	1–50	In-house validation	-	No
LC-010	>5	1–50	Please Select	-	Please Select
LC-011	<1	Never	NORDTEST	-	Yes
LC-012	<1	Never	In-house validation	-	Yes

Table 17: Information on the used analytical methods (Part III).

Lab. Code	5. Did you test a blank sample?	if YES specify here	6. Did you substract these blank values?	7. Did you apply any special treatment to the samples provided?	if YES specify here
LC-001	Yes	-	No	Yes	derivatization with Heptafluorbutyrylimidazole
LC-002	Yes	Complete sample preparation using pure water	No	No	-
LC-003	Yes	Cold water extract with water, processed as sample	Yes	Yes	29.25 g of NaCl in 250 mL volumetric flask
LC-004	Yes	Bi-demin. water used for CWE is analyzed with the same procedure.	Yes	No	-
LC-005	Yes	Bi-dest. water was prepared (addition of NaCl) and analyzed analogously to CWEs (but not stored for 24 h at RT like CWEs). Blank values for 3-MCPD were at approx. 0.2 to 0.3 µg/L.	No	No	-
LC-006	Yes	solution blank	Yes	No	-
LC-007	Yes	Water, same treatment like the samples	No	Yes	SPE with Extrelut, Elution with ethylacetate, Concentrate 5 mL to 0.5 mL
LC-008	Yes	water	Yes	No	-
LC-009	Yes	a cold water extract without sample is performed and analyzed	No	No	-
LC-010	Yes	Just water, the same used for the preparation of the extracts	Yes	No	-
LC-011	Yes	Milli-Q water with ISTD, the whole extraction step was done	No	No	-
LC-012	Yes	We carried out the method procedure with a sample and substracted that obtained blind value of 3.3 µg/L for 3-MCPD analysis of the samples	No	Yes	derivatization with MSTFA

Table 18: Information on the used analytical methods (Part IV).

Lab. Code	8. Did you encounter any problems with the sample analysis?	if YES specify here
LC-001	Please Select	-
LC-002	No	-
LC-003	No	-
LC-004	Yes	In Sample 2 and Solution 1 we could not confirm the presence 3-MCPD because the qualifier ions (m/z = 116 and 101) we use for confirmation were obstructed by background signals. Despite this we found 3-MCPD with an average of 3.04 µg/L in Sample 2 and with an average of 1.35 µg/L (below calibration/LOQ) in Solution 1 because the quantifier (m/z = 239) was not obstructed. In the case of 1,3-DCP we always substract blank values.
LC-005	Yes	In Solution 1, the quantifier for 3-MCPD was detected and estimated at approx. 1.2 µg/L. However, 3-MCPD could not be clearly identified because the qualifier ratios significantly differed from the standard substance in calibration solutions. Thus, the LOD for 3-MCPD in Solution 1 was set to 2 µg/L instead of 1 µg/L and results for 3-MCPD in Solution 1 are reported as <2 µg/L. 2-MCPD is not part of our accredited method anymore. We tried to identify/quantify 2-MCPD in Solution 1, but there were problems with co-elution and qualifier ratios compared to the standard substance in calibration solutions. Thus, results for 2-MCPD in Solution 1 are reported as “not analyzed”.
LC-006	Yes	2-MCPD qualifier not always conclusively.
LC-007	No	-
LC-008	No	-
LC-009	Yes	We had a reduced instrument response (needs to be cleaned) and therefore our sensitivity was not at its best.
LC-010	No	-
LC-011	No	-
LC-012	Please Select	We couldn’t get ethylacetate in the required purity. For that reason, we detect the following blind values 3-MCPD: 3.3 µg/L and for 2-MCPD (below LOQ (0.7 µg/L)). So couldn’t evaluate 2-MCPD, because the detected values were 2 and 2.1 µg/l and we can’t substract the blind value as it is 0.7 µg/L (that means below LOQ 2 µg/L).

13.4.3 Cold water extract

Table 19: 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-001	Yes, glass-fiber filter (size C)	-	11–50 mL
LC-002	Yes, glass-fiber filter (other size)	-	more than 50 mL
LC-003	No (Specify)	Fritted glass, porosity 3	0–10 mL
LC-004	Yes, glass-fiber filter (other size)	-	11–50 mL
LC-005	Yes, glass-fiber filter (size C)	-	0–10 mL
LC-006	Yes, glass-fiber filter (other size)	-	11–50 mL
LC-007	Yes, glass-fiber filter (size C)	-	0–10 mL
LC-008	No (Specify)	-	Please Select
LC-009	Yes, glass-fiber filter (other size)	125 mm diameter	0–10 mL
LC-010	Yes, glass-fiber filter (size C)	-	11–50 mL
LC-011	Yes, glass-fiber filter (size C)	-	11–50 mL
LC-012	Yes, glass-fiber filter (size C)	-	11–50 mL

13.4.4 Analysis

Table 20: Information on the analysis.

Lab. Code	1. Did you use a derivatization agent?	if YES, please specify here which derivatization agent did you use:	2. Did you use internal standards?	if YES, please specify here which internal standard did you use for quantification of:		
				3-MCPD	2-MCPD	1,3-DCP
LC-001	Yes (Specify)	Heptafluorbutyrylimidazole	Yes (Specify)	Methoxy-1,2-propandiol	-	Methoxy-1,2-propandiol
LC-002	Yes (Specify)	MSTFA	Yes (Specify)	3-MCPD-d5	3-MCPD-d5	3-Chlor-1-methoxy-2-propanol (DCP-Methox)
LC-003	Yes (Specify)	MSTFA	Yes (Specify)	3-MCPD-d5	3-MCPD-d5	3-Chlor-1-methoxy-2-propanol
LC-004	Yes (Specify)	MSTFA	Yes (Specify)	d5-3-MCPD	d5-3-MCPD	d5-1,3-DCP
LC-005	Yes (Specify)	3,3-*N*-Methyl-*N*-trimethylsilyl-trifluoracetamid (MSTFA)	Yes (Specify)	3-MCPD-d5	-	1-Chloro-3-methoxy-propan-2-ol (CMP)
LC-006	Yes (Specify)	MSTFA	Yes (Specify)	(±)-3-Chlor-1,2-propan-1,1,2,3,3-d5-diol	(±)-3-Chlor-1,2-propan-1,1,2,3,3-d5-diol	1,3-Dichloro-2-propanol-d5

Continuation Table 20: Information on the analysis.

Lab. Code	1. Did you use a derivatization agent?	if YES, please specify here which derivatization agent did you use:	2. Did you use internal standards?	if YES, please specify here which internal standard did you use for quantification of:		
				3-MCPD	2-MCPD	1,3-DCP
LC-007	Yes (Specify)	MSTFA	Yes (Specify)	d5-3-MCPD	n.a.	CMP
LC-008	Yes (Specify)	*p*-Toluenesulfonic acid	Yes (Specify)	3-MCPD d5	3-MCPD d5	-
LC-009	Yes (Specify)	MSTFA	Yes (Specify)	MCPD-D5	MCPD-D5	MCPD-D5
LC-010	Yes (Specify)	*N*-heptafluoro-butyrylimidazole	Yes (Specify)	3-chloro-1,2-propanediol-D5	3-chloro-1,2-propanediol-D5	1,3-dichloro-isopropyl-D5
LC-011	Yes (Specify)	*N*-Methyl-*N*-trimethylsilyltrifluoracetamid (MSTFA)	Yes (Specify)	(±)-3-Chloro-1,2-propan-1,1,2,3,3-d5-diol	(±)-3-Chloro-1,2-propan-1,1,2,3,3-d5-diol	3-Chlor-1-Methoxy-2-propanol
LC-012	Yes (Specify)	MSTFA	Yes (Specify)	3 MCPD-d5	3 MCPD-d5	3-Chlor-1-methoxy-2-propanol