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Determination of styrene in milk

FCM-23/02 Proficiency Test Report

268-PT

Valzacchi, S., Bratinova, S.P., Gutiérrez Linares, A., Robouch, P., Hoekstra, E.

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Abstract

The implementation of the EU legislation on food contact materials (FCM) requires the EU member states to conduct tests ensuring that the legal requirements of the FCM are met. These tests are performed by laboratories designated for official controls. In line with Regulation (EU) 2017/625 on official controls for food and feed, the European Union Reference Laboratory for Food Contact Materials (EURL-FCM) organised a proficiency testing (PT) round for the determination of styrene in milk. The scope of this PT was to assess the proficiency of the participants in quantifying styrene in milk at a concentration that is proposed to be the new specific migration limit (SML) of styrene from plastics into food, as outlined in the future amendment of to the Commission Regulation (EU) 10/2011 on plastic materials and articles intended to come into contact with food. This PT was open to EU National Reference Laboratories (NRLs) and interested Official Control Laboratories (OCLs).

This report summarises the results of the PT "FCM-23/02" for the determination of styrene in milk. The two test items analysed in this PT consisted of a commercial milk (T1) and the same milk spiked with styrene (T2). Twenty-seven laboratories registered, but twenty-six submitted results to the PT round, including 17 National Reference Laboratories (NRLs) from 19 EU Member States and Switzerland and 9 EU Official Control Laboratories (OCLs). The evaluation of the analytical performance confirms that most laboratories are able to identify and accurately quantify styrene in milk.

Acknowledgements

The twenty-six laboratories listed hereafter are kindly acknowledged for their participation in the PT.

Organisation	Country
Austrian Agency for Health and Food Safety (AGES),Department Consumer Goods and Cosmetics *	Austria
Sciensano *	Belgium
Testing Centre ALMI TEST	Bulgaria
State General Laboratory *	Cyprus
Croatian Institute of Public Health, Laboratory of General Use Items *	Croatia
Technical University of Denmark, National Food Institute *	Denmark
National Institute of Public Health *	Czech Republic
Laboratoire National De Metrologie Et D'essais (LNE) *	France
Bundesinstitut fuer Risikobewertung (BFR) *	Germany
Chemisches- und Veterinäruntersuchungsamt (CVUA) Stuttgart	Germany
Thüringer Landesamt für Verbraucherschutz	Germany
Landesuntersuchungsanstalt für das Gesundheits - und Veterinärwesen Sachsen (LUA)	Germany
Landesamt für Verbraucherschutz Sachsen-Anhalt	Germany
Chemisches und Veterinäruntersuchungsamt Münsterland-Emscher-Lippe (CVUA-MEL)	Germany
General Chemical State Laboratory, Chemical Service of Athens *	Greece
Dublin Public Analyst's Laboratory, Sir Patrick Dun`s *	Ireland
Istituo Zooprofilattico Statale della Sardegna, S.C. Chimica	Italy
Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Reparto Chimico degli Alimenti	Italy
Laboratoire National de Santé, Service Alimentaire *	Luxembourg
Handelslaboratorium Dr. A. Verwey	Netherlands
Escola Superior de Biotecnologia-Universidade Católica Portuguesa *	Portugal
Regional Public Health Authority *	Slovakia
National Laboratory of Health, Environment and Food *	Slovenia
National Center for Food - Spanish Food Safety and Nutrition Agency *	Spain
Swedish Food Agency *	Sweden
Official Food Control Authority of the Canton of Zürich *	Switzerland

(*) NRL FCM: National reference laboratory for food contact materials

Authors

Valzacchi S., Bratinova S., Gutierrez Linares A., Robouch P. and Hoekstra E.

This proficiency test report has been authorised by Ursula Vincent, Head of the Food and Feed Compliance Unit, JRC.F.5.

Executive summary

The European Union Reference Laboratory for Food Contact Materials (EURL-FCM) organised a proficiency testing (PT) round (FCM-23/02) for the determination of styrene in milk. The scope of this PT was to assess the proficiency of National Reference Laboratories (NRLs) and Official Control Laboratories (OCLs) in quantifying styrene in milk at a concentration suggested to be the new specific migration limit (SML) for styrene - of 0.040 mg kg^{-1} food - in a future amendment to the Commission Regulation (EU) 10/2011 on plastic materials and articles intended to come into contact with food [1]. As the conventional food simulants are not suitable for migration testing (due to swelling of polystyrene FCMs), it is foreseen that foods, such as milk, should be used instead of food simulants.

Considering the perishability of UHT milk after the opening of the package, the EURL decided not to prepare the spiked milk test item. Instead, participants received a methanolic solution of styrene and a commercial UHT milk. They had to prepare the spiked milk test item following the provided instructions. The milk was previously analysed for a potential background concentration of styrene. The assigned value of the spiked milk and the homogeneity and stability of the styrene solution were evaluated by the EURL-FCM.

The concentration of styrene in milk (test item A, blank) was below 0.1 µg L^{-1} and was not considered significant. The assigned value of the spiked milk (test item B) was 0.023 mg L^{-1} assuming a density of 1 L kg^{-1} for food and food simulant and was derived from formulation. This concentration was deliberately set to be significantly lower than the expected SML, yet higher than 20 % of the SML being the maximum of the lower calibration point. The relative standard deviations for proficiency assessment ($\sigma_{pt,rel}$) was set at 15 % of the assigned value, based on the judgment of experts.

The styrene solution in methanol was proven to be suitably homogeneous and stable throughout the entire duration of the PT.

At first, participants were asked to determine the styrene concentration in milk (test item A). Subsequently, they were instructed to spike the milk with the styrene in a methanolic solution and promptly quantify styrene in the resulting test item B using their routine procedure. Participants were asked to report the styrene concentration in both test items A and B together with the associated expanded uncertainties (expressed in µg L^{-1}), the coverage factor, and the analytical technique used.

Eighteen NRLs and 9 OCLs from 19 Member States and Switzerland registered to the exercise. One NRL did not submit their results. Laboratory results were rated using z, and zeta (ζ) scores in accordance with ISO 13528:2022 [2].

Twenty-one laboratories out of 26 had a satisfactory z-score and 20 laboratories a satisfactory zeta-score. Nineteen laboratories reported a realistic measurement uncertainty. Most of the laboratories used various techniques based on gas chromatography mass spectrometry.

The overall performance of the participants was satisfactory (above 80 % expressed as z score), indicating the competence of the participants to meet the proposed SML for styrene.

1 Introduction

The European Union Reference Laboratory for Food Contact Materials (EURL-FCM), hosted by the Joint Research Centre of the European Commission, organised a proficiency testing (PT) round for the determination of styrene in milk. Milk was selected as substitute of food simulant D1 (ethanol 50%) responsible for swelling effect on styrenic-polymers. The PT was organised in view of the adoption of a specific migration limit for styrene in the Commission Regulation (EU) No 10/2011 on plastic materials and articles intended to come into contact with food [1].

This PT was agreed with the Directorate General for Health and Food Safety (DG SANTE) as part of the EURL-FCM annual work programme 2023, thus complying with the mandate set in Regulation (EU) 2017/625 [3]. The PT was mandatory for all National Reference Laboratories (NRLs) and open to interested Official Control Laboratories (OCLs) (under certain conditions).

This report summarises the outcome of the PT.

2 Scope

The present PT aimed to assess the performance of NRLs and OCLs in the quantification of styrene in milk.

This PT, organised in line with ISO 17043:2010 [4], was identified as "FCM-23/02".

3 Set up of the exercise

3.1 Quality assurance

The JRC Unit hosting the EURL-FCM is accredited according to:

- ISO/IEC 17043:2010 (certificate number: BELAC 268-PT, proficiency test provider)

The reported results were evaluated following the relevant administrative and logistic procedures.

3.2 Confidentiality

The procedures used for the organisation of PTs guarantee that the identity of the participants and the information provided by them is treated as confidential. The participants in this PT received a unique laboratory code used throughout this report. However, the laboratory codes of NRLs appointed in line with Regulation (EU) 2017/625 [2] may be disclosed to DG SANTE upon request for the purpose of an assessment of their (long-term) performance. Similarly, laboratory codes of appointed OCLs may be disclosed to their respective NRL upon request.

3.3 Time frame

The following time frame was followed:

Invitation letter to NRLs (Annex 1):	October 4, 2023.
Registration deadline:	October 18, 2023.
Instruction letter (Annex 2):	October 23, 2023.
Samples shipment:	October 30, 2023.
Deadline for reporting of results:	December 1, 2023. extended to December 6, 2023 (upon request of some participants).

3.4 Sample shipment

Each participant received:

- 0.5 L pack of UHT semi-skimmed milk (item 1);
- One vial containing 20 mL of methanol spiked with styrene (item 2);
- the "Accompanying letter" (Annex 2);

Samples were sent under normal transport conditions at ambient temperature.

3.5 Instructions to participants

Detailed instructions were given to participants in the "Instructions to the participants" letter (Annex 3).

Participants were asked to check whether the bottles and vial were undamaged after transport.

They were instructed to store item 2 in the refrigerator at 4 °C ± 2 °C in the dark, upon reception and until analysis.

At first, they had to analyse test item A (item 1) for the styrene content. Successively, they had to spike the milk (item 1) with the styrene solution (item 2) and quantify the styrene immediately after the spiking in the resulting "test item B", according to their routine procedure.

Participants were asked to report the styrene content (expressed in μg L^{-1} assuming a density of 1 g cm^{-3} for milk) determined in test items A and B, the associated expanded uncertainties (in μg L^{-1}), the coverage factor of the uncertainty, and the analytical technique used.

Results had to be reported in the same format (e.g. number of significant figures) as normally reported to customers.

Participants received also the link to the reporting website, the password to access the reporting interface and their individual Laboratory code (Annex 3). They were then asked to fill in a questionnaire with additional information related to measurements and laboratories (Annex 4).

4 Test item

4.1 Preparation

UHT semi-skimmed milk (1.6 % fat content) (item 1) was obtained from a local vendor in boxes containing 10 half-liter packs, all identified with the same stock number to guarantee the homogeneity of the milk. The milk was analysed to confirm the absence of any styrene contamination.

A stock solution of styrene was prepared by weighing around 50 mg of styrene (analytical standard, Merck, Darmstadt, Germany) in a 100 mL volumetric flask subsequently filled to the mark with methanol. The final bulk solution was prepared by transferring 2.0 mL of stock solution in 2 L volumetric flask filled to the mark with methanol and thoroughly mixed. The bulk solution was transferred into 22 mL glass vials with screw caps and PTFE liner for the distribution to the participants (item 2).

4.2 Homogeneity and stability

The homogeneity and stability studies and the statistical treatment of data were performed by the EURL-FCM.

The assessment of homogeneity involved spiking 0.50 mL of item 2 (styrene in methanol solution) into 10.0 mL milk (item 1), following the prescribed procedure for the PT. Ten spiked milk samples (test item B), prepared from randomly selected vials of styrene solution, were then analysed in duplicate soon after spiking. Results were evaluated according to ISO 13528:2022 [2]. The test item B proved to be homogeneous for the styrene content (Annex 5.A), thus indirectly proving the homogeneity of the dispatched test item 2 at a level of 0.5 mL.

For the stability study, solutions of styrene in methanol (item 2) were subject to thermal treatments for specific duration. This included two vials stored at 40 °C for 7 days to simulate transport conditions, and two vials stored at 4 °C for 4 weeks to cover the entire duration of the PT. Aliquots (0.5 mL) from each vials were then spiked into 10.0 mL milk samples (item 1) – thus resulting in test item B samples – which were then analysed soon after spiking. The stability was assessed according to ISO 13528:2022 [2] and test item 2 proved to be stable (Annex 5.B).

5 Assigned value and corresponding uncertainty

5.1 Assigned value

The concentration of styrene in the milk sample (test item A) was determined to be below the limit of quantification (LOQ) of 0.1 µg L^{-1}. Consequently, it was considered negligible in comparison to the assigned value of test item B. Hence, no assigned value was established, and the corresponding reported results were not scored.

The assigned value for the test item B (x_{pt}) was derived from formulation and is provided in Table 1. This value was further confirmed by the experimental results obtained during the homogeneity study (Annex 5.A)

Table 1: Assigned value (x_{pt}), associated standard uncertainty of the assigned value ($u(x_{pt})$), standard deviation for the PT assessment (σ_{pt}) and other relevant parameters for the assessment of results.

	x_{pt}	u_{char}	u_{hom}	u_{st}	**$u(x_{pt})$, k=1**	σ_{pt} (15%)	$u(x_{pt})/\sigma_{pt}$	scoring
µg L^{-1}	**23.43**	0.063	0.066	0	**0.092**	3.51	0.03	z, ζ

5.2 Associated uncertainties

The associated standard uncertainty of the assigned value ($u(x_{pt})$) was calculated following the law of uncertainty propagation, combining the standard measurement uncertainty of the characterization (u_{char}) with the standard uncertainty contributions from homogeneity (u_{hom}) and stability (u_{st}) (Eq. 1), in compliance with ISO 13528:2022 [2]:

$$u(x_{pt}) = \sqrt{u_{char}^2 + u_{hom}^2 + u_{st}^2} \qquad \text{Eq. 1}$$

The uncertainty u_{char} was estimated from the formulation according to the recommendations of ISO 13528:2022 [2].

5.3 Metrological traceability of the assigned value

Item 2 was prepared gravimetrically using styrene analytical standard purchased from Sigma-Aldrich with the related certificate of analysis and purity.

The SI-traceable calibration of balance and a thorough control of the weighing procedure for the preparation of the test item ensured traceability of the weighing itself.

5.4 Standard deviation for proficiency assessment, σ_{pt}

The relative standard deviation for PT assessment ($\sigma_{pt,\ rel}$) was set, based on expert judgment, at 15 % of the assigned value (x_{pt}) of test item B (Table 1).

6 Evaluation of results

6.1 Scores and evaluation criteria

The individual laboratory performance was expressed in terms of z and ζ scores (Eq. 2 and 3) according to ISO 13528:2022 [2]:

$$z = \frac{x_i - x_{pt}}{\sigma_{pt}} \qquad \text{Eq. 2}$$

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

Where: x_i is the measurement result reported by a participant;
$u(x_i)$ is the standard measurement uncertainty reported by a participant;
x_{pt} is the assigned value;
$u(x_{pt})$ is the standard measurement uncertainty of the assigned value;
σ_{pt} is the standard deviation for proficiency test assessment.

The interpretation of the *z* and *ζ* performance scores was done according ISO 13528:2022 [2]:

|score| ≤ 2	satisfactory performance	(green in Annexes 7 - 20)
2 < |score| < 3	questionable performance	(yellow in Annexes 7 - 20)
|score| ≥ 3	unsatisfactory performance	(red in Annexes 7 - 20)

The *z* scores compare the participant's deviation from the assigned value with the standard deviation for proficiency test assessment (σ_{pt}) used as common quality criterion.

The *ζ* scores state whether the laboratory's result agrees with the assigned value within the respective uncertainty. The denominator is the combined uncertainty of the assigned value $u(x_{pt})$ and the measurement uncertainty as stated by the laboratory $u(x_i)$. The *ζ* score includes all parts of a measurement result, namely the expected value (assigned value), its measurement uncertainty in the unit of the result as well as the uncertainty of the reported values. An unsatisfactory *ζ* score can either be caused by an inappropriate estimation of the concentration, or of its measurement uncertainty, or both.

The standard measurement uncertainty of the laboratory $u(x_i)$ was obtained by dividing the reported expanded measurement uncertainty by the reported coverage factor*, k.* When no uncertainty was reported, it was set to zero ($u(x_i) = 0$) by the PT coordinator. When *k* was not specified, the reported expanded measurement uncertainty was considered by the PT coordinator as the half-width of a rectangular distribution; $u(x_i)$ was then calculated by dividing this half-width by √3, as recommended by Eurachem [6].

Uncertainty estimation is not trivial, therefore an additional assessment was provided to each laboratory reporting measurement uncertainty, indicating how reasonable their measurement uncertainty estimation has been. The relative standard measurement uncertainty was calculated based on the absolute values of the assigned values [$u_{rel}(x_{pt}) = 100*(u(x_{pt})/x_{pt})$] and of the reported values [$u_{rel}(x_i)=100*(u(x_i)/x_i)$].

The relative standard measurement uncertainty from the laboratory $u_{rel}(x_i)$ is most likely to fall in a range between a minimum and a maximum allowed uncertainty (case "a": $u_{min,rel} \leq u_{rel}(x_i) \leq u_{max,rel}$). $u_{min,rel}$ is set to the standard uncertainty of the assigned values $u_{rel}(x_{pt})$. It is unlikely that a laboratory carrying out the analysis on a routine basis would determine the measurand with a smaller measurement uncertainty than the expert laboratories chosen to establish the assigned value (ISO 13528:2022 §7.6) or, if applicable, by formulation (ISO 13528:2022 §7.3) or than the certified measurement uncertainty associated with a certified reference material property value (ISO 13528:2022 §7.4). $u_{max,rel}$ is set to the standard deviation accepted for the PT assessment, σ_{pt} (expressed as a percentage of the assigned value). Consequently, case "a" becomes: $u_{rel}(x_{pt}) \leq u_{rel}(x_i) \leq \sigma_{pt,\%}$.

If $u_{rel}(x_i)$ is smaller than $u_{rel}(x_{pt})$ (case "b") the laboratory may have underestimated its measurement uncertainty. Such a statement has to be taken with care as each laboratory reported only measurement uncertainty, whereas the measurement uncertainty associated with the assigned value also includes contributions for homogeneity and stability of the test item. If those are large, relative measurement uncertainties smaller than $u_{rel}(x_{pt})$ are possible and plausible.

If $u_{rel}(x_i)$ is larger than $\sigma_{pt,\%}$ (case "c") the laboratory may have overestimated its measurement uncertainty. An evaluation of this statement can be made when looking at the difference between the reported value and the assigned value: if the difference is

smaller than the expanded uncertainty $U(x_{pt})$ then overestimation is likely. If the difference is larger but x_i agrees with x_{pt} within their respective expanded measurement uncertainties, then the measurement uncertainty is properly assessed resulting in a satisfactory performance expressed as a ζ score, though the corresponding performance, expressed as a z score, may be questionable or unsatisfactory.

It should be pointed out that "$u_{max,rel}$" is a normative criterion when set by legislation.

6.2 General observations

Eighteen NRLs and 9 OCLs, representing 19 Member States and Switzerland (listed in the Acknowledgements), registered to the exercise. While 1 NRL did not report results, the remaining 26 laboratories reported results for both the raw milk sample (test item A) and the spiked milk they prepared (test item B).

All participants (26) submitted the questionnaire (Annex 4). Analyses were performed using different analytical approaches (Table 2), with the vast majority applying gas chromatography mass spectrometry (GC-MS) based techniques. The experimental details are provided in Annex 9 and 10.

Table 2: Analytical techniques applied by the participants

Analytical technique	Number of laboratories
HS-SPME-GC-MS/MS	7
HS-SPME-GC-MS	4
HS-GC-MS	6
HS-GC-FID	1
P&T-GC-MS	1
GC-MS/MS *	1
GC-MS *	1
HPLC-DAD *	1
HPLC-FLR *	2
LC-GC-FID	1
LC-GC-MS/MS	1

(*) After liquid extraction

6.3 Laboratory results and scorings

Performances

Annex 6 presents the reported results for test item B in a table and a graph. NRLs and OCLs are denoted as N-xx and O-xx, respectively.

The majority of laboratories achieved satisfactory scores for the spiked milk (test item B), with 81 % and 77 % expressed as z and zeta scores, respectively (Table 3).

Table 3: Overview of laboratory performances for the determination of the concentration of styrene in milk (test item B). The total number of reported results (n = 26) is compared to the number of Satisfactory (S), Questionable (Q),

Unsatisfactory (U) *z* and *zeta* (ζ) scores, and realistic (a), underestimated (b) and overestimated (c) measurement uncertainties (MU).

	z score	zeta score	MU	
S	21	20	19	a
Q	3	1	0	b
U	2	5	7	c
	26	26	26	

Limit of quantification (LOQ)

Since the EURL measured the concentration of test item A below the LOQ it was expected that this would also be valid for the participating laboratories. Therefore the results reported by the participants served to get an indication about the LOQ of the methods applied (Figure 1). Only participant O17 and O25 reported a value for the concentration of styrene in blank milk (test item A).

Almost all participants reported styrene levels in test item A below 8 µg L^{-1}, which corresponds to 20 % of the target SML of 40 µg L^{-1}. However, two participants declared a LOQ of 20 µg L^{-1} (well above 20 % SML) when performing an extraction of styrene prior to HPLC-FLD analysis.

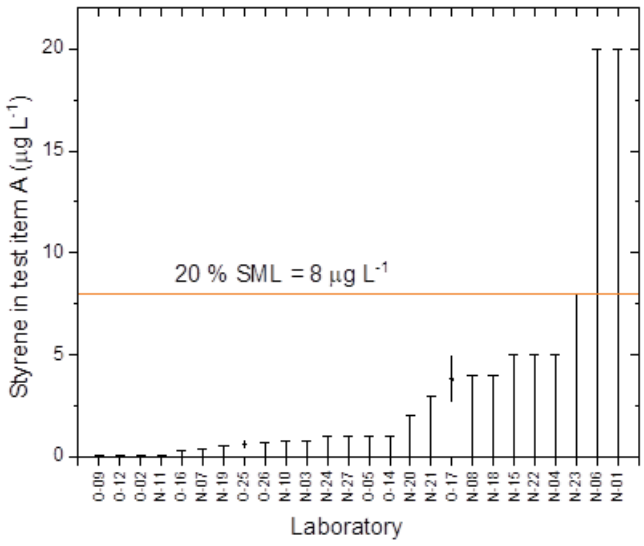


Figure 1. Styrene concentration in milk (test item A): most of the participants reported values below their limit of quantification (vertical lines). The orange line represents 20 % of the expected SML**.**

Measurement uncertainties

The majority of the participants reported realistic measurement uncertainty (MU) values as described in Section 6.1 (see "a" in Table 3).

All, except one, laboratories that used a validated method, reported realistic MUs. Those laboratories that overestimated their MU were found not to have used fully validated methods (Annex 9).

The average relative measurement uncertainties for different analytical techniques are listed in Table 4, with SPME showing the lowest one.

Table 4: Average relative standard measurement uncertainties of the different analytical techniques

Analytical technique	Number of laboratories	Average relative MU
SPME	11	6.9 %
Headspace	7	12 %
Solvent extraction	4	18 %
Purge and trap	1	18 %
Quechers	1	12 %

Additional information extracted from the questionnaire

The questionnaire was answered by 26 participants giving information on their analytical methods. Annex 9 summarises the experimental details, the analytical technique used and the limits of quantification (LOQ). Some small discrepancies were observed (N10, N11, N19 and O26) in the LOQs reported in the questionnaire comparing with the respective result for test item A.

Based on the questionnaire responses, eight laboratories (4 NRLs and 4 OCLs) applied an in-house validated method for quantifying styrene in milk. Thirteen laboratories (8 NRLs and 5 OCLs) do not have a validated method for styrene in milk, while 4 NRLs and 1 OCL are currently undergoing the validation process. It is worth noting that all the laboratories with a validated method achieved satisfactory results for both z and zeta scores.

Three participants are accredited according to ISO/IEC 17025 [5] for the quantification of styrene in dairy products, one participant is accredited for food simulants and food, six participants are accredited only for food simulants and 16 participants do not have an accredited method.

Fourteen participants used deuterated styrene as an internal standard. Other participants opted for xylene, chlorobenzene or cycohexanone as internal standard, while four other participants did not use an internal standard. No correlation was observed between the IS used and the respective participants' z-scores.

7 Conclusion

The proficiency testing round FCM-23/02 was organised to assess the analytical proficiency of NRLs and OCLs in determining the concentration of styrene in milk. A total of 17 NRLs and 9 OCLs submitted results.

Most participants demonstrated satisfactory performance with a percentage of satisfactory z scores above 80 %. The fact that this good performance relates to a concentration of the styrene in milk lower than the expected SML and higher then 20 % of the calibration range around the proposed SML, indicates that the majority of laboratories have methods fit for the enforcement. As the usual food simulants are not suitable for migration testing of polystyrene FCMs due to the swelling, it is foreseen that food (e.g milk) could be used instead of food simulants. The proposed SML for the migration of styrene will be

implemented in a future amendment to Commission Regulation (EU) 10/2011 on plastic materials and articles intended to come in contact with food, following EFSA priority settings for substances without SML [6].

References

[1] Commission Regulation (EU) No 10/2011 on plastic materials and articles intended to come into contact with food, Off. J. Eur. Communities L12/1 (2011). (CELEX 32011R0010)

[2] ISO 13528:2022 *"Statistical methods for use in proficiency testing by interlaboratory comparison"*, International Organisation for Standardization, Geneva (CH).

[3] 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 (CELEX 32017R0625)

[4] ISO/IEC 17043:2010 *"Conformity assessment – General requirements for proficiency testing".*International Organisation for Standardization, Geneva (CH).

[5] ISO/IEC 17025:2017 "General requirements for the competence of testing and calibration laboratories. International Organisation for Standardization, Geneva (CH).

[6] Review and priority setting for substances that are listed without a specific migration limit in Table 1 of Annex 1 of Regulation 10/2011 on plastic materials and articles intended to come into contact with food. https://doi.org/10.2903/j.efsa.2020.6124

List of abbreviations and symbols

Item 1 or Test item A	UHT raw semi-skimmed milk
Item 2	Styrene in methanol, spiking solution
Test item B	Milk (test item 1 or A) spiked with styrene (test item 2)
DG SANTE	Directorate General for Health and Food Safety
EU	European Union
EURL	European Union Reference Laboratory
FCM	Food Contact Materials
HPLC-DAD	High-Performance Liquid Chromatography with Diode-Array Detection
ISO	International Organization for Standardization
JRC	Joint Research Centre
LC-MS/MS	Liquid chromatography tandem mass spectrometry;
HS	Head space injection technique
SPME	Solid phase microextraction
P&T	Purge and Trap
DAD	Diod array detector
FLD	Fluorescence detector
LOQ	Limit of Quantification
NRL	National Reference Laboratory
OCL	Official Control Laboratory
PT	Proficiency Test
k	coverage factor
σ_{pt}	standard deviation for proficiency test assessment
$\sigma_{pt,\ rel}$	relative standard deviation for proficiency test assessment
$u(x_i)$	calculated standard measurement uncertainty (of participant "i")
$u(x_{pt})$	standard uncertainty of the assigned value
u_{char}	(standard) uncertainty contribution due to characterisation
u_{hom}	(standard) uncertainty contribution due to homogeneity
u_{st}	(standard) uncertainty contribution due to stability
$U(x_i)$	reported expanded uncertainty by participant "i"
$U(x_{pt})$	expanded uncertainty of the assigned value
x_i	reported mean value by participant "i"
x_{pt}	assigned value
z	z score
ζ	zeta score

Annex 1: Invitation letter

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate F – Health and Food
Food and Feed Compliance

EURL
European Union Reference Laboratory
for Food Contact Materials

Ispra, 4 October 2023
JRC.F.5/SV/mt/ARES(2023)23-074

Subject: **Invitation to participate in Proficiency Testing round "FCM-23/02"**

Dear NRL colleagues,

The European Union Reference Laboratory for Food Contact Materials (EURL-FCM) will organise a proficiency test round (FCM-23/02) for the "Determination of styrene in milk". This PT is organised in view of the expected adoption of an SML for styrene in food in Regulation (EU) No 10/2011.

There is no charge for participation.

Two items will be provided:
- one 0.5 L Tetra Pak© containing 1.5 % fat UHT milk;
- one 22 ml glass vial containing a solution of styrene in methanol.

You will be asked to analyse (i) the virgin milk for traces of styrene and (ii) the milk spiked with the styrene solution. Spiking the milk with the styrene solution should be performed according to the instructions that you will receive with the samples.

The PT on migration of styrene from polystyrene articles, reported in the pre-announcement letter, is planned as a follow-up for 2024.

Performance score (z-scores and ζ-scores) will be assigned for the quantification of styrene in the spiked milk.

The procedure used for the organisation of PTs are accredited according to ISO/IEC 17043:2010 and guarantee that the identity of the participants and the information provided by them is treated as confidential. However, laboratory codes of national Reference Laboratories (NRLs) – appointed in line with Regulation (EU) 2017/625 – will be disclosed to DG SANTE upon request for (long-term) performance assessment. Similarly, laboratory codes of appointed Official Control Laboratories (OCLs) may be disclosed to their NRL upon request.

Please forward this invitation to the Official Control Laboratories (OCLs) in your network that would be interested in participating.

If you intend to participate, register electronically as soon as possible by using the link below and following the instructions provided.
https://web.jrc.ec.europa.eu/ilcRegistrationWeb/registration/registration.do?selComparison=2961

Commission européenne/Europese Commissie, Retieseweg 111, 2440 Geel, BELGIQUE/BELGIË – Tel. +32 14571705

Commissione europea, Via Enrico Fermi 2749, 21027 Ispra VA, ITALIA – Tel. +39 0332789111
Office: 26 00/004 – Tel. direct line +39 033278-5293

Sandro.valzacchi@ec.europa.eu

The **deadline for registration is set to**	18 October 2023
Samples will be dispatched by	the end of October 2023
The deadline for submission of results will be on	1st December 2023

Do not hesitate to contact us if you have any question.

Kind regards,

e-signed

Sandro Valzacchi
PT coordinator

Annex 2: Test item accompanying letter

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Directorate F - Health, Consumers and Reference Materials
Food and Feed Compliance

EURL
European Union Reference Laboratory
for Food Contact Materials

Geel/Ispra, 13 February 2024

Subject: **Participation in FCM-23/02 - Determination of styrene in milk**

Dear participant,

Thank you for participating in the FCM-23/02 proficiency test (PT) for the **" Determination of styrene in milk ".**

The parcel you received contains, in addition to this letter:

- item 1: 1 commercial pack of UHT milk (0.5 L)
- item 2: 20 mL crimp cap glass vial containing a solution of styrene in methanol;

Upon arrival of this parcel, please check whether the items are undamaged after the transport and promptly inform us if this is not the case. There is no need to send proof of delivery to the EURL-FCM.

Item 2 should be stored refrigerated, while item 1 could be stored at room temperature before opening (start of the analyses).

Further instructions on this PT round, your individual lab code and passcode for entering the results have been provided by e-mail to the person that register for this round.

Do not hesitate to contact us for all issues related to this PT.

Thank you for your collaboration.

Your sincerely,

e-signed

Sandro Valzacchi/Stefanka Bratinova
PT Coordinators
European Union Reference Laboratory for Food Contact Materials

Cc: Eddo Hoekstra – Manager EURL-FCM;

Annex 3: Instruction letter

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate F – Health and Food
Food and Feed Compliance

EURL
European Union Reference Laboratory
for Food Contact Materials

Ispra/Geel, 23 October 2023
JRC.F.5/XX/xx/ARES(2023) 23-

Attn.: «Title» «Firstname» «Surname»
«Organisation»
«Department»
«Zip» «Town», «Country»

Reporting website	https://web.jrc.ec.europa.eu/ilcReporting Web
EU login	For help, see the Participant's guidelines
Password for reporting	**«Part_key»**
LabCode	**«LCode»**

Subject: Instructions to participants in Proficiency Testing round "FCM-23/02"

Dear «Title» «Surname»,

Thank you for participating in the FCM-23/2 proficiency test (PT) **"Determination of styrene in milk"**.

The parcels will be dispatched Monday 30th October. Each parcel contains:
(1) item 1 - 1 commercial pack of UHT milk (0.5 L).
(2) item 2 - 20 mL screw cap glass vial containing a solution of styrene in methanol;

Upon arrival of this parcel, please check whether the items are undamaged after the transport and promptly inform us if this is not the case. There is no need to send proof of delivery to the EURL-FCM.

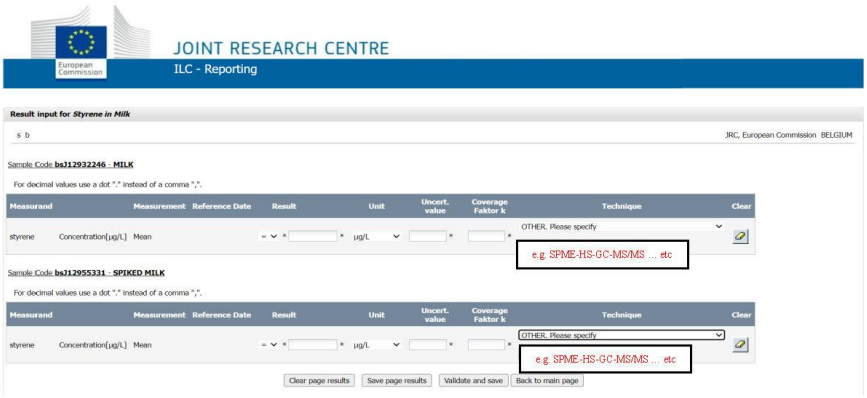
Item 2 should be stored refrigerated, while item 1 could be stored at room temperature before opening (start of the analyses).

To start the analyses open the UHT milk pack and prepare the test items A and B:
Test item A - raw milk as provided (item 1);
Test item B - add 500 µL of styrene solution (item 2) to 10 ml of milk (item 1) and consider for calculation the total volume. You could prepare bigger amount of test item B, provided that the ratio is respected.

Test items A and B should be analysed immediately after the preparation and stored in a refrigerator afterwards. The analysis of test items A should be carried out before test items B to avoid cross contaminations. Both test items should be analysed according to your routine procedure.

Test results and the expanded method uncertainty (MU) should be expressed in µg/L of milk. Please add/type your instrumental technique after selecting OTHER as illustrated in the reporting table below:

1



The laboratory performance will be z and ζ-scored for evaluation.

You can find the MILC reporting website at https://web.jrc.ec.europa.eu/ilcReportingWeb. You need first to login with your EU login account (see detailed guideline) and then enter the personal password. Your unique password is indicated above in the box under your address data. The system will guide you through the reporting procedure. Do not forget to submit and confirm when required.

Directly after submitting your results and the questionnaire online, you will be requested to print the completed report form. Please check carefully this report form and send it back via mail to us. In case mistakes are detected, contact the PT coordinator as soon as possible before the reporting deadline.

The deadline for submission of results is 06/12/2023. It will not be possible to submit your results after the deadline.

The procedures used for the organisation of PTs are accredited according to ISO/IEC 17043:2010 and guarantee that the identity of the participants and the information provided by them is treated as confidential. However, lab codes of National Reference Laboratories appointed in line with Regulation (EU) 2017/625 will be disclosed to DG SANTE upon request for (long-term) performance assessment. Lab codes of appointed Official Control Laboratories may be disclosed to their National Reference Laboratory upon request.

Remember that collusion is contrary to professional scientific conduct and serves only to nullify the benefits of proficiency tests to customers, accreditation bodies and analysts alike.

Your participation in this PT is greatly appreciated. Please be aware of the existence of an appeal procedure in case you disagree with your scores.

Do not hesitate to contact me for further information.

With kind regards,

/signed electronically in Ares/

Sandro Valzacchi
PT Coordinator

/signed electronically in Ares/

Stefanka Bratinova
Deputy PT Coordinator

Annex 4: Questionnaire

Milc questionnaire

Comparison for Styrene in Milk

Please answer the following questions

Submission Form

1. **Please indicate your status as a laboratory** *

- ☐ NRL
- ☐ OCL
- ☐ other

2. **Is the method you applied for analyses of styrene in milk validated?** *

3. **Are you accredited for the analyses of styrene?** *

- ☐ no
- ☐ yes

3.1. Please specify in which matrices

4. **How much of the test item B you prepared?** *

5. **What was the milk aliquot you used for the analyses?** *

6. **Any sample preparation before the instrumental analyses? Please describe shrotly** *

7. **Analytical techniques used?** *

- ☐ GC-MS
- ☐ HPLC-GC-FID
- ☐ HPLC-GCxGC-FID
- ☐ HS-GC-MS
- ☐ HS-GC-MS/MS
- ☐ Other, please specify
- ☐ SPME-HS-GC-MS
- ☐ SPME-HS-GC-MS/MS

7.1. Please specify other analytical techniques used for the quantification of styrene

8. **Did you use IS** *

- ☐ **no**
- ☐ yes

8.1. Please specify

9. **LOQ for styrene in milk?** *

Annex 5: Homogeneity and stability results

A. Homogeneity study (all values in µg L^{-1})

	Styrene (µg L^{-1})	
1	23.21	23.15
2	23.15	23.55
3	23.55	23.35
4	23.35	23.40
5	23.40	23.34
6	23.34	23.45
7	23.45	23.63
8	23.63	23.31
9	23.31	23.29
10	23.29	23.48
Mean	23.38	
u_{hom}	0.28 %	
σ_{pt}	15 %	
0.3 σ_{pt}	4.5 %	
u_{hom} < 0.3 σ_{pt}	passed	

Where: σ_{pt} is the standard deviation for the PT assessment,
u_{hom} is the standard uncertainty contribution due to homogeneity

B. Stability study

Temperature (°C)	Days	Y_o (µg L^{-1})	Y_{end} (µg L^{-1})	$\lvert Y_0 - Y_{end} \rvert$ (µg L^{-1})	O.3 σ_{PT} (µg L^{-1})	Stability test
4	60	23.38	23.88	0.5	1.04	Stable [a]
40	7		23.67	0.3		Stable [a]

[a] Stability criteria according to ISO 13528:2022 § B.5: $|Y_0 - Y_{end}| < 0/3 * \sigma_{pt}$

Annex 6: Results for styrene in the test item B (spiked milk) reported by the participants

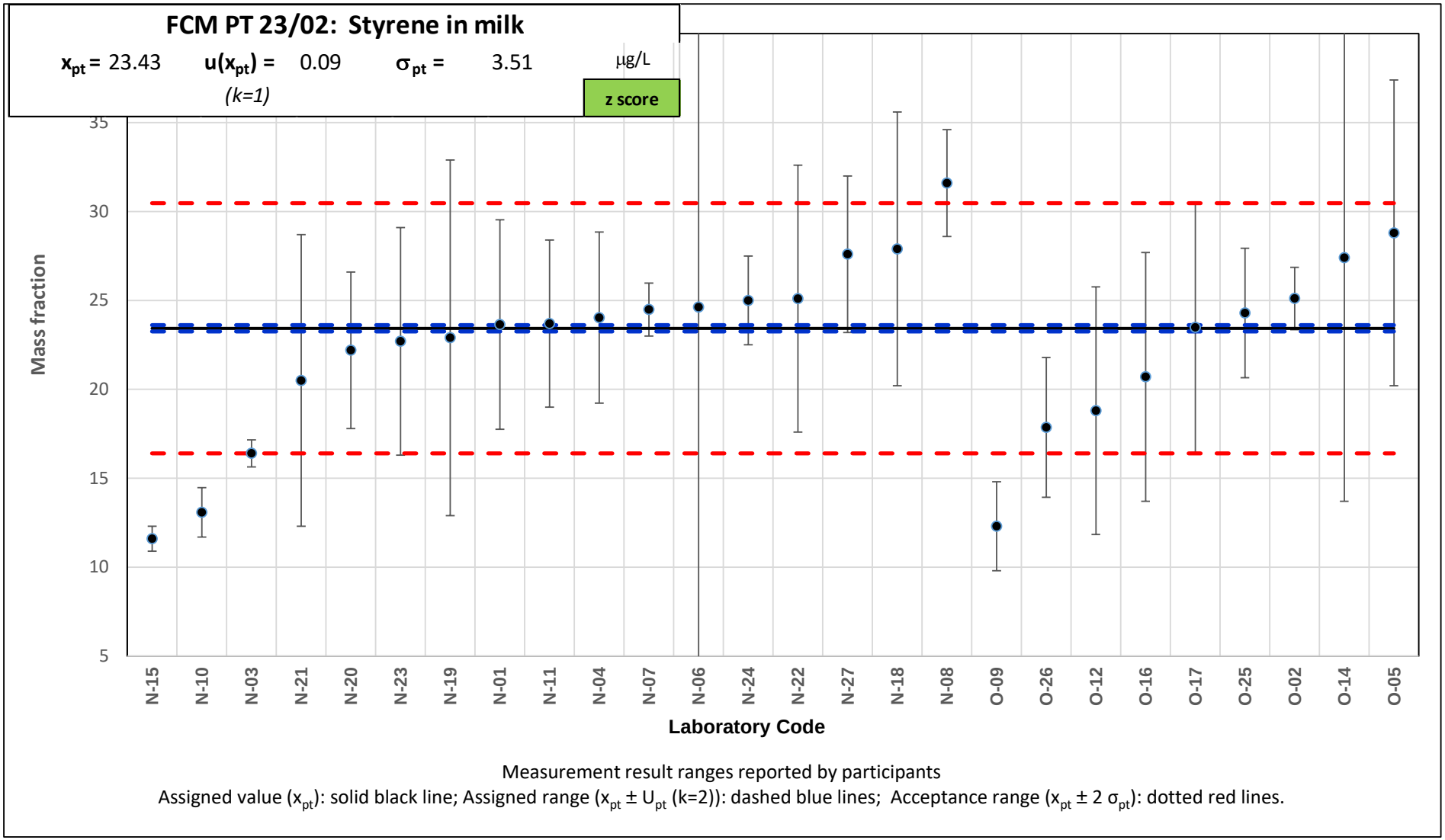
Assigned range: x_{pt} = 23.43 ± 0.09 $U(x_{pt}, k = 1.0)$; σ_{pt} = 3.515 (all values in µg L^{-1})

LabCode	x_i	±	k	Technique	z score[b]	ζ score[b]	unc.[c]
N-01	23.64	5.89	2	HPLC-FLD	0.06	0.07	a
O-02	25.11	1.75	2	SPME-GC-MS	0.48	1.91	a
N-03	16.4	0.76	2	HS-SPME-GC-MS/MS	-2.00	-18.00	a
N-04	24.03	4.81	2	HS-SPME-GC-MS	0.17	0.25	a
O-05	28.8	8.6	2	LC-GC-MS/MS	1.53	1.25	a
N-06	24.63	24.9	1.73[a]	HPLC-FLD	0.34	0.08	c
N-07	24.49	1.49	2	HS-SPME-GC-MS/MS	0.30	1.41	a
N-08	31.6	3	2	HS-SPME-GC-MS/MS	2.32	5.44	a
O-09	12.3	2.5	2	HS-GC-MS	-3.17	-8.88	a
N-10	13.08	1.39	2	HS-SPME-GC-MS	-2.95	-14.77	a
N-11	23.7	4.7	2	HS-SPME-GC-MS/MS	0.08	0.11	a
O-12	18.8	6.96	2	P&T-GC-MS	-1.32	-1.33	c
O-14	27.4	13.7	2	GC-MS/MS	1.13	0.58	c
N-15	11.6	0.7	1	HS-GC-MS	-3.37	-16.76	a
O-16	20.7	7	2	HS-GC-MS	-0.78	-0.78	c
O-17	23.49	7.05	2	HPLC-DAD	0.02	0.02	c
N-18	27.9	7.7	2	HS-SPME-GC-MS/MS	1.27	1.16	a
N-19	22.9	10	2	GC-MS	-0.15	-0.11	c
N-20	22.2	4.4	2	HS-SPME-GC-MS	-0.35	-0.56	a
N-21	20.5	8.2	2	HS-GC-MS	-0.83	-0.71	c
N-22	25.1	7.5	2	LC-GC-FID	0.47	0.44	a
N-23	22.7	6.4	2	HS-GC-FID	-0.21	-0.23	a
N-24	25	2.5	2	HS-SPME-GC-MS/MS	0.45	1.25	a
O-25	24.29	3.64	2	HS-GC-MS	0.24	0.47	a
O-26	17.86	3.93	2	HS-GC-MS	-1.59	-2.83	a
N-27	27.6	4.4	2	HS-SPME-GC-MS/MS	1.19	1.89	a

[a] √3 is set by the PT coordinator when no coverage factor *k* is reported. The reported uncertainty was assumed to have a rectangular distribution with *k* = √3,

[b] Performance scoring: satisfactory (green), questionable (yellow), unsatisfactory (red),

[c] a: $u(x_{pt,rel}) \leq u(x_{i.\ rel}) \leq \sigma_{pt,rel}$; *b:* $u(x_{i,rel}) < u(x_{pt,rel})$; *and c:* $u(x_{i,rel}) > \sigma_{pt,rel}$; NP*: not provided*



Measurement result ranges reported by participants

Assigned value (x_{pt}): solid black line; Assigned range ($x_{pt} \pm U_{pt}$ (k=2)): dashed blue lines; Acceptance range ($x_{pt} \pm 2\ \sigma_{pt}$): dotted red lines.

Annex 7: Styrene in milk (test item A)

LabCode	x_i	±	k	Technique
N-01	< 20			HPLC-FLR
O-02	< 0.1			SPME-GC-MS
N-03	< 0.8			HS-SPME-GC-MS/MS
N-04	< 5			HS-SPME-GC-MS
O-05	< 1			LC-GC-MS/MS
N-06	< 20			HPLC-FLD
N-07	< 0.37			HS-SPME-GC-MS/MS
N-08	< 4			HS-SPME-GC-MS/MS
O-09	< 0.05			HS-GC-MS
N-10	< 0.79			HS-SPME-GC-MS
N-11	< 0.1			HS-SPME-GC-MS/MS
O-12	< 0.1			Purge&Trap-GC-MS
O-14	< 1			GC-MS/MS
N-15	< 5			HS-GC-MS
O-16	< 0.3			HS-GC-MS
O-17	3.82	1.15	2	HPLC-DAD
N-18	< 4			HS-SPME-GC-MS/MS
N-19	< 0.5			GC-MS
N-20	< 2			HS-SPME-GC-MS
N-21	< 3			HS-GC-MS
N-22	< 5			LC-GC-FID
N-23	< 8			HS-GC-FID
N-24	< 1			HS-SPME-GC-MS/MS
O-25	0.6	0.09	2	HS-GC-MS
O-26	< 0.7			HS-GC-MS
N-27	< 1			HS-SPME-GC-MS/MS

All values expressed in µg L^{-1}

Annex 8: Results of the questionnaire

Lab Code	Is your method for styrene in milk validated?	Are you accredited for the analysis of styrene?	If yes, specify in which matrice.	How much test item B did you prepare?	What was the milk aliquot you prepared for the analysis?	Any sample preparation before the instrumental analysis? Please describe shortly.	Which analytical technique did you apply?
N-01	yes	yes	Dairy products	10 mL	10 mL (no dilution)	QUECHERS method	LC-FLR
O-02	yes	no		10 mL per Analysis/Sample	10 mL	no	GC-MS, SPME-HS
N-03	no	yes	migration solutions (simulant A, C, D1)	10.5 ml (10 ml milk + 0.5 ml spiking solution supplied)	1 g, diluted with 7 ml water (1:8)	none	GC-MS/MS, SPME-HS
N-04	yes	no		2 x 10.5 mL	Aliquot of 1mL, Dilution factor 10	Dilution with water 1:9, addition of 2.5g NaCl and internal standard Styrene-d8	GC-MS, SPME-HS
O-05	no	yes	food simulants (10% and 50% ethanol, vegetable oil, Tenax), food (cheese, fish, meat products)	2 x 5 mL	5 mL, not diluted	extraction using hexane and ethanol	LC-GC-MS/MS
N-06	yes	yes	Dairy products	10 mL	10 mL, no dilution	Quechers method	LC-FLR
N-07	Partly validated.	no		We prepared 10 ml of milk + 500 µL of spiking styrene solution, 5 times.	We used aliquot of 1 ml for every milk analyses.	1 g of milk (or spiked milk) + 2 g NaCl + 8 ml water was shaked intensively.	GC-MS/MS, SPME-HS
N-08	no	no		10 ml	10 ml	Add 2 gram NaCl, whirlmixe for 10 sec.	SPME-HS
O-09				10 ml	5 ml	no	GC-MS, static HS
N-10	No	Yes	In food	2 times 10 mL of milk	1 mL diluted in 7	2g NaCl + 7 ml H20 + 1 ml of	GC-MS, SPME-HS

Lab Code	Is your method for styrene in milk validated?	Are you accredited for the analysis of styrene?	If yes, specify in which matrice.	How much test item B did you prepare?	What was the milk aliquot you prepared for the analysis?	Any sample preparation before the instrumental analysis? Please describe shortly.	Which analytical technique did you apply?
			simulants ethanol 10%, 20%, 50% and acetic acid 3%	plus 0.500 mL of test item B (we repeat the analysis in two different days)	mL of water	sample (either Item A or B) + 10 µL of IS	
N-11	Yes	No		10 mL	1 g of milk was diluted with 8 mL of ultra-pure water (in the headspace vial)	No	GC-MS/MS, SPME-HS
O-12	Yes	No		60 ml	4.2 ml; yes; 1:10	Other sample milk spiked with our MRS and also Sample standards	GC-MS, Purge and Trap
N-13							
O-14				20 mL	2 mL, extracted with 10 mL of Ethylacetate	Blank sample and spiked sample at 25 ppb and 50 ppb	GC-MS/MS
N-15	No	no		1	10 mL no dilution	No	GC-MS SIM, static HS
O-16	No	Yes	Migration in food simulant	500 µl	10 mL	none	static HS
O-17	Method is under development and final validation is still pending	No		4x (5 ml milk + 0.25 ml Styrene-Solution) = 20 ml milk + 1 ml Styrene-solution	5 ml milk + 0.25 ml Styrene-Solution = 5.25 ml spiked milk used for extraction without dilution	5.25 ml spiked milk extracted with 8 ml ACN for 10 min, Centrifugation of the Milk-ACN-Mixture at 3000 rpm for 5 min, collect ACN-supernatant, repeat extraction and centrifugation with the milk residue, mixture of both ACN-supernatants and dillution to 25 ml with water (sample), analyses of this sample with HPLC-DAD	HPLC-DAD (determination wavelength: 245 nm)
N-18	Method validation in	No		10,5 mL	1 g of Test item B and Test item A	After dilution with ultrapure water, 1 g of NaCl was added in	GC-MS/MS, SPME-HS

Lab Code	Is your method for styrene in milk validated?	Are you accredited for the analysis of styrene?	If yes, specify in which matrice.	How much test item B did you prepare?	What was the milk aliquot you prepared for the analysis?	Any sample preparation before the instrumental analysis? Please describe shortly.	Which analytical technique did you apply?
	progress.				was weighted. All the samples (matrix matched calibration curve, quality control samples and unknown samples) were diluted with 4 mL of ultrapure water.	order to promote the extraction of styrene (salting-out effect).	
N-19	No	No		8	no dilution; 10 mL milk and 500 µl styrene	extraction, centrifugation and filtration 0.25 µm	GC-MS
N-20	No	No		3	No	No	GC-MS, SPME-HS
N-21	No	Yes	water	10 mL of milk + 500 µL of styrene solution	Dilution factor: 2 and 5	3g of NaCl + 10mL of diluted sample	GC-MS, staic HS
N-22	Yes	Yes	milk products, PS FCM	2 x 10 mL	10 mL each	+5 ml KOH (50 % water), + 10 ml ethanol, + 10 ml C6 + ISD, saponification 1 h 60 °C while shaking, remove C6 phase, extract again with 10 ml C6, inject 100 ul of combined C6 phases into LC-GC-FID	on-line LC-GC-FID
N-23	No	No		none	5 mL	homogenation of the sample	GC-FID, static HS
N-24	No	No		2 test (1000 µl)		1g milk + 8 ml water and 2g NaCl to SPME-HS Vials	GC-MS/MS, SPME-HS
O-25	Yes	Yes	Specific migration of styrene in food simulants A, B, C, D1, D2, 95% ethanol	10,5 mL	10,5 ml, no dilution	No	GC-MS, static HS
O-26	No	Yes	Food contact	5 times, according to	no dilution,	No	GC-MS, static HS

Lab Code	Is your method for styrene in milk validated?	Are you accredited for the analysis of styrene?	If yes, specify in which matrice.	How much test item B did you prepare?	What was the milk aliquot you prepared for the analysis?	Any sample preparation before the instrumental analysis? Please describe shortly.	Which analytical technique did you apply?
			material	instruction: 10 ml milk/500 µl solution of styrene	according to instruction: 10 ml milk/500 µl solution of styrene used for HS-GC-MS		
N-27	The method is partially validated at present	No	Accreditation applied for, visit due May 2024	10 mL	4 mL no dilution	Addition of 2 g NaCl into HS vial	GC-MS/MS, SPME-HS

Lab Code	Time and temperature for the equilibration	Time and temperature for the adsorbtion	Type of SPME fiber	Did you use an Internal standard?	Which one?	LOQ of styrene in milk	Problems?	Comments?
N-01	NA	NA	NA	No		20 µg/L	No	
O-02	0 min	30 min 70°C	DVB/CAR/PDMS	Yes	Styrene-d8	0.5 µg/L	No	
N-03	NA	NA	NA	Yes	styrene-d8	0.8 µg/L	None	
N-04	10 min	15 min	PDMS/DVB	Yes	Styrene-d8	5 µg/L	None	None
O-05	NA	NA	NA	Yes	styrene-d8	1 µg/L	phase separation during extraction was difficult due to the formation of a milk coagulum	control experiments using GC-MS with static HS (equilibration: 35 min at 80°C) and styrene-d8 as an internal standard led to comparable results (method is not validated)
N-06	NA	NA	NA	No		20 µg/L	No	
N-07	10 minutes, 50 °C	30 minutes, 50 °C	PDMS/DVB	Yes	Styrene d8	0.37 ug/L	Broken SPME fiber, calculation of expressing units, milk thickened to cream after some time	
N-08	10 sec, 50°C	10 min, 50°C	DVB/PDMS	Yes	Styrene-D8	4 µg/ml		
O-09	80°C / 10 minutes	80°C / 30 minutes	NA	Yes	Styrene-d8	0.05µg/L	No	
N-10	10 min at 50C	30 min at 50C	DVB/CAR/PDMS	Yes	Styrene-D8	2.65	No	
N-11	50°C/10 min	50°C/30 min	polydimethylsiloxane/divinylbenzene (PDMS/DVB) - 65 µm thickness	Yes	styrene-d8 (CAS No: 19361-62-7)	1 µg/L	No	
O-12	NA	NA	NA	Yes	Chlorobenzene-D5	0.1 ug/L	any	
N-13								
O-14	NA	NA	NA	No		1 ppb	No	This is the first time the method has been applied. Styrene recovery was corrected for recovery
N-15	15 minutes at	NA	NA	No		5 µg/L	No	None

Lab Code	Time and temperature for the equilibration	Time and temperature for the adsorbtion	Type of SPME fiber	Did you use an Internal standard?	Which one?	LOQ of styrene in milk	Problems?	Comments?
	90°C							
O-16	15 min, 70°C	0,5 min load time, 70°C		Yes	Cyclohexanon	not validated in milk but in water 1 µg/l		
O-17	NA	NA	NA	No		0.82 µg/L	The milk samples measured as part of the previous method development did not show a blank value for styrene. In the present measurement, the styrene content determined in the blank value (milk) (unequivocal identification not possible) was subtracted from the styrene content determined in the spiked milk.	Determination of styrene on the fluorescence detector (HPLC-FLD) is significantly more sensitive. In the present case, however, it leads to lower contents.
N-18	SPME fiber conditioning step: 10 minutes, 250 ºC and sample vial pre-incubation: 10 minutes, 50 ºC	SPME process: 30 minutes, 50 ºC and desorption process: 5 minutes, 240 ºC (injection port).	SPME fiber: DVB/PDMS 65/10	Yes	Styrene-d8	LOQ = 4 micrograms/L	No difficulties were encountered during analyses of milk samples.	
N-19	NA	NA	NA	Yes	styrene d8	2.5 µg/L	the separation of the phases after extraction	
N-20	5 min, 50 °C	20 min, 50 °C	PDMS-DVB	Yes	Styrene D6	2.0 µg/L	No	
N-21	30 min	NA	NA	Yes	o-Xylene D10	3 µg/L	No	
N-22				Yes	p-xylene	5 ug/kg	No	
N-23	30 min, 80°C	30 min, 80°C		Yes	m-xylene	0.008 mg/l	no experience with testing in real food	we don´t have an experiences with testing styrene in food
N-24	10 min/50 0C	30 min/50 0C	PDMS/DVB	Yes	D8-Styrene	1 µg/Kg		
O-25	30 min at 90 deg C	0,5 min 100 deg C		No		0,6	No	

Lab Code	Time and temperature for the equilibration	Time and temperature for the adsorbtion	Type of SPME fiber	Did you use an Internal standard?	Which one?	LOQ of styrene in milk	Problems?	Comments?
O-26	30 min / 70 °C	NA	NA	Yes	Benzene D6	0,15 µg/L	No	
N-27	10 minutes at 50 °C	20 mins at 50 °C	65µ PDMS/DVB	Yes	Styrene D8	1 µg/Kg	No	

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