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Report of an inter-laboratory comparison from the European Union Reference Laboratory for Food Contact Materials

ILC02 2015 – Specific migration from a multilayer in food simulant A

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Abstract

This report presents the results of an inter-laboratory comparison on the specific migration of caprolactam (CAP) and 2,4-di-tert butyl phenol (2,4-DTBP) from a plastic food contact material in food simulant A (ethanol 10 %, v/v) organised by the EURL-FCM, Ispra (Italy). Homogeneity and stability studies were conducted. Participants had to carry out a migration test by immersion with food simulant A for 10 days at 60°C, to quantify the migration of caprolactam (CAP) in food simulant A, and to provide details of the analytical and sample extraction procedure. In addition a by-product 2,4-di-tert butyl phenol (2,4-DTBP) was also present and had to be quantified. The assigned values of the migration of the migrant were calculated as the robust mean of the results reported by the participants by applying the Q/Hampel method robust statistics. The participation to the ILC was satisfactory with 28 out of 29 participating laboratories submitting results. The z-score values of the laboratories for each substance were calculated based on the assigned value. In the case of CAP and also for the more challenging non intentionally added substances 2,4-DTBP more than 82% of the results were fully satisfactory (|z-score|<2). This percentage rose to close to 90% for z-scores <3 in the compliance evaluation of the regulated substance CAP.

Report of the inter-laboratory comparison

ILC 02 2015 – Specific migration in food simulant A

EC-JRC-IHCP, CAT Unit

2016

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1. *Summary*

The Institute for Health and Consumer Protection (IHCP) of the European Commission's Directorate-General Joint Research Centre hosts the EU Reference Laboratory for Food Contact Materials (EURL-FCM). One of its core tasks is to organise Inter-Laboratory Comparisons (ILCs) among appointed National Reference Laboratories (NRLs).

This report presents the results of an ILC which focused on the quantification of selected substances from a plastic food contact material into food simulant A.

Regulation (EU) No 10/2011 established food simulant A which is assigned for foods that have a hydrophilic character and are able to extract hydrophilic substances [1].

The EURL-FCM selected a multilayer plastic food contact material that had been tested for potential migrants. Caprolactam (CAP) and 2,4-di-tert-butylphenol (2,4-DTBP) were selected for quantification. The homogeneity and stability studies of the material were performed by the EURL-FCM.

The participating laboratories received the test material and were asked to perform a migration by immersion with food simulant A, 10% ethanol v/v, at 60^{o}C for 10 days [1].

There were 29 participants from 22 countries to whom samples were dispatched. Twenty eight laboratories submitted results, of which 24 were NRLs and 6 OCLs. The participants were invited to report four replicates, under repeatable conditions, for the quantification of target migrants.

The assigned values of the migration of the migrant were calculated as the robust mean of the results reported by the participants by applying the Q/Hampel method robust statistics. Laboratory results were rated with z-scores in accordance with ISO 13528:2015 and the reproducibility standard deviations were calculated [2].

The participation in the ILC was satisfactory. 28 out of 29 laboratories submitted their results.

In the case of CAP 82% of the results were satisfactory (|z-score|<2), 7% were questionable (2<|z-score|≤3) and 11% were unsatisfactory (|z-score|>3).

In the case of 2,4-DTBP 1 laboratory did not perform a quantitative analysis and 2 laboratories could not quantify it, because the limit of quantification (LOQ) of their analytical method was higher than the concentration of 2,4-DTBP. 84% of the results were satisfactory (|z-score|<3), whilst 16% of the results were unsatisfactory (|z-score|>4).

Laboratories analysed CAP predominantly by direct injection to the analytical system or by a Liquid-Liquid Extraction (LLE) prior to analysis. The main analytical technique for quantification of CAP was GC-MS followed by HPLC-DAD and LC-MS/MS with positive electrospray ionization (ESI+). For 2,4-DTBP, the majority of the participants applied mainly a LLE extraction prior to analysis. The predominant analytical technique was GC-MS followed by LC-MS/MS with electrospray ionization operating in negative mode (ESI-).

2. *Introduction*

Inter-laboratory Comparisons (ILCs) are widely used to evaluate the performance of an analytical method, as well as the ability of official control laboratories to deliver results within agreed accuracy. ILC studies are also an essential element of laboratory quality assurance, which allows individual laboratories to compare their analytical results with those from other laboratories while providing them objective standards to perform against.

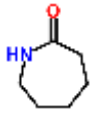
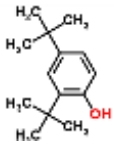
Regulation (EC) No 882/2004 of the European Parliament and of the Council stipulated the organisation of ILC as one of the principal responsibilities of the European Union Reference Laboratories (EURLs) [3].

In accordance with the above requirements the European Union Reference Laboratory for Food Contact Materials (EURL-FCM) organised inter-laboratory comparison (ILC) tests for the network of National Reference Laboratories (NRLs) in 2015.

The scope of the ILCs for 2015 were discussed and established in the plenaries with all NRLs. It was agreed that the objective of this ILC02-2015 would be the identification and quantification of migrants into food simulant A.

The choice of the test material for the ILC was based on the additives it contained that were able to migrate. Compositional substances were sought from their reported presence in the literature as potential migrants from FCM into different food simulants [4-7]. Caprolactam is a FCM substance (FCM no. 212) which is regulated by the EU Regulation No.10/2011 and is the monomer of polyamide-6. The substance 2,4-di-tert-butylphenol (2,4-DTBP) is a degradation product from the antioxidant Irgafos 168 [tris (2,4-di-tert-butylphenyl) phosphate] (FCM no. 760). 2,4-DTBP has been characterised as NIAS (Non-Intentionally Added Substances) [4-7]. Chemical structures and other details of the selected substances are presented in Table 1.

Table 1. Selected migrating substances for the ILC 02-2015.

Substance	IUPAC name	CAS	FCM No.*	SML** (mg/kg) [1]	Chemical structure
Caprolactam	Azepan-2-one	105-60-2	212	15 (Total)	
2,4-di-tert-butyl phenol	2,4-bis(2-methyl-2-propanyl)phenol	96-76-4	-	-	

* EU Reg. 10/2011
** SML=Specific Migration Limits

The participants were asked to perform a migration test by immersion in food simulant A (10 % ethanol in water, v/v) and to quantify the specific migrants in mg/kg taking into account that 1 dm^2 equals to 6 kg of food [1]. The laboratories were free to use their own analytical methods, since this ILC was a Proficiency Test (PT). Participants were invited to report four replicates under repeatable conditions.

3. Scope

The scope of this ILC was a PT to test the performance of appointed NRLs and of guest official control laboratories (OCLs) to quantify the substances CAP and 2,4-DTBP after migration into food simulant A (10% ethanol v/v) at 60^{o}C for 10 days.

The assessment of all measurement results was undertaken on the basis of requirements laid down in international standards [2, 9, 14].

4. Time frame

Invitation letters were sent by e-mail to all NRL's and interested OCLs on the 16th December 2015 (see Annex 1). Laboratories were asked to fill in a letter of confirmation of their participation (see Annex 2).

In the end of December 2015 the samples were dispatched to the participants including a shipping and instruction form in (see Annex 3), a sample acknowledgement receipt (see Annex 4), the instructions and requested results (see Annex 5), lab code (see Annex 6), a results reporting form (see Annex 7) and a questionnaire form to be filled (see Annex 8).

The deadline to report results was set on the 31st January 2016.

5. Test material

5.1. Homogeneity assessment

Before sample kit dispatch, a sample set (Sample code: CAT.028/EURL/2015/049) was tested for homogeneity by the EURL-FCM.

Based on ISO13528:2015 [2] twelve randomly selected test specimens of the sample were analysed for caprolactam (CAP) and 2,4-di-tert-butyl phenol (2,4-DTBP), by GC-MS/MS. The random selection was based on statistical sampling procedure realised by the "R" statistical program [11].

The EURL-FCM evaluated the homogeneity using the ProLab Software [10] according to IUPAC International Harmonized Protocol F-test [9] and by checking for significant and adequate heterogeneity method based on ISO 13528:2015 [2]. The homogeneity results and their statistical evaluation are presented in Annex 9. The test material showed sufficient homogeneity.

5.2. Distribution

The sample kits were dispatched to the participants by the EURL-FCM in the end of December 2015 along with a shipping and instruction form (see Annex 3). Each participant received:

- 1 piece of food packaging material;
- Vial containing caprolactam analytical standard
- Vial containing 2,4-di-tert-butyl phenol analytical standard
- Vial containing 4-ethylphenol (internal standard)
- Sample receipt acknowledgement form
- Instructions for compilation of results
- Laboratory code
- Results reporting form
- Questionnaire form

5.3. Stability test

The sample was monitored for its stability for the selected substances by the EURL-FCM. Based on ISO13528:2015 [2], three randomly selected test specimens from the sample were analysed for CAP and 2,4-DTBP, by GC-MS/MS. The random selection was based on statistical sampling procedure based on the "R" statistical program [11]. The randomly selected sample specimens were stored at 20^{o}C.

The EURL-FCM evaluated the stability by the ProLab Software [10] according to IUPAC International Harmonized Protocol F-test [9] and with the expanded criterion of ISO 13528:2015 [2]. The homogeneity results and their statistical evaluation are presented in Annex 10. The test material showed sufficient stability and no significant trend was observed for the tested samples.

6. *Instruction to participants and requested results*

Detailed instructions were given to all the participating laboratories in the letters that accompanied the sample kit. Laboratories were asked to measure 1 dm^2 of the food contact material, divide the specimen into small equal surface pieces, place them into a metallic support and perform a migration by immersion with food simulant A at 60^{o}C (±2^{o}C) for 10 days. The surface area had to take into account that 1 dm^2 comes into contact with 1 kg of food, the sample-to-food simulant volume ratio was 1 dm^2/100 mL food simulant A and also had to take into account that the substances were migrating only from one side of the material.

The participants had to report 4 quantitative results for each substance using the indicated unit of measure (mg/kg). As the exercise was a PT, the participants were free to use any suitable method of their own choice for the analysis and quantification (Annexes 1 and 5).

Additionally, regarding the divided specimen portions, it was suggested to the participating laboratories as an option to use a metallic stainless steel thin grid or another appropriate mean in order to avoid the potential sticking together of the portions.

7. Evaluation of results

7.1. General observations

In this ILC, 29 participants to whom the samples were dispatched (23 NRLs, 5 OCLs from Germany and 1 OCL from Italy). Results were submitted by all NRLs and 5 OCLs.

7.2. Statistical evaluation of results

The statistical evaluation of the results was performed by the EURL-FCM by using the ProLab software [10].

7.2.1. Assigned values

A robust mean of the results reported by the participants was chosen as a consensus for the assigned value for the selected substances in the ILC02-2015. The assigned value was evaluated according to Q-method/Hampel estimator (ISO 13528:2015) as one of the most robust, indicated especially when many outliers are present [2].

7.2.2. Target standard deviation

The value of the target standard deviation for proficiency assessment (σ_{pt}) determines the limits of satisfactory performance in an ILC test. In most cases Horwitz standard deviation (SD), a general model commonly used for the chemical field, is a good compromise but does not reflect different levels of complexity of a given analytical method. If the target standard deviation is not chosen realistically the interpretation “satisfactory”, “questionable” and “unsatisfactory” would not be valid. On the other hand, the standard deviation of the reproducibility in collaborative trials can be considered as an appropriate indicator of the best agreement between laboratories. The σ_{pt} of each substance evaluated in this ILC02-2015 was its reproducibility SD [2].

7.2.3. Kernel density

A kernel density plot (KDE-plot) is a way of presenting graphically the general distribution shape of a dataset. The KDE could be used additionally to identify possible multi-modality in the reported datset distribution. In certain cases, the results are not "normally" distributed or contain values giving rise to multiple distribution modes. These modes can be visualised by KDE-plots [12,13]. Kernel density plots were computed by ProLab software based on the analytical data provided by the participating laboratories, by representing the individual numeric values each as a normalised Gaussian distribution, centered on the respective analytical value. The sum of these normal distributions formed the KDE-plot for each of the studied substance [10].

7.2.4. z-score

Individual laboratory performance was expressed in terms of z-score in accordance with ISO 13528 [2] and the International Harmonised Protocol [9]. The z-scores compared the participant's deviation from the assigned value with the target standard deviation accepted for the PT:

$$z = \frac{x_i - x_{pt}}{\sigma_{pt}}$$

Where:

x_i	is the measurement result (mean value) reported by a participant;
X_{pt}	is the assigned value;
σ_{pt}	is the target standard deviation for proficiency assessment.

The conventional interpretation of z-scores is as follow [14]:

$\|z\| \leq 2$	indicates "satisfactory" performance;
$2 < \|z\| \leq 3$	indicates "questionably" performance;
$\|z\| > 3$	indicates "unsatisfactory" performance (action signal).

In this exercise the z-scores calculated with the reproducibility SD as σ_{pt} were used to assess the performance of the laboratories.

8. Results

8.1. Preliminary considerations

There were 29 participants from 22 countries to whom samples were dispatched. 28 laboratories submitted results, of which 23 were NRLs and 5 OCLs. The participants were invited to report four replicate measurements, under repeatable conditions, for the target analytes.

8.2. Laboratories performance and z-scores

The participation of the laboratories in that ILC can be considered as very satisfactory, where 29 laboratories from 22 countries participated, among them 23 NRLs and 6 OCLs and 28 laboratories reported results (96%). The overview is presented in Table 2.

In the case of CAP all the 28 laboratories reported results. In the case of the Non intentionally Added Substance (NIAS) 2,4-DTBP, 25 laboratories were also able to report results. One laboratory did not analyse the NIAS and 2 laboratories could not quantify them as the limit of quantification (LOQ) of their analytical method was greater than the substance's concentration. The results submitted by the participants for the selected substances, along with information regarding the analytical method used and the sample preparation, are available in Annex 11.

Table 2. Participation, results and successful qualitative analysis of the substances

ILC02-2015	CAP	2,4-DTBP
Participants	29	29
Submitted results	28	28
% participation	96	96
Total reported results	28	25
Correct results	23	21
% Correct results	82	84

Concerning the statistical evaluation, the summary of the results obtained by ProLab software [10] for all the substances with assigned values, target standard deviations, robust repeatability SD and robust reproducibility SD calculated according to Q-method/Hampel estimator (ISO13528:2015) [2] are given in Table 3. The summary of z-scores results of the participants, are presented in Table 4.

Table 3. Summary of the results for the studied substances calculated according to ISO13528:2015

ProLab Results	SUBSTANCES	
	CAP	*2,4-DTBP*
Assigned value = Robust Mean, mg/kg	3.021	0.018
Robust Repeatability s.d., mg/kg	0.146	0.002
Robust Reproducibility s.d., mg/kg	0.885	0.019
Target SD (Reproducibility s.d.), mg/kg	0.885	0.019
Target SD (Horwitz), mg/kg	0.409	0.005
Rel. target SD (Reproducibility s.d.), %	29.31	108.17
Rel. target SD (Horwitz), %	13.55	29.37
Rel. Repeatability s.d., %	5.00	13.84
Lower limit of tolerance, mg/kg	1.250	-0.021
Upper limit of tolerance, mg/kg	4.791	0.056
Laboratories	28	25
Test results	111	98

Table 4. Laboratories z-scores for CAP and 2,4-DTBP

Laboratory	CAP	2,4-DTBP
LC0002	0.115	-0.008
LC0004	-0.114	3.907
LC0005	2.784	1.720
LC0006	0.060	-*
LC0010	1.089	-0.545
LC0011	-1.927	0.315
LC0013	-0.102	-*
LC0016	-3.352	-0.911
LC0017	0.588	1.275
LC0018	-1.244	-0.388
LC0020	5.523	126.211
LC0024	-1.540	-0.571
LC0025	0.046	-0.008
LC0028	-0.111	0.359
LC0031	0.390	1.380
LC0032	-*	-*
LC0037	0.138	-0.152
LC0040	0.435	-0.767
LC0043	-0.461	0.332
LC0044	-0.030	-0.785
LC0047	0.226	14.722
LC0049	-0.300	-0.322
LC0050	-1.818	-0.466
LC0055	0.342	-0.217
LC0056	3.814	0.293
LC0059	-0.344	-0.846
LC0061	0.623	-*
LC0064	0.615	-0.060
LC0113	2.590	172.561
** Values not reported by the participants*		

The overall performance of the laboratories was regarded as satisfactory. The results of the statistical evaluation are presented graphically in Figures 1 and 2 for CAP and 2,4-DTBP, respectively. Those figures present the results of mean values, assigned mean, along with robust repeatability SD and robust reproducibility SD, target standard deviations, calculated according to Q-method/Hampel, z-scores and the kernel density plot (KDE-plot) of CAP and 2,4-DTBP, respectively.

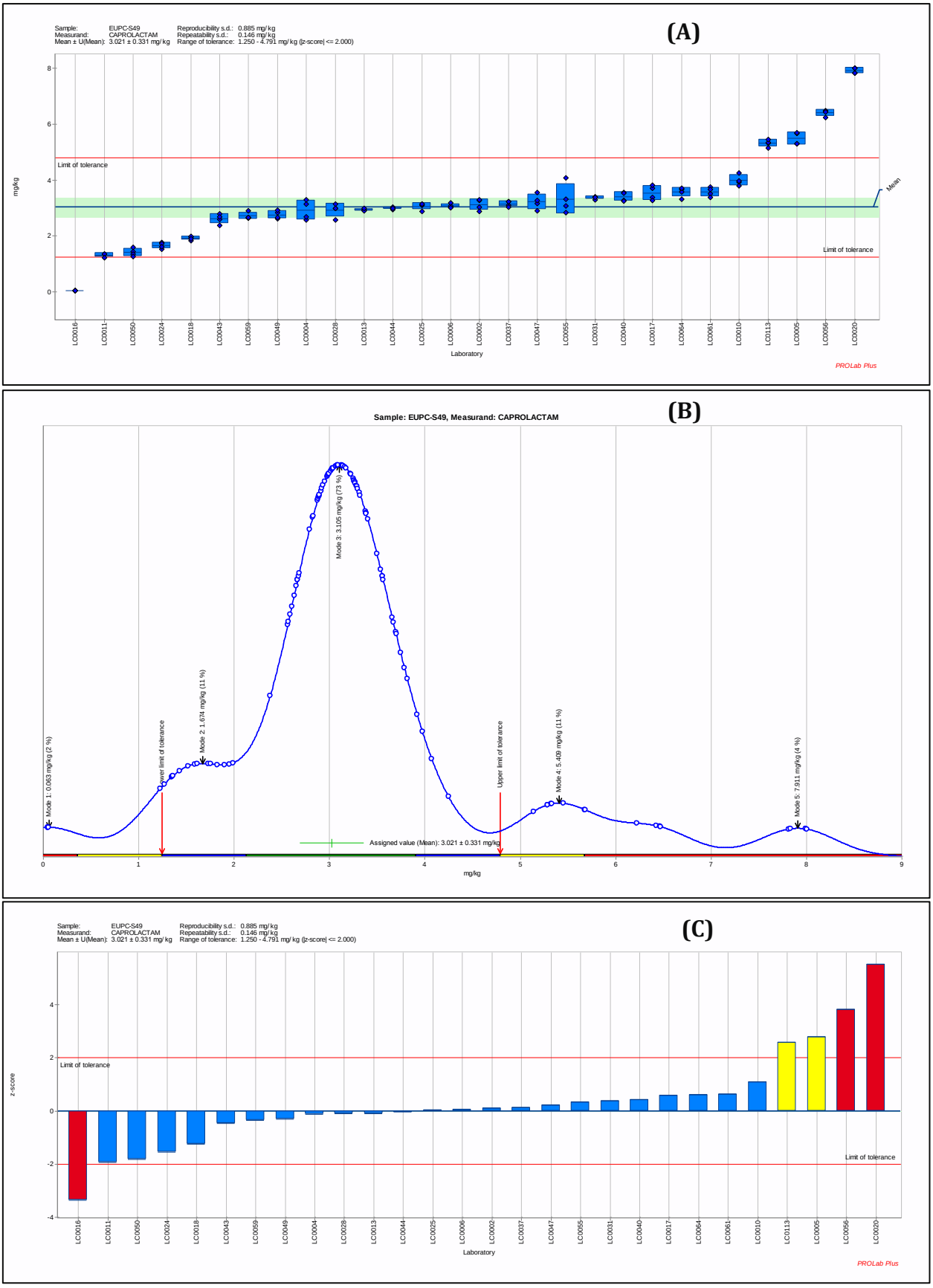


Figure 1. Summary of the laboratories test results for CAP with repeatability SD (A), Kernel desity plot (B) and z-scores (C).

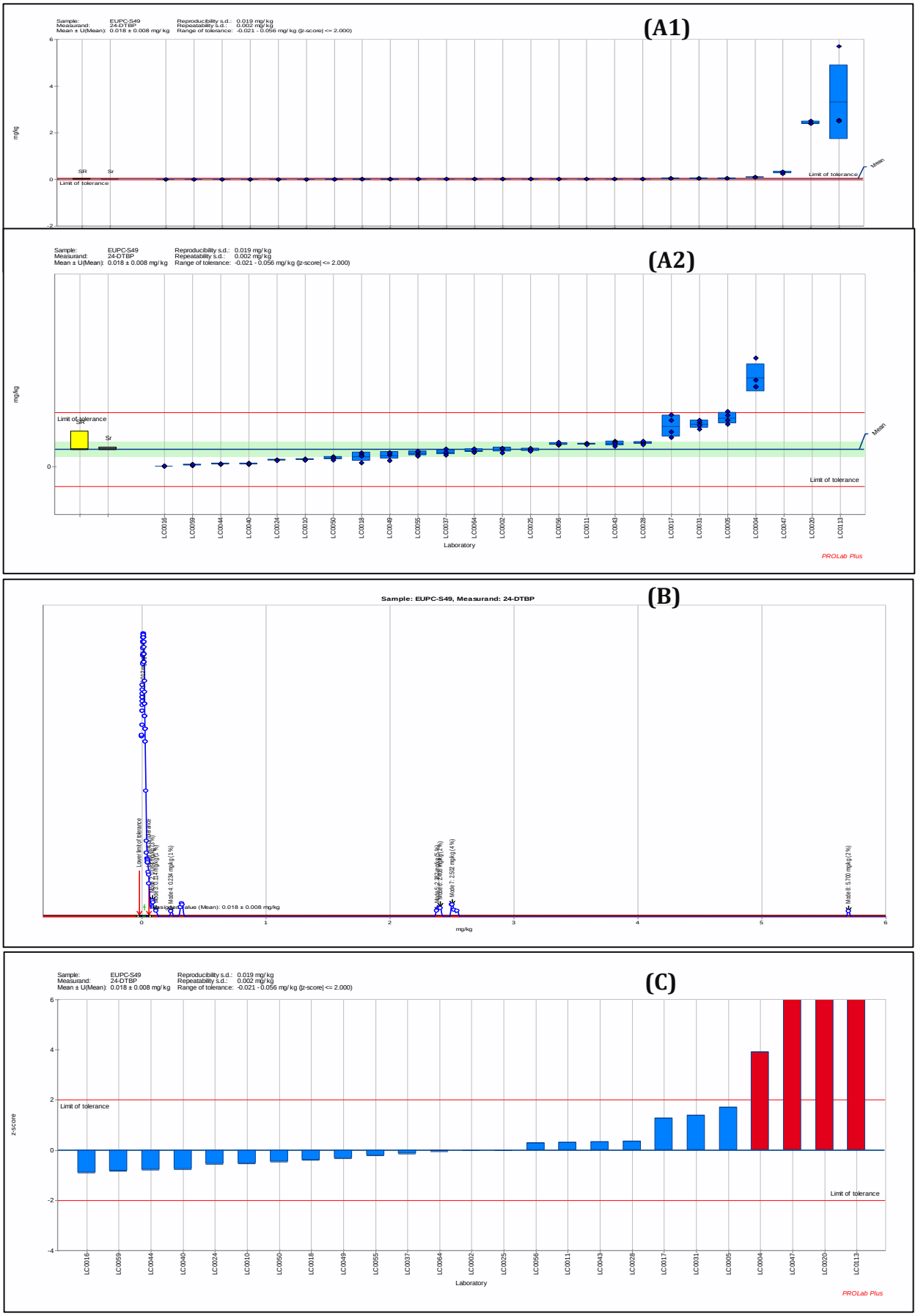


Figure 2. Summary of the laboratories test results for 2,4-DTBP with their repeatability SD (A1; A2), Kernel desity plot (B) and z-scores (C).

In the case of CAP 28 laboratories reported results, from which 82% of the results were satisfactory (|z-score|≤2), 7% were questionable (2<|z-score|≤3) and 11% were unsatisfactory (|z-score|>3). In the case of 2,4-DTBP, 28 laboratories reported results, of which 1 laboratory did not analyse the migration and 2 laboratories could not quantify 2,4-DTBP because the limit of quantification (LOQ) of their analytical method was higher than its concentration. 84% of the results were satisfactory (|z-score|≤2) and 16% were unsatisfactory (|z-score|>3).

In the case of CAP the majority of the participants applied a direct injection technique, followed by Liquid-Liquid Extraction (LLE) prior to analysis using appropriate solvent, dilution with a polar organic solvent prior to injection, solvent change by evaporating the food simulant A, reconstitution with other solvent, and the application of Solid Phase Extraction (SPE). For 2,4-DTBP, the laboratories applied predominantly LLE with appropriate solvent followed by direct injection, SPE and solvent change. The results for the applied sample preparation techniques are reported graphically in Figure 3.

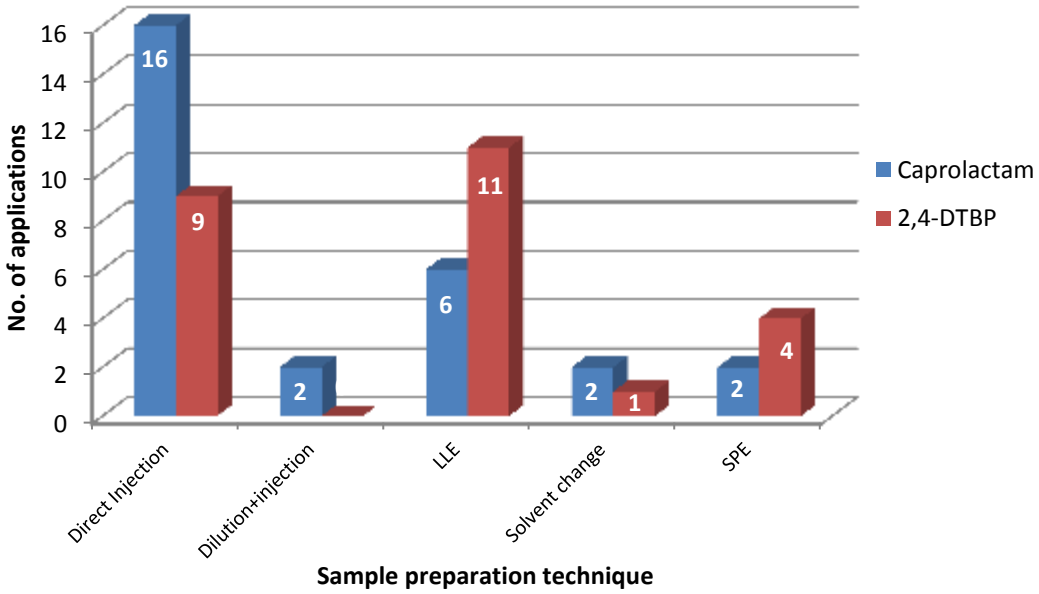


Figure 3. Sample preparation preparation for analysis of CAP and 2,4-DTBP in ILC 02-2015

For CAP the main analytical technique used was gas chromatography with mass spectrometry detector (GC-MS) followed by high pressure liquid chromatography with diode array detector (HPLC-DAD), liquid chromatography-tandem mass spectrometry (LC-MS/MS) with electro spray ionization operating in positive mode (ESI+), high pressure liquid chromatography with UV detector (HPLC-UV), gas chromatography with flame ionization detector (GC-FID) and finally liquid chromatography with mass spectrometry detection (LC-MS). For 2,4-DTBP the predominant analytical technique was GC-MS followed by LC-MS/MS with electro spray ionization operating in negative mode (ESI-), HPLC with fluorescence detector (HPLC-FLD), HPLC-UV and finally GC-FID.

The results for the applied analytical techniques are reported graphically in Figure 4.

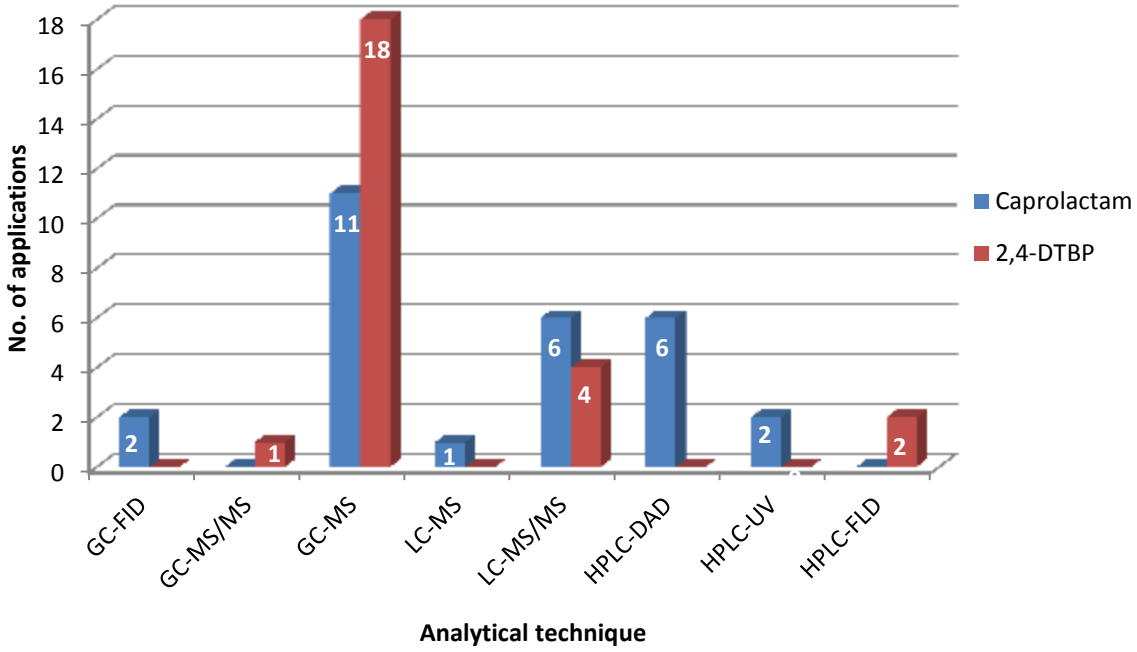


Figure 4. Analytical techiques applied for the determination of CAP and 2,4-DTBP in ILC02-2015

9. Conclusions

The overall participation in the ILC02-2015 was satisfactory. 28 laboratories out of 29 invited laboratories have reported results which represented a 96% of participation. Concerning NRLs, the participation was 77% (23 out of 30 laboratories).

Regarding the quantification of CAP 82% of the results were satisfactory (|z-score|≤2), 7% were questionable (2<|z-score|≤3) and 11% were unsatisfactory (|z-score|>3). , in the majority of the cases the sample was analysed by direct injection or cleaned prior to analysis by a Liquid-Liquid Extraction (LLE). The main analytical technique was GC-MS followed by HPLC-DAD and LC-MS/MS with positive electrospray ionization (ESI+).

In the case of the NIAS 2,4-DTBP 28 laboratories reported results of which 1 laboratory did not analyse the migration solution and 2 laboratories could not quantify because their limit of quantification (LOQ) was higher than the concentration of 2,4-DTBP. 84% of the results were satisfactory (|z-score|≤2) and a 16% were unsatisfactory (|z-score|>3). The majority of the participants applied a LLE followed by the direct injection and the predominant analytical technique was GC-MS followed by LC-MS/MS with negative electrospray ionization (ESI-).

10. Acknowledgements

The NRLs and OCLs who participated in this exercise (see list below) are kindly acknowledged.

NRLs

Austria	Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES) Abt. Gebrauch-sgegenstände, Vienna
Belgium	Scientific Institute of Public Health, Consumer Safety, Bruxelles
Croatia	Croatian National Institute of Public Health Food Contact Materials and Articles, Zagreb
Czech republic	National Institute of Public Health, Unit for Chemical Safety of Products, Prague
Cyprus	Laboratory for Control of Food Contact Materials and Control of Toys Ministry of Health, State General Laboratory (SGL), Nicosia
Denmark	Technical University of Denmark, National Food Institute Analytical Food Chemistry, Søborg
Denmark	Danish Veterinary and Food Administration Laboratory Århus, Lystrup
Estonia	Health Board Central Chemistry Laboratory, Tallinn
Finland	Finnish Customs Laboratory, Espoo
Germany	Bundesinstitut für Risikobewertung (BfR) (Federal Institute for Risk Assessment), Berlin
Greece	General Chemical State Laboratory, Laboratory of Articles and Materials in Contact with Foodstuffs, Athens
Hungary	National Food Chain Safety Office Food and Feed Safety Directorate Food Toxicological NRL, Budapest
Ireland	Public Analyst Laboratory, Sir Patrick Dun's, Dublin
Italy	Istituto Superiore di Sanità, Laboratorio Esposizione e rischio da materiali, c/o Dipartimento ambiente e connessa prevenzione primaria, Roma
Lithuania	National Public Health Surveillance Laboratory of Chemistry Department, Vilnius
Luxembourg	Laboratoire National de Santé Service de Surveillance Alimentaire, Luxembourg

Poland	National Institute of Public Health - National Institute of Hygiene, Laboratory of Department of Food Safety, Warsaw
Portugal	Escola Superior de Biotecnologia Universidade Católica Portuguesa CINATE, Porto
Slovakia	National Reference Centre and Laboratory for materials and articles intended to come into contact with food, Regional Public Health Authority In Poprad (RUVZ), Poprad
Slovenia	National Laboratory of Health, Environment and Food Center for Environment and Health Laboratory for Consumer Products, Ljubljana
Spain	Agencia Espanola de Seguridad Alimentaria y Nutrición (AECOSAN) Centro Nacional Alimentación, Madrid
Sweden	National Food Agency Department of Chemistry Division of Science, Uppsala
United Kingdom	The Food and Environment Research Agency (FERA), York

OCLs

Germany	Chemisches und Veterinäruntersuchungsamt Fellbach Abt. Bedarfsgegenstände, Fellbach
Germany	Laudesant für Verbraucheschutz Sachsen-Anhalt, Halle
Germany	Bayerisches Landesamt für Gesundheit und Lebens-mittelsicherheit R 5 Bedarfsgegenstände, Erlangen
Germany	Landesuntersuchungsanstalt für das Gesundheits-und Veterinärwesen Sachsen, Dresden
Germany	LAVES -Institut für Bedarfsgegenstände, Lüneburg
Italy	Istituto Zooprofilattico Sperimentale LER, Laboratorio Chimico, Bologna

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[14] ISO/IEC 17043:2010(E). Conformity assessment – General requirements for proficiency testing

12. Annexes

Annex 1. Invitation accompanying the sample ILC02 2015

EUROPEAN COMMISSION
GENERAL DIRECTORATE JRC
JOINT RESEARCH CENTRE
Institute for Health and Consumer Protection – IHCP
Chemical Assessment and Testing Unit

INVITATION LETTER

Inter-laboratory Comparison (ILC) Exercise on Specific migration in Simulant A – ILC02-2015

Dear Sir or Madam,

On behalf of the EURL for food contact materials, I would like to invite you to participate in an ILC exercise for the determination of substances in food simulant A.

This ILC aims at the extraction and quantification of substances released from food packaging material in food simulant. It consists in 1) a migration experiment and 2) analysis of selected migrating substances. The scope of this ILC exercise is a Proficiency Testing, you are free to use a method of your choice for the analytical determination.

You will receive a food packaging film. You will perform a migration experiment in Simulant A in 60°C for 10 days. You will be asked to quantify a food contact substance and a NIAS. The test uses a market materials and thus the quantification may not be related to the levels of SMLs.

I would like to remind you that it is a requirement for NRLs to participate in the ILCs organised by the EURL-FCM. Due to the limited amount of available material, there is limited possibility for OCL's to participate to that ILC02-2015. Their participation would be evaluated upon request and material availability.

Please confirm your participation until **17th December 2015** by sending back the completed participation form to Emmanouil TSOCHATZIS (Emmanouil.TSOCHATZIS@ec.europa.eu).

Once we have received your confirmation of participation, we will send a sample kit to you. The shipment of the sample kits is foreseen for **18th December 2015**. You will find detailed instructions concerning the requested results in the sample kits (and ex ante in the attached documents).

The **deadline for submission of results** is **31st January 2016**.

If you have any question, please contact E. Tsochatzis (Emmanouil.Tsochatzis@ec.europa.eu).

Sincerely yours,

Dr. Catherine Simoneau
Operating Manager, EU Reference Laboratory for Food Contact Materials
European Commission, DG-Joint Research Centre
Institute for Health and Consumer Protection
Chemical Assessment and Testing Unit, T.P. 260
Ispra (VA) 21020, Italy

Cc: P. Aguar (JRC), D. Rembges (JRC), B. Schupp (SANTE)

Annex 2. Confirmation of participation to ILC02 2015

EUROPEAN COMMISSION
GENERAL DIRECTORATE JRC
JOINT RESEARCH CENTRE
Institute for Health and Consumer Protection – IHCP
Chemical Assessment and Testing Unit

CONFIRMATION OF PARTICIPATION

ILC 02 2015: Specific migration in Simulant A

To participate in the exercise, complete the form and return it until **21st December 2015** by fax (+39 0332 785707) or e-mail (Emmanouil.TSOCHATZIS@ec.europa.eu)

DETAILS OF THE INTERLABORATORY COMPARISON EXERCISE	
ILC code	ILC 02 2015
ILC Title	Special migration
Year	2015
Sample type	Multilayer plastic film
Parameters for determination	Migration experiment (immersion)
Sample quantity	1 piece (35 X 10 cm)
Packaging	padded cardboard box
Shipment conditions	no special precautions
Sample dispatch	18th December 2015
Deadline for results	31st January 2016

PARTICIPATING INSTITUTION	
Organisation	
Laboratory	
CONTACT INFORMATION	
Contact person	
Address for sample dispatch	
Telephone	
Fax	
e-mail	

Annex 3. Shipping and Instruction form

EUROPEAN COMMISSION
GENERAL DIRECTORATE JRC
JOINT RESEARCH CENTRE
Institute for Health and Consumer Protection – IHCP
Chemical Assessment and Testing Unit

SHIPPING and INSTRUCTION FORM

ILC 02 2015: Specific migration in Simulant A

Material/samples sent :
• 1 piece of food packaging material
• Vial containing caprolactam analytical standard
• Vial containing 2,4-di-tert-butyl phenol analytical standard
• Vial containing 4-ethylphenol (internal standard)
• JRC.I.1.Form.FIT-EURL.03 ver.1_Sample Receipt Acknowledgement • JRC.I.1.Form.FIT-EURL.04 ver.1_Instructions for compilation of results • JRC.I.1.Form.FIT-EURL.07 ver.1_Laboratory code • JRC.I.1.Form.FIT-EURL.06 ver.1_Results Reporting Form • JRC.I.1.Form.FIT-EURL.05 ver.1_Questionnaire Form

Instructions :
1) All samples should be stored at room temperature. Analytical standards must be stored in a refrigerator (4^{o}C)
2) Caprolactam (CAS: 105-60-2) and 2,4-di-tert butyl phenol (CAS: 96-76-4) concentration (mg kg^{-1})
3) Please submit your results using the JRC.I.1.Form.FIT-EURL.06 ver.1_Results Reporting Form.
4) Closing date: 31/01/2016
5) Other instruction if needed: -

Sincerely yours,

Dr. Catherine Simoneau
Operating Manager, EU Reference Laboratory for Food Contact Materials
European Commission, DG-Joint Research Centre
Institute for Health and Consumer Protection
Unit Chemical Assessment and Testing, T.P. 260
Ispra Va 21020 Italy

Annex 4. Sample acknowledgement receipt

EUROPEAN COMMISSION
GENERAL DIRECTORATE JRC
JOINT RESEARCH CENTRE
Institute for Health and Consumer Protection – IHCP
Chemical Assessment and Testing Unit

Please complete the present form to acknowledge the sample receipt and return it by fax (+ 39 0332 785707) or e-mail (Emmanouil.TSOCHATZIS@ec.europa.eu) **within 14 days** after the sample receipt

SAMPLE RECEIPT ACKNOWLEDGEMENT FORM

ILC 02-2015: Specific migration in Simulant A

LABORATORY NAME:	
LABORATORY CODE:	
SAMPLE CODE:	CAT.028/EURL/2015/049
DATE OF RECEIPT:	
STATE OF SAMPLE:	

COMMENTS:

Date Name/Signature

Annex 5. Instructions and requested results

EUROPEAN COMMISSION
GENERAL DIRECTORATE JRC
JOINT RESEARCH CENTRE
Institute for Health and Consumer Protection – IHCP
Chemical Assessment and Testing Unit

INSTRUCTIONS AND REQUESTED RESULTS

ILC 02 2015: Specific migration in Simulant A

Measure 1 dm^2 of the food contact material that you have received from the EURL. Divide into small equal surface pieces and place them into a metallic support, add Simulant A and perform migration (immersion) in **60°C (±2 °C)** for **10 days** according to EN standards. Please note that we optionally suggest that the test specimen strips could be separated from one another by a metallic stainless steel thin grid or another appropriate means, as to avoid the potential sticking..

You will be asked to quantify caprolactam (FCM 212) and 2,4-di-tert-butyl-phenol (NIAS). Calculate of the substance concentration in mg kg^{-1}. The exercise uses a market material so the quantification is not targeting around SMLs. For the extraction and analysis you are free to use the method of your choice.

Please report your results in the provided Word file "JRC.I.1.Form.FIT-EURL.06 ver.1_Results Reporting Form.docx" or fill in the print copy. Please also fill in the **questionnaire** and provide as much details as possible on the extraction and analytical procedure that you have applied. Send back your results and the completed questionnaire by fax (+39 0332 78 5707) or by e-mail to Emmanouil TSOCHATZIS (Emmanouil.TSOCHATZIS@ec.europa.eu) until **31st January 2016**.

For further information, please contact Emmanouil TSOCHATZIS (phone: +39 0332 78 9548, e-mail: Emmanouil.TSOCHATZIS@ec.europa.eu).

Sincerely yours,

Dr. Catherine Simoneau
Operating Manager, EU Reference Laboratory for Food Contact Materials
European Commission, DG-Joint Research Centre
Institute for Health and Consumer Protection
Chemical Assessment and Testing Unit, T.P. 260
Ispra (VA) 21020, Italy

Annex 6. Laboratory code

EUROPEAN COMMISSION
GENERAL DIRECTORATE JRC
JOINT RESEARCH CENTRE
Institute for Health and Consumer Protection – IHCP
Chemical Assessment and Testing Unit

LABORATORY CODE

ILC 02-2015: Specific migration in Simulant A

LABORATORY NAME:	
LABORATORY CODE:	

Sincerely yours,

Dr. Catherine Simoneau
Operating Manager, EU Reference Laboratory for Food Contact Materials
European Commission, DG-Joint Research Centre
Institute for Health and Consumer Protection
Chemical Assessment and Testing Unit, T.P. 260
Ispra (VA) 21020, Italy

Annex 7. Results reporting form

EUROPEAN COMMISSION
GENERAL DIRECTORATE JRC
JOINT RESEARCH CENTRE
Institute for Health and Consumer Protection – IHCP
Chemical Assessment and Testing Unit

Use this form to submit your results by entering data in the space provided and return it by 31st January 2016 (deadline) by fax (+390332785707) or e-mail (Emmanouil.Tsochatzis@ec.europa.eu)

RESULTS REPORTING FORM – ILC 02 2015: Specific migration in Simulant A

LABORATORY CODE	

CONCENTRATION IN THE SAMPLE (mg kg^{-1})*						
SAMPLE CODE	ANALYTE	REPLICATE 1*	REPLICATE 2*	REPLICATE 3*	REPLICATE 4*	REMARKS
CAT.028/EURL/2015/049	Caprolactam (CAS: 105-60-2)					
CAT.028/EURL/2015/049	2,4-DTBP (CAS: 96-76-4)					

*Three (3) number of decimals e.g. 0.001

PLACE AND DATE	LABORATORY MANAGER	SIGNATURE

Annex 8. Questionnaire

EUROPEAN COMMISSION
GENERAL DIRECTORATE JRC
JOINT RESEARCH CENTRE
Institute for Health and Consumer Protection – IHCP
Chemical Assessment and Testing Unit

QUESTIONNAIRE FORM

ILC 02-2015: Specific migration in Simulant A

Complete the form and return it until **31st January 2016** (deadline) by fax (+39 0332 785707) or e-mail (Emmanouil.TSOCHATZIS@ec.europa.eu)

LABORATORY CODE:	

METHOD DESCRIPTION

Is the method validated? If YES, indicate the analytes it has been validated for
Reference
Is the method accredited? If YES, indicate the analytes it is accredited for
Did you analyze the sample according to an official method? If YES, please specify

EXPERIMENTAL PART

Sample amount used for analysis (mL) :	
Extraction solution used :	
Extraction procedure (please specify all the conditions used):	
Did you apply any special treatment to the samples provided? If YES, please specify	

Which analytical technique did you use?
Please provide your method details? (e.g. injection vol., temperature program, eluents etc.. if applicable)
Does your laboratory carry out this type of analysis (same matrix, analytes) on a routine basis?
Did you encounter any problems with sample analysis? If YES, please specify
Other Comments

Annex 9. Results of the homogeneity study

Analyte	Mean (mg/kg)	Mode s(target)	s(analytical) [%]	s for proficiency assessment [%]	HORRAT	ISO 13528:2015 Check for adequate heterogeneity	13528:2015 Check for significant heterogeneity	Harmonized Protocol Test on significant heterogeneity
CAP	2.929	Horwitz	1.5	13.608	1	Ok	Ok	Ok
2,4-DTBP	0.018	Horwitz	6.2	29.173	1	Ok	Ok	Ok

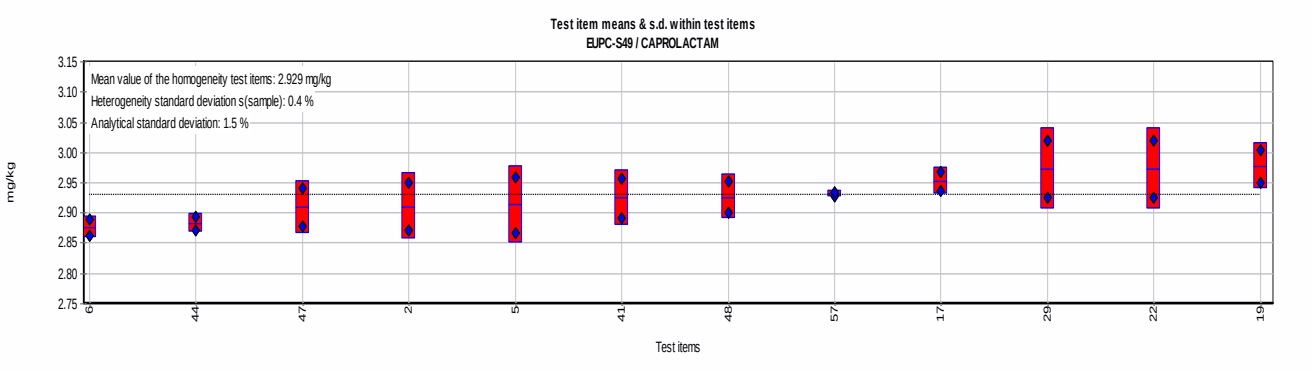

Figure 4. Homogeneity test results for Caprolactam (CAP) with Prolab software.

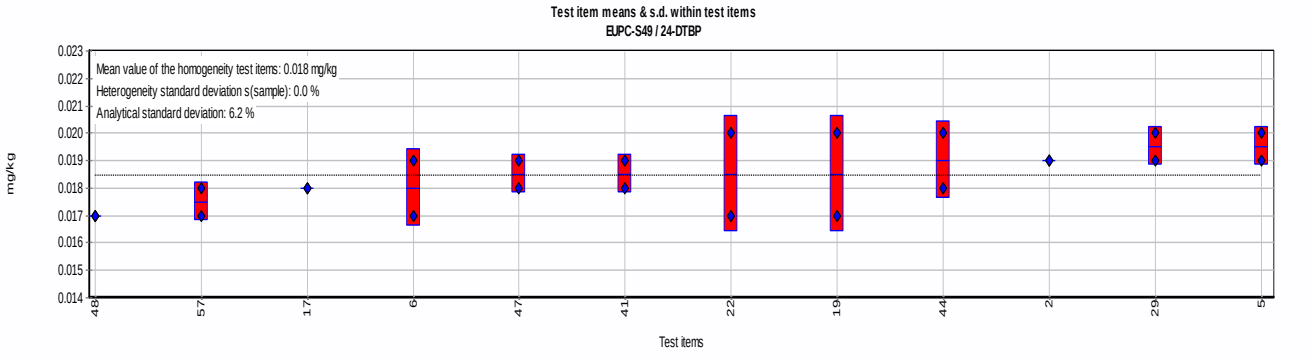

Figure 5. Homogeneity test results for 2,4-di-tert butyl phenol (2,4-DTBP) with Prolab software.

Annex 10. Results of the stability study

Analyte	Mean (mg/kg)	Mean homogeneity measurement	Mean stability measurement	s for proficiency assessment [%]	Mode s(target)	ISO 13528:2015 Expanded criterion	Harmonized Protocol / ISO13528:2015 – t-test
CAP	2.929	2.929	2.937	13.608	Horwitz	Ok	Ok
2,4-DTBP	0.018	0.018	0.018	29.173	Horwitz	Ok	Ok

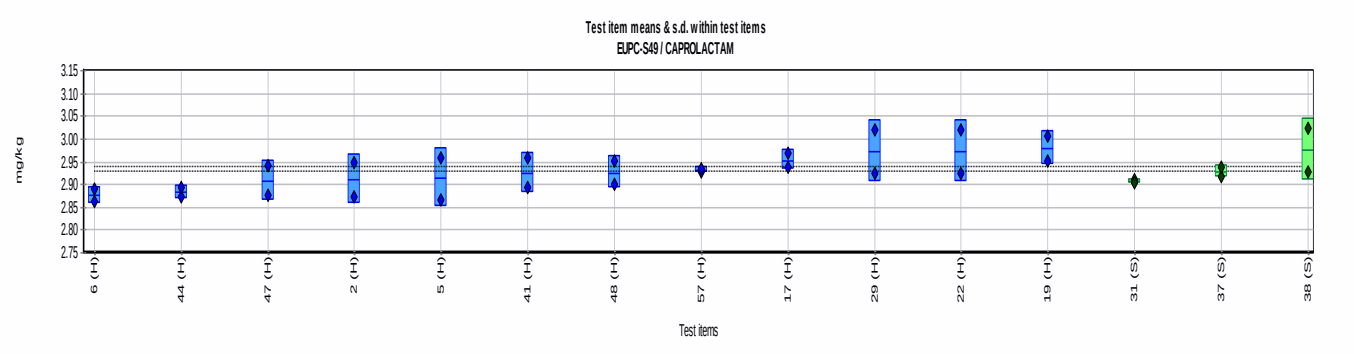

Figure 6. Stability test results for Caprolactam (CAP) with Prolab software.

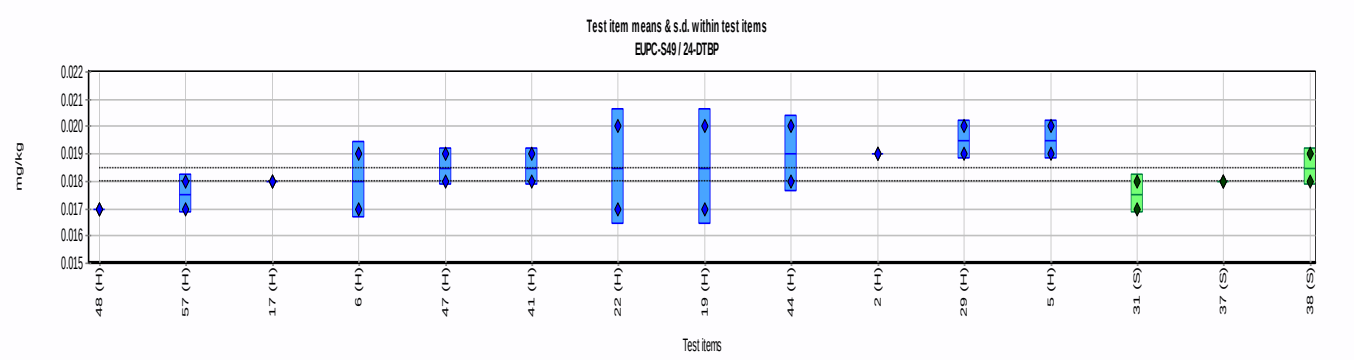

Figure 7. Stability test results for 2,4-di-tert butyl phenol (2,4-DTBP) with Prolab software.

Annex 11. Caprolactam analysis results reported by the participants

CAP (mg/kg)							
Laboratory	**Replication1**	**Replication2**	**Replication2**	**Replication4**	**Sample Preparation***	**Solvent/ Sorbent used**	**Analytical technique**
LC0002	3.040	2.880	3.270	3.300	Direct injection	-	HPLC-DAD
LC0004	3.285	2.573	3.143	2.678	LLE	CH_2Cl_2	GC-MS
LC0005	5.288	5.292	5.678	5.684	Direct injection	-	HPLC-UV
LC0006	3.038	3.066	3.014	3.178	Direct injection	-	GC-FID
LC0010	3.922	3.977	4.250	3.789	Solvent change	methanol	GC-MS
LC0011	1.354	1.360	1.230	-	LLE	Hexane, CH_2Cl_2	GC-MS
LC0013	2.912	2.936	2.895	2.977	Dilution+injection	methanol	HPLC-UV
LC0016	0.052	0.053	0.056	0.051	LLE	CH_2Cl_2	GC-MS
LC0017	3.702	3.386	3.261	3.818	Dilution+injection	Simulant+ITSD** sol.	LC-MS/MS
LC0018	1.953	1.829	1.995	1.902	Direct injection	-	LC-MS/MS
LC0020	7.830	7.815	7.992	8.004	Solvent change	n-hexane	GC-MS
LC0024	1.757	1.734	1.522	1.617	Direct injection	-	LC-MS/MS
LC0025	3.090	3.131	3.139	2.886	LLE	CH_2Cl_2	GC-MS
LC0028	2.567	2.986	3.139	2.998	Direct injection	-	LC-MS/MS
LC0031	3.385	3.289	3.404	3.387	SPE	SDVB	GC-MS
LC0037	3.223	3.096	3.027	3.227	Direct injection	-	HPLC-DAD
LC0040	3.558	3.274	3.252	3.541	LLE	EtAc***/toluene 1:1 v/v	GC-MS
LC0043	2.793	2.590	2.686	2.382	Direct injection	-	LC-MS/MS
LC0044	2.994	2.954	3.030	2.997	Direct injection	-	HPLC-DAD
LC0047	3.558	3.166	3.262	2.899	Direct injection	-	LC-MS
LC0049	2.831	2.609	2.656	2.924	Direct injection	-	GC-FID
LC0050	1.430	1.592	1.349	1.273	LLE	CH_2Cl_2	GC-MS
LC0055	2.827	3.314	3.078	4.074	SPE	C18/Envicarb	GC-MS
LC0056	6.225	6.471	6.464	6.431	Direct injection	-	HPLC-DAD
LC0059	2.892	2.634	2.670	2.670	Direct injection	-	HPLC-DAD
LC0061	3.500	3.380	3.750	3.660	Direct injection	-	LC-MS/MS
LC0064	3.325	3.673	3.566	3.696	Direct injection	-	HPLC-DAD
LC0113	5.142	5.328	5.331	5.453	Direct injection	-	GC-MS

* LLE=Liquid-Liquid Extraction; SPE= Solid-Phase Extraction
** ITSD= Internal Standard
*** EtAC: Ethyl acetate

Annex 12: 2,4-DTBP analysis results reported by the participants

2,4-DTBP (mg/kg)							
Laboratory	**Replication1**	**Replication2**	**Replication2**	**Replication4**	**Sample Preparation***	**Solvent/ Sorbent used**	**Analytical technique**
LC0002	0.019	0.014	0.018	0.019	Direct injection	-	HPLC-FLD
LC0004	0.113	0.083	0.090	0.083	LLE	CH_2Cl_2	GC-MS
LC0005	0.057	0.053	0.044	0.047	LLE	Cyclohexane	GC-MS
LC0006	ND;(<LOQ)**	ND;(<LOQ)**	ND;(<LOQ)**	ND;(<LOQ)**	Direct injection	-	GC-FID
LC0010	0.007	0.008	0.007	0.007	LLE	Isooctane	GC-MS
LC0011	0.024	0.024	0.023	-	LLE	Hexane, CH_2Cl_2	GC-MS
LC0013	ND;(<LOQ)**	ND;(<LOQ)**	ND;(<LOQ)**	ND;(<LOQ)**	Dilution+injection	methanol	HPLC-UV
LC0016	0.0003	0.0003	0.0003	0.0002	LLE	CH_2Cl_2	GC-MS
LC0017	0.036	0.054	0.048	0.030	Dilution+injection	ITSD sol.	LC-MS/MS
LC0018	0.014	0.012	0.011	0.004	Direct injection	-	LC-MS/MS
LC0020	2.520	2.40	2.410	2.380	Solvent change	n-hexane	GC-MS
LC0024	0.007	0.007	0.007	0.006	SPE	C18	GC-MS/MS
LC0025	0.017	0.018	0.019	0.016	LLE	CH_2Cl_2	GC-MS
LC0028	0.024	0.025	0.026	0.023	Direct injection	-	LC-MS/MS
LC0031	0.046	0.048	0.043	0.039	SPE	SDVB	GC-MS
LC0037	0.014	0.018	0.012	0.015	LLE	isooctane	GC-MS
LC0040	0.0036	0.0033	0.0024	0.0024	LLE	EtAc***/toluene 1:1 v/v	GC-MS
LC0043	0.026	0.025	0.021	0.024	Direct injection	-	LC-MS/MS
LC0044	0.002	0.003	0.003	-	LLE	Hexane/EtAc	GC-MS
LC0047	0.326	0.311	0.236	0.322	LLE	Cyclohexane	GC-MS
LC0049	0.012	0.006	0.014	0.014	LLE	hexane	GC-MS
LC0050	0.010	0.009	0.009	0.007	LLE	CH_2Cl_2	GC-MS
LC0055	0.015	0.012	0.016	0.011	SPE	C18/Envicarb	GC-MS
LC0056	0.023	0.022	0.023	0.025	Direct injection	-	HPLC-FLD
LC0059	0.002	0.001	0.001	0.002	SPE	-	GC-MS
LC0061	-	-	-	-	-	-	-
LC0064	0.015	0.015	0.018	0.018	LLE	Cyclohexane	GC-MS
LC0113	2.544	2.702	2.503	2.501	Direct injection	-	GC-MS

* LLE=Liquid-Liquid Extraction; SPE= Solid-Phase Extraction
** ND= not detected; concentration <LOQ of the method
*** EtAC: Ethyl acetate

European Commission
EUR 27828 EN – Joint Research Centre – Institute for Health and Consumer Protection

Title: **Report of an inter-laboratory comparison from the European Union Reference Laboratory for Food Contact Materials: ILC02 2015 – Specific migration from a multilayer in food simulant A**

Author(s): Emmanouil Tsochatzis, Anja Mieth and Catherine Simoneau

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