TESTING METHODOLOGY
FOR PRINTED CORRUGATED BOARD IN FOOD CONTACT

FEFCO
Corrugated Packaging

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INTRODUCTION

There is no specific harmonized EU measure which regulates food contact paper and board. Hence, at EU level, the general requirements for all food contact materials set out in the Framework Regulation (EC) 1935/2004, and in Regulation (EC) 2023/2006 on good manufacturing practice for materials and articles intended to come into contact with food are applied to paper and board (P&B), resp. corrugated board (CB).

The lack of EU harmonized regulations bear a potential risk for P&B and CB manufacturers.

To close this gap FEFCO initiated a study to identify test criteria for compliance testing for corrugated board. It is a known fact that the typical simulant Tenax, used for P&B gives overestimation. Therefore, a two-step approach was introduced by using Tenax and a real food - Infant food (milk powder) -as unique confirmation method. In article 18 (6) of EU Regulation 10/2011 is it clearly written "The results of specific migration testing obtained in food shall prevail over the results obtained in food simulant. The results of specific migration testing obtained in food simulant shall prevail over the results obtained by screening approaches". In the absence of a harmonized regulation for P&B, we believe the same principle could be applied. The infant food (milk powder) was selected as it is:

- relevant for the most critical consumer group
- in its powdered form it is highly comparable to Tenax, especially as the infant food contains a significant fat fraction
- more realistic in terms of particle size compared to Tenax
- treatable in the same way as Tenax in terms of time/temperature conditions.

The tests performed as part of the FEFCO study demonstrated clearly that exposing printed corrugated board from the non-printed side at 10d 40° C identifies all relevant IAS and NIAS. This procedure led to avoiding the formation of analytical artefacts/chemical degradation products. As part of the two-step approach infant food was introduced as a confirmatory method at 10d 60° C to verify and demonstrate that with Tenax an overestimation is possible.

As a result, the following procedure has been developed:

- Exposing printed corrugated board to Tenax at 10d 40°C to identify IAS and NIAS
- In parallel or subsequently a test is started with commercial infant food at 10d 60° C for target and unknown fingerprints (defined mass-to-charge ratios) in real food
- Tenax is tested by GC/MS and LC/MS in the target mode, to identify substances disclosed by printing ink companies, and in the non-target mode, to cover potential NIAS.
- In case the Tenax results do not show any compliance issue, further testing could be stopped
- In case the Tenax results show an exceeding of migration limits and/or do show a huge amount of NIAS, a verification can be done with the infant food.

The above procedure can be used to clearly demonstrate compliance with simulation and real food applications and is explained in details in the testing methodology.

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METHOD

Determination of printing ink migration from printed corrugated board after simulant exposure by GC/MS and LC/MS.

SCOPE

The scope of the document is to provide a guideline how printed corrugated board could be best analysed to evaluate possible migration of substances from the printing inks. Printed corrugated carboard samples are exposed to food simulants –Tenax® and infant food- to determine the migration level of printing ink substances with focus on IAS (intentionally added substances) and NIAS (non-intentionally substances).

SAMPLE PREPARATION

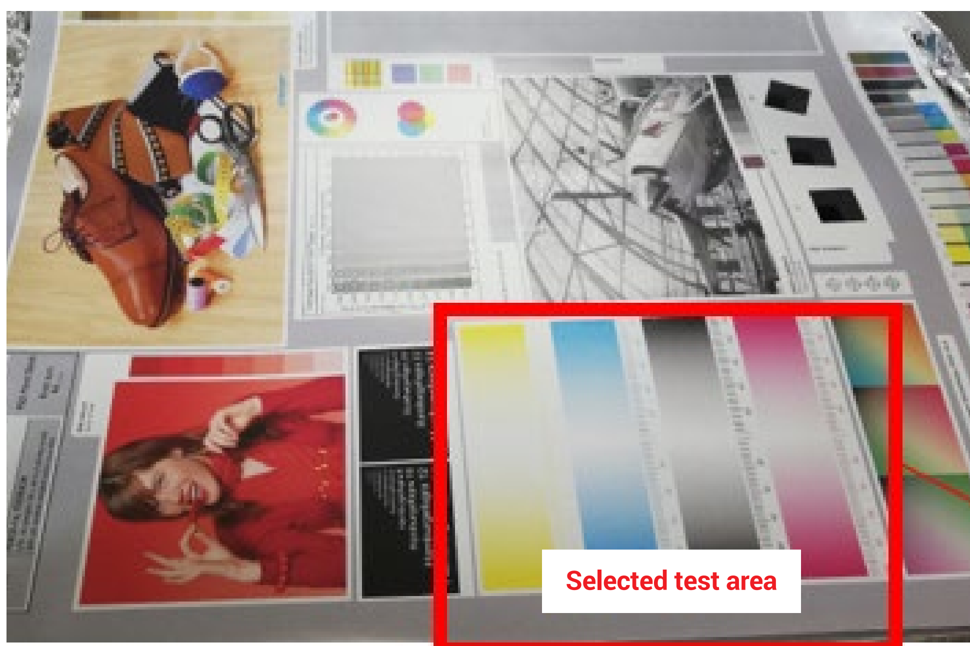
Sample shipment

All samples taken after printing trial for migration tests should be wrapped in aluminium foil to avoid any cross-contamination during transport face. It is also highly recommended that samples are not touched with fingers – wearing gloves is key.

Sample Preparation

Representative printed samples – The scale tone area is recommended to have 4 color stripes (CMYK) – see Figure 1 – are exposed to Tenax® and/or infant food. The samples should be min A4 or max A3 format.

Figure 1: Printing Design



- Tenax® exposure is performed as described in EN 14338:2003.
- 4 g MPPO are evenly distributed in the smaller Petri dish. Then the Petri dish is covered with the test sample and the system is closed with the larger Petri dish. If the two sides of the paper or cardboard are not the same, the surface intended for food contact should face the MPPO. The test system is assembled and turned over for testing. Empty Petri dishes are used to determine the blank value, into which the same mass of MPPO is added as for the test samples.
- Infant food exposure is performed in the same way as Tenax®.
- In both cases 4 g powder are exposed to 1dm^2 material.
- Tenax® is the trade name for MPPO - Poly(2,6-diphenyl-p-phenylenoxid) – CAS 24938-68-9.
- As Infant food (buy milk powder) any brand can be used – the fat content should vary between 25 to 40%.
- After having applied Tenax® /Infant food to the not-printed side of corrugated board the samples should be stored in an oven for 10 d 40° C (Tenax®) and 10d 60° C (infant food).
- Tenax® /infant food is afterwards extracted with ethylacetate/cyclohexane (1:1) for GC/MS or with acetonitrile for LC/MS.

EVALUATION METHODS

The following parameters have been used for the FEFCO study. Any similar system delivering the same performance criteria as described under the section on page 10 can be applied as well.

GC/MS-QToF

Principle

Various migration solutions and/or extracts are produced to investigate food contact materials (FCM). Such a migrate or extract is screened for non-target substances (analytes of this method) after concentration by means of GC-QTOF-MS / FID. Non-target substances are substances to be expected in the food contact materials area (e.g. hydrocarbons, antioxidants, oligomers of the polymers used, degradation products of photoinitiators, etc.) which are assigned by means of the internal library and/or the spectrum library NIST. Non-target peaks greater than 10 ppb based on the standard conversion (standard O / V: 6 dm² / 1 kg food (EU cube)) are compared with an internal library and the NIST spectrum library.

The internal standards (IS) are added as an IS mix (IS 1: heptadecane; IS 2: D4-DBP; IS 3: D4-BBP and IS 4: D4-DnNP) to each sample and to each blank value sample. IS 1, 3 and 4 have a concentration of 100 ppb based on the standard conversion. They are intended to ensure that an evaluation can take place even if one or two IS co-elute with an ingredient. Internal standard 2 has a converted concentration of 10 ppb and is used to check whether the method’s determination limit of 10 ppb can be observed. Both identifiable and non-identifiable substances are quantified using the internal standards.

Substances that cannot be identified are reported as “unknown” and described with their m / z fragments and the relative retention time.

Material

Ultrasonic bath:	Branson 2510
Laboratory shaker:	Gerhardt, Laboshake 215
Centrifuge:	Heraeus Megafuge 1.0
Precision balance:	Mettler Toledo XSE205Du, FCMWG01
Evaporation station:	Barkey-Vapotherm with nitrogen from the house pipe
Migration cells:	Migration chamber “Sieg-Mi-Flex” from LABC Labortechnik
Welding device:	Polystar 110 GE
Piston-operated pipettes:	GILSON - Microman M10, M100 and M1000, with corresponding tips
GC vial:	1.5 ml crimp neck bottle, clear glass
Insert vial:	approx. 0.3ml TopSert TPX snap ring bottle
GC-Cap:	aluminum cap painted red 5.5 mm hole silicone white / PTFE red, UltraClean
HS-Vial:	Headspace bottle 20 ml with thread, amber glass
HS cap:	Headspace UltraClean screw cap 18 mm, magnetic
Membrane filter:	Whatman Syringe Filter 0.45 µm
Disposable syringes:	HWS Henke Sass Wolf GmbH, plastic syringes 1 ml & 5 ml
Pasteur pipettes:	disposable made of glass
Test tubes:	10 ml graduated test tubes narrow neck NS 12/21 10 ml graduated test tube wide neck NS 14/23 and appropriate glass stoppers
Glass funnel:	4 cm diameter, approx. 4 cm length
Tilting bottle dispenser:	with 10 ml and 5 ml glass cut
	Poly spoons, spatulas, paper scissors, tin scissors, scalpel and carpet knife

Chemicals

- Handling of the substances and solutions according to internal procedures.
- Granulated sodium sulfate anhydrous
- Sodium chloride p.A.

Solvents

- Ethyl acetate> 99.8% or p.A.
- Cyclohexane> 99.8% or p.A.
- Acetonitrile> 99.8% or p.A.

Reagents

Extraction mixture : ethyl acetate / cyclohexane V1: V1 (EtAc / Cyh). This mixture is freshly prepared weekly in a Glas Erlenmeyer.

Reference substances (internal standards)

CAS 629-78-7	heptadecane> 98%
CAS 93952-11-5	Di-n-butylphthalate-D4 (D4-DBP)> 98%
CAS 93951-88-3	Benzylbutylphthalate-D4 (D4-BBP)> 98%
CAS 1202865-43-7	Di-n-nonylphthalate-3,4,5,6-D4 (D4-DnNP)> 98%

Equipment

Autosampler:	PAL autosampler and headspace option, via Gerstel
Injection system:	Gerstel PTV injector KAS 4 and external cooling
Gas chromatograph:	Agilent 7890B Network GC with flame ionization detector and Parallel detection set from Gerstel
Mass detector:	Agilent 7200 GC / MS-QTOF mainframe
Separation column:	DB-5MS 30 m x 0.25 mm ID x 0.1 µm film Agilent J&W or comparable, service life at least 4 months
PDS:	Transfer-Line: deactivated 1.27 m x 0.15 mm I.D. = 1.5 ml / min
FID connection:	deactivated 1.21 m x 0.20 mm I.D. = 1.5 ml / min
GC flow:	helium 6.0, approx. 3 ml / min
Injector:	PTV with UPC cooling mixture EtOH/H2O V70%: V30% at 40°C Liner “buffled” glass - 40°C - 315°C solvent vent 0.2 min then splitless for 1.5 min
Oven program:	55°C - 320°C
Injection volume:	2 µl, rinsing solutions A and B are EtAc / Cyh 1: 1
Transfer line:	300°C
FID temperature:	300°C
FID gases:	30 ml / min H2, 300 ml / min synthetic air, 20 ml / min N2 from generator
Spring temperature:	230°C
TOF Quad heating:	150°C
Collision gas:	nitrogen 6.0, 1.5 ml
Measuring range scan:	45 - 800 m/z

Disclaimer: the above mentioned materials, chemicals and equipment were used in the study. However, similar items can be used if the same performance criteria (as described on page 10) can be achieved.

LC/MS-QToF

Principle

A migration solution is screened for substances from databases and possible additional substances (substances that can be identified or unknown via precise mass) using LC-QTOF-MS.

A screening mix and a blank value are analyzed for each measurement series. 4-Morpholinobenzoic acid (4-MB) and Irganox 565 from the screening mix are used to monitor the performance of the device. They also serve to validate the method. Urethane acrylate is used to quantify unknown substances.

In order to quantify the substances detected in the LC-QTOF-MS screening method and listed in the database, an external calibration with the respective substance must be carried out after the screening. Independent of the screening or the database, this method can also be used to quantify substances for which a reference substance is available.

If an unknown substance is detected in the screening, it can be assumed as a worst case that its response is ≥ that of the urethane acrylate, since the response of the mass spectrometer to urethane acrylate is extremely low under the given conditions. The urethane acrylate found in the screening mix can thus be used to quantify unknown substances.

Material

Ultrasonic bath	Branson 2510
Shaking machine	Gerhardt, Laboshake 215
Centrifuge	Heraeus Megafuge 1.0
Falcontubes	TPP 50ml
0.45 µm filter	Whatman Syringe Filter
Vials	brown glass, Schmidlin Labor + Service AG
Pipettes	10-100 µl, 100-1000 µl, 500-5000 µl pipettes, Eppendorf
Pasteur pipettes, spatula	

Chemicals

Acetonitrile 99.95%	HPLC Supra-gradient, Biosolve
Millipore Water	
Formic acid 98%	Fluka (56302)
Methanol absolute	99.95%, Biosolve
Leucine-Enkephalin 95%	Sigma Aldrich (L9133)
Ethanol absolute, 99.95% Biosolve	
Sodium hydroxide solution 0.1 mol / l, Reagecon	

Reference Standard

Urethane acrylate	CAS 63225-53-6, Sigma-Aldrich Chemie GmbH (496952)

Equipment

Chromatographic parameters

UPLC	Acquity UPLC, Waters
Column	Acquity UPLC BEH C18 1.7 µm, 2.1 mm x 100 mm
Pressure	1000 bar
UPLC gradient	eluent A: 0.1 % formic acid in deion. water eluent B: 0.1 % formic acid in acetonitrile flow 0.5 ml/min
Time gradient	see below

Time gradient:

Time [min]	A [%]	B [%]
0	95	5
3	40	60
4	40	60
6	5	95
15	5	95

Chromatographic parameters

MS	Synapt G2-S, Waters
Ionisation	electrospray ionisation (ESI), positive mode
Detector	Time-of Flight
Acquisition mode	high resolution
m/z range [Da]	50 – 1200
Capillary [kV]	0.50
Sampling cone [V]	30
Source offset [V]	60
Source temp. [°C]	120
Desolvation [°C]	550
Cone gas (N2) [l/h]	30
Desolv. gas (N2) [l/h]	950
Nebuliser (N2) [bar]	6.0
Runtime MS [min]	15

Disclaimer: the above mentioned materials, chemicals and equipment were used in the study. However, similar items can be used if the same performance criteria (as described on page 10) can be achieved.

PERFORMANCE CRITERIA

All solution yielded from the extraction of Tenax® and/or infant food have to be concentrated by factor 10 to achieve the following performance criteria:

Parameter	System	
	LC/MS	GC/MS
Accuracy (%) – based on internal standards	70-120	70-120
Linearity– based of Surface-to-volume ratio of $6dm^2/kg$ (if deviating S/Vs are used more material needs to be exposed or higher concentration factor is needed	0.005 – 1 (mg/kg food)	0.005 – 1 (mg/kg food)
Precision (%)	± 20%	± 20%
Limit of Quantification (LoQ) Minimum criterium based on internal standards	0.01 - 0.02 (mg/kg food)	0.01 - 0.02 (mg/kg food)
Limit of Detection (LoD) Minimum criterium based on internal standards	0.005 – 0.01 (mg/kg food)	0.005 – 0.01 (mg/kg food)

ASSESSMENT

Any signal detected above 0.01 mg/kg food should be identified as much as possible. If the identification is positive, the quantification is performed according an external calibration as long as a suitable reference standard is commercially available.

These results are clearly marked by giving a CAS number as identifier for a substance in the report.

All other substances, which cannot be identified via references and/or exact masses will be semi-quantified via the used internal standards. The result will be either expressed by giving the most abundant exact masses as identifier or a group specific name or a typical mass fragment loss.

Table 1 and 2 show examples how to express the results.

Table 1: GC/Ms result expression as example

			Conz.	Standard S/V	SML
RRT	**Substance**	**CAS No**	**[mg/dm²]**	**[mg/kg LM]**	**[mg/kg LM]**
[min]	Limit of Detection		0.0017	0.010	
0.73	unknown (m/z 327.169+55.053+299.138)		0.013	0.078	< 0.01
1.28	Erucasäureamid	112-84-5	0.011	0.064	60
–	cyclic Oligomere	–	–	–	–
1.50	IPHA/EG/IPHA/EG		0.0025	0.015	

Table 2: LC/MS result expression as example

RT [min]	Fragment m/z	Ion	Possible Structure/ Substance	CAS No	Conz. (UA) [mg/dm²]	Standard S/V [mg/kg LM]	SML [mg/kg LM]
			Limit of Detection		0.010	0.060	
3.03	505.265	[M+Na]+	C22H42O11		0.50	3.0	
6.64	369.301	[M+Na]+	Ethoxylated Subst.		2.0	12	
8.02	265.123	M+	Known Substance	111-22-33	0.02	0.12	0.5

For Tenax® as well as for Infant food it is necessary to have blank values. This does mean exposing Tenax® /Infant food to time/temperature without having contact to any packaging material, undergoing the same extractions and subtract the signals therefrom.

FEFCO
Corrugated Packaging

FOR MORE INFORMATION CONTACT

Avenue Louise 250
B - 1050 Brussels

Phone: +32 2 646 40 70
Email: info@fefco.org

www.fefco.org
@Corrugated_OC
Twitter @FEFCO