EUROPEAN COMMISSION
DIRECTORATE-GENERAL
Joint Research Centre

Compilation of analytical methods for model migrants in foodstuffs:
Review of analytical methodologies

This publication corresponds to project deliverable D2 of the

EU-Project QLK1-CT2002-2390

"FOODMIGROSURE"

Modelling Migration from Plastics into Foodstuffs as a Novel and Cost Efficient Tool for Estimation of Consumer Exposure from Food Contact Materials.

P. Paseiro (author)

C. Simoneau and R. Franz (editors)

2006 **EUR 22552 EN**

Mission of the IHCP

The mission of the Institute for Health and Consumer Protection is to support EU policies for health and consumer protection. The institute carries out research to improve the understanding of the hazards, exposure and risk posed by food contaminants, drugs, chemicals, products, services and to develop, validate and apply advances methods and strategies of high scientific quality.

ISSN 1018-5593
EUR 22552 EN

Printed in Italy

Compilation of analytical methods for model migrants in foodstuffs: Review of analytical methodologies

This publication corresponds to project deliverable D2 of the EU-Project QLK1-CT2002-2390

"FOODMIGROSURE"

Modelling Migration from Plastics into Foodstuffs as a Novel and Cost Efficient Tool for Estimation of Consumer Exposure from Food Contact Materials.

P. Paseiro (author)

R. Franz and C. Simoneau (editors)

Co-Authors

- Sendón García, Raquel
- Sanches Silva, Ana
- Lopez Hernandez, Julia

Universidad de Santiago de Compostela,

Dpt. Química Analítica, Nutrición E Bromatoloxia,

Facultad de Farmacia

Santiago de Compostela, ES

INITIAL CONSIDERATIONS

1. Model migrants chosen for this project and their interesting physical and chemical properties are in table 1.

2. An abstract of the revised literature on analytical methods in polymers, food simulants and foodstuffs is collected in table 2; it can be summarized in three points.

   a) The literature on this subject is scant, and most has placed more emphasis on results concerning, migration in simulants and amount in polymers than on food analysis.

   b) Analytical methods are still in the research and development stage, and no particular set of methods has attracted widespread agreement. No two studies on the use of compounds appear to have used the same procedure to determine a given compound in a given type of sample.

   c) Some of the protocols that have been used are not described in the literature in sufficient detail to allow confident replication by other laboratories. This is a common situation in the early stage of the development of the analytical methodology.

   To sum up, in general it is not at present possible to draw up reliable analytical protocols for the determination of all the selected compounds in food. For most migrant/food combinations, any protocol drawn up on the basis of the literature and general analytical principles and experience is necessarily a tentative proposal requiring exhaustive evaluation.

3. Results of preliminary experiments carried out by us about properties of interest (ultraviolet, fluorescence, atmospheric pressure chemical ionization-mass spectroscopy, GC possibility and acetonitrile/olive oil extraction) to prepare analytical methods are included in table 3. A short description of experimental procedures is in Annex I.

4. General recommendations, focused in the preparation of the sample and the determination stage, to prepare analytical procedures for model migrants in foods are suggested in table 4.

ANALYSIS OF MODEL MIGRANTS. SAMPLE PREPARATION

Polymer materials

In general, the first stage in the determination of the migrants in polymers is their separation from the matrix. For this, the two most widely used methods are as follows.

1) Extraction with solvents. It is widely accepted that the solvent used should both dissolve the target compound well and also swell the polymer matrix. Polymer swelling data are readily available in the literature, but the solubilities of the selected surrogates in plausible extraction solvents are only incompletely documented and, pending determination, must be estimated on the basis of the nature of analyte and extractant. Several combinations solvent/analyte/polymer has been used. Dichloromethane for Irganox 1076 and DEHA in multilayer materials (PP/EVA/EVOH); acetonitrile for Chimassorb 81, BHT and Irganox 1076 in PET; ethanol 95% and 2-propanol for Caprolactam in polyamide and PVC; heptane for BHT from LDPE; methanol for Limonene in LDPE; and THF for Chimassorb 81 and Uvitex OB in HDPE and PP. Extraction procedures are usually carried out by hand shaking, although sometimes ultrasonic maceration or Soxhlet were used to improve the process (Vargo, J. D., 1985; Ulsaker, G. A., 1992; Nerin, C., 1996; O' Brien, A. P., 1997; Marque, D., 1998; Monteiro, M., 1998; Wessling, C., 1998; Avison, S. J., 2001; EU project G6RD-CT200-00411). Regarding other food contact materials, Laurolactam, DIPN and Benzophenone were extracted from paper and board with absolute ethanol (Summerfield, W., 2001; Triantafyllou, V. I., 2002). Extractions with Supercritical Fluid or Microwave-Assisted were used for Irganox 1076 and Chimassorb 81 in PE (Salafranca, J., 1999), and DEHA and ATBC in PVC (Cano, J. M., 2002).

2) Use of solvents that swell and/or dissolve both selected compound and polymer, followed by precipitation of the polymer. Toluene for polyolefins containing Irganox 1076 followed by precipitation of the polymer with methanol (EU project G6RD-CT200-00411). DEHA was determined by total dissolution of polymers with chloroform and then precipitating with methanol (O' Brien, A. P., 1997). Polycarbonates containing Bisphenol A were dissolved in dichloromethane or chloroform and then precipitated with 2-propanol (Mountfort, K. A., 1997) or re-extracted with a sodium hydroxide solution (Howe, S. R., 1998).

Food simulants

1) Aqueous official food simulants (distilled water, acetic acid 3%(w/v) and ethanol 10%(v/v))

   Usually they are directly injected when the analysis is carried out by RP-HPLC. DEHA, Irganox 1076 and Bisphenol A were determined following this methodology (O'Brien, A. P, 1997, Howe, S. R., 1998), and Chimassorb 81 and ε-Caprolactam after dilution with miscible organic solvents (Spyropoulos, D.V., 1998; EU project G6RD-CT200-00411),

   Other times, a concentration step or change of solvent is necessary before the chromatographic analysis. Irganox and DEHA were extracted with hexane (Simoneau, C., 1999), Chimassorb 81 with dichloromethane (Nerin, C., 1996), Uvitex OB with chloroform and DEHA with heptane (O' Brien, A. P., 1997). Benzophenone was extracted using an activated silica-bound-phase cartridge, which was eluted with a methanolic solution (Papillound, S., 2002). After drying Bisphenol A residue was redissolved with acetonitrile (Munguia-Lopez, E., 2001).

   For volatiles substances specific techniques were applied. Solid Phase Micro Extraction (SPE) for the determination of styrene (Silva, F. C., 2000) and BHT (Tombesi, N., 2002) and thermal desorption extraction for determination of Limonene (Linssen, J. P. H., 1993).

2) Official fatty food simulants (olive oil, synthetic mixture of triglycerides, sunflower oil and corn oil).

Dilution with solvents followed by direct injection in a chromatographic system. Acetone was used in the determination of the migration of Irganox 1076 (Helmroth, I. E., 2002; O'Brien, A. P., 1997), Chimassorb 81 and Uvitex OB (EU project G6RD-CT200-00411), dichloromethane or THF to determine Chimassorb 81 (Nerin, C., 1996; Spyropoulos, D. V., 1998), heptane was the solvent when migration of DEHA was evaluated (O' Brien, A. P., 1997) and chloroform was used for Bisphenol A (Howe, S. R., 1998).

Extraction with solvents. Acetonitrile was used for DEHA and Irganox 1076 (Simoneau, C., 1999), and methanol to extract Chimassorb 81 (Spyropoulos, D. V., 1998), although very poor recovery was achieved.

Size exclusion chromatography was used in the evaluation of the Laurolactam migration (Stoffers, N. H., 2003).

For volatiles substances specific techniques were applied again. Styrene migration into olive oil was determined extracting the sample with hexane using a Likens-Nikerson distillation apparatus (Jickells, S. M., 1993). A Tenax absorption column followed by extraction with hexane has been employed to determine DEHA and ATBC (Van Lierop, J. B. H., 1988).

3) Substitute fat simulants

Direct injection of the simulant, after migration tests, in a chromatographic system was used to determine the migration of Irganox 1076, Chimassorb 81 and Uvitex OB (EU project G6RD-CT200-00411), ε-Caprolactam (EU project G6RD-CT200-00411) and Chimassorb 81 after dilution with THF (Spyropoulos, D. V., 1998).

Evaporation of the simulant to dryness followed by dissolution in chloroform, methanol, acetonitrile or heptane, was used to determine Irganox 1076 (Garde, J. A., 2001), Laurolactam (Stoffers, N. H., 2003), Benzophenone (Papillound, S., 2002) and BHT (Wessling, C., 1998) respectively.

Extraction with hexane/water was used to determine DEHA and Irganox 1076 in ethanol 95% (v/v) (Simoneau, C., 1999).

Foodstuffs.

Due to the complexity of food samples, the procedure more broadly used with simulants, the direct injection, it doesn't seem the most appropriate to evaluate migration in foods. In spite of the lack of abundant bibliographic support, the available information related with the study of migration into foodstuffs reveals that the scientific community focuses mainly the problem as follow.

Extraction with solvents. This procedure is one of the most generally used, as much for fatty foods as non fatty foods. In non fatty foods, DEHA was extracted from jelly, candy, fruit juices, wines and beers with cyclohexane-dichloromethane (1:1) or hexane and dichloromethane (10:1) (Oi-wah, L., 1996; Page, B. D., 1995), Limonene from kiwi fruit with methylene chloride (Jordan, M. J., 2002), DIPN from dry food with absolute ethanol using ultrasonics bath (Summerfield, W., 2001). In fatty foods, DIPN was extracted from pastry, cake and pizza with acetonitrile (Summerfield, W., 2001). BHT from smoke flavoured sausage with acetonitrile (Yankah, V., 1998) and good recoveries were achieved. Methanol was used to extract BHT according the IUPAC method (Dieffenbacher, D., 1998).

After solvent extraction a clean-up step may be necessary.

Solid Phase Extraction. It was used in sample preparation of DEHA and other plasticizers in fatty food (animal tissues, fats and high fat content cheese) after blending with dichloromethane and passing the extract through a Florisil column (Page, B. D., 1995). Bisphenol A was extracted from the solid portion in cans of vegetables and fruit extracting with acetonitrile, evaporating to dryness, dissolving with acetone-heptane and purifying with a Sep-Pak Florisil cartridge. Good recoveries and relative standard deviation were achieved. The aqueous portion was applied to an OASIS HLD extraction cartridge (Yoshida, T., 2001). In addition, Bisphenol A from coffee or infant feed was extracted with water or water-ethanol,

followed by a SPE purification/concentration step (Mountfort, K. A., 1997; Kang, J., 2002).

Size Exclusion Chromatography or Gel Permeation Chromatography were used to clean up fatty matrix containing non polar analytes. DEHA in homogenized foods was extracted with acetone/hexane, extracts were dried, evaporated to dryness and redissolved in dichloromethane/cyclohexane and Biobeads S-X3 column was used to clean-up (Startin, J. R., 1987, Oi-Wah, L., 1996). ATBC in food (cheese, fruit, vegetables, soups, cakes, puddings and meal dishes) was determined using similar procedure (Castle, L., 1988).

Volatile compounds were separated by volatilisation with or without concentration step (static or dynamic Headspace, SPME or TENAX). Styrene was determined in sliced potatoes with grated cheese and minced beef fried with tomato sauce (Jickells, S. M., 1993). 1-octene in pure beef fat (Umano, K., 1987) and menhaden fish oil (Horiuchi, M., 1998), and Limonene in cheese (Valero, E., 2001, Peres, C., 2002) or water (Linssen, J. P. H., 1993). 1-octene in ham was extracted using a 75 µm carboxen-poly (dimethylsiloxane) coating fiber (Andres, A. I., 2002) and Limonene in cheese with a 75 µm Carboxen/PMDS or PDMS/DVB (Peres, C., 2001). ATBC and DEHA migration was determined in fatty food extracting samples with diethyl ether in a Soxhlet apparatus and then using Tenax (Van Lierop, J. B. H., 1988).

ANALYSIS OF MODEL MIGRANTS. DETERMINATION

A large variety of chromatographic methods were found in the literature. The following revision was sorted by chromatographic technique and then by detection system.

HPLC

Most of analyses were performed on reversed phase, with C18 columns and using appropriate gradient and composition of mobile phase, mainly acetonitrile/water or alcohols/water, with or without modifiers. Some analyses were performed with normal phase on silica columns, and then mobile phases used were hexane (EU project G6RD-

CT200-00411), hexane / 2-propanol / dichloromethane (Mountfort, K. A., 1997), dichloromethane/hexane (Nerin, C., 1996).

Fluorescence detection:

Different wavelengths of excitation (λex) and emission (λem) were involved. For Irganox 1076, a λex of 282 nm and a λem of 308 nm or 342 nm were used (Helmroth, I. E., 2002, O'Brien, A. P., 1997, EU project G6RD-CT200-00411). For DIPN the λex was of 232 nm and λem of 338 nm (Summerfierld, W., 2001). For BHT were selected a ex. of 280 nm and λem of 310 nm, followed by a GC/MS confirmation (Yankah, V., 1998). For Bisphenol A several wavelengths were selected, λex of 275 nm and λem of 300 nm (Simal Gandara, J., 1993, Kang, J., 2002), λex of 224 nm and λem of 310 nm (Munguia-Lopez, 2001), λex of 235 nm and λem of 317 nm (Howe, S. R., 1998) or λex of 285 nm and λem of 300 nm (Mountford, 1997). For DPBD a λex of 347 nm and λem of 375 nm were chosen (EU project G6RD-CT200-00411).

Our preliminary experiments (respecting to λex and λem selected) were basically in agreement with the wavelengths chosen in the case of DPBD, and Bisphenol A. However, Irganox 1076 and BHT did not show a good sensibility in the fluorescence spectrometer.

Ultraviolet detection:

Irganox 1076 was measured at 275 nm (O'Brien, A. P., 1997, Marque, D., 1998) or 230 nm (EU project G6RD-CT200-00411, Vargo, 1985). This compound had showed a good response in the UV. Chimassorb 81 was determined at 330 nm or 290/280 nm (Nerin, C., 1996; Syropoulos, D. V., 1998, EU project G6RD-CT200-00411, Salafranca, J., 1999). For Uvitex OB wavelengths of 330 or 374 nm were used (EU project G6RD-CT200-00411). Ours results showed in this case that fluorescence response was better and more selectively than UV response. DEHA, after its analysis by GC/FID was confirmed at λ of 209/211 nm (Page, B. D., 1995). Caprolactam was determined at 220/230 nm (Barky, C. T., 1993) or 210 nm (Bonifaci, L., 1991; Ulsaker, G. A., 1992; EU project G6RD-CT200-00411), and Laurolactam at 207 nm (Stoffers, N. H., 2003). For BHT a λ of 280 nm was selected (Dieffenbacher, D., 1998). Bisphenol A was analysed at 228 nm (Howe, S. R., 1998, Yoshida, T., 2001).

Mass detection:

It was employed to determine Chimassorb 81, BHT, Irganox 1076 and other additives in acetonitrile extracts by methane chemical ionisation (Vargo, J. D., 1985). Caprolactam was qualitatively determined by FAB ionisation (positive) (Barky, C. T., 1993) and Laurolactam (with oligomers) by APcI (Stoffers, N. H., 2003).

Experiments carried out in our laboratory using the APcI in both negative and positive mode demonstrated that HPLC-MS can be a very effective technique for the determination of those compounds that presents low response by UV or fluorescence.

Refractive index detection: Caprolactam (Bonifaci, L., 1991) was determined using this detector.

Gas Chromatography

Most of analyses were carried out on apolar columns, type 5% phenyl-95%dimethylpoysiloxane, with a length of 15 to 30 m, and a beta phase of intermediate values, although in some cases others kinds of columns were used. Split/splitless injection is the widely used technique.

Flame Ionisation Detection:

To determine Irganox 1076 a high temperature is required. Oven program reached 310ºC (Helmroth, I. E., 2002), or 350º (Garde, J. A., 1998; Simoneau, C., 1999). For DEHA the ramp reached 350ºC (Simoneau, C., 1999), 360ºC (O'Brien, A. P., 1997), 295ºC (Page, B. D., 1995) or 260ºC (Cano, J. M., 2002) and for Caprolactam the temperatures achieved were 240ºC or 280ºC (EU project G6RD-CT200-00411) depending on the column used. DIPN was determined using an oven temperature from 40 to 200 ºC (Marianni, M. B., 1999) or from 60 to 270 ºC (Triantafyllou, V. I., 2002) followed in both cases by confirmation by GC-MS. The analysis of ATBC was conducted using an oven program that

reached 260ºC (Cano, J. M., 2002) while Laurolactam ramp reached 170ºC and 240ºC (Stoffers, N. H., 2003).

The oven program for separation of volatile compounds started obviously with lower temperatures. For Styrene the ramp was from 50 ºC to 150 ºC (Silva, F. C., 2000), for Limonene was from 50ºC to 250ºC (Linssen, J. P. H., 1993) while for 1-octene the oven program was from 40 to 200 ºC (Umano, K., 1987).

Mass Detection:

Chimassorb 81 was determined by single ion monitorizing (SIM) at 213(m/z) and using an oven program from 100 to 280ºC (Monteiro, M., 1998). DEHA was determined at 129 (m/z) with an oven temperature of 220ºC (Startin, J. R., 1987) or a ramp from 100 to 300ºC (Oi-Wah, L., 1996). ATBC was determined by on column injection and using a ramp from 70 to 210 ºC at 185 (m/z) (Castle, L., 1988), another author used an oven program from 60 to 300 ºC in SIM mode (Van Lierop, J. B. H., 1988). Benzophenone was determined with a ramp temperature from 50 to 250 ºC in full scan mode (Papillound, S., 2002). BHT was separated using a ramp temperature from 100 to 290 ºC in SIM mode at 205 (m/z) (Wessling, C., 1998) or with an oven program from 40 to 250 ºC in full scan mode (Tombesi, N., 2002).

Headspace GC/MS was used to determine Styrene at 104/112 m/z and using an oven temperature of 35ºC (Jickells, S.M., 1993), for 1-octene, after SPME extraction, the separation was performed with a temperature gradient from 40 to 200 ºC by full scan (Andres A. I., 2002). A similar oven program was used by other authors (Horiuchi M., 1998). To determine Limonene similar oven program from 40 to 250ºC in full scan mode was used (Bentivenga, G., 2001; Avison, S. J., 2001; Peres, C., 2001; Valero, E., 2001; Peres, C., 2002; Jordan, M. J., 2002).

This detector was also used when confirmation was required for Caprolactam (Ulsaker, G. A., 1992), Styrene (Silva, F. C., 2000), DIPN (Summerfierld, W., 2001, Boccaci, M., 1999, Triantafyllou, V. I., 2002), BHT (Yankah, V., 1998), Bisphenol A (Munguia-Lopez, E., 2001) and for 1-octene (Umano, K., 1987) after their determination by other techniques.

Other techniques as Supercritical Fluid Chromatography or Thin Layer Chromatography were used to determine Irganox 1076 (Doehl, J., 1987) and Uvitex OB (Lawrence, A. H., 1980) respectively.

GENERAL COMMENTS ON TABLE 4

Irganox 1076, Chimassorb 81 and Uvitex OB have a very low solubility in water (<0.03 mg/L), therefore it can be expected that migration to non fatty food, especially aqueous food, also will be very low. Both extraction and concentration steps are necessary, extraction with weakly or non polar solvents seems better option than polar solvents, in which they are not very soluble.

Although data on fat solubility are not available, the migration is expected much higher than into water. In this case, the better way seems the extraction with weakly or non polar solvents, followed by a GPC-SEC clean-up step to separate bulk lipids and then a concentration step. The extraction with polar solvents immiscible with fat seems more difficult, because it will involve a high number of extraction steps and/or big extraction volumes, although laborious it can be a simpler way for analytic laboratories

Regarding the determination, the HPLC-fluorescence combination is the best for Uvitex OB and HPLC-(UV or APcI(+)) and GC-(FID or MS) are possible for the three compounds.

Diphenylbutadiene, benzophenone, diphenylphthalate, bisphenol A, BHT and triclosan can be extracted from foods with polar solvents immiscible with fat, although DPBD and BHT with some difficulty. All the migrants exhibit good properties in the ultraviolet region and furthermore DPBD and Bisphenol A show very good response in fluorescence. Therefore HPLC with UV-FI detection seems an appropriate determination technique. Except for diphenylbutadiene, HPLC coupled with APcI-MS detector can be also used. GC-(FID or MS) is also a common possible technique for most of them.

Caprolactam and triacetin are two compounds soluble in water and not very soluble in oils. They can be extracted from foods with polar solvents immiscible with fat. They can be determined by GC-(FID or MS) or by HPLC only with an APcI (+)-MS detector because they do not show interesting properties in fluorescence or ultraviolet region.

DEHA, laurolactam and ATBC have low solubility in water and they present a problematic similar to the first group. They do not show interesting properties in UV or fluorescence and they need to be determined by GC-(FID or MS) or by HPLC only with an APcI-MS detector.

Styrene, 1-octene and limonene can be separated from food matrix by volatilization or distillation and it seems the better way to remove them from other food components. Analysis by Static or dynamic headspace-GC (FID or MS) is an excellent and common determination for the three compounds. Other ways also are possible as extraction from non fatty foods with weakly polar solvents immiscible with water, or using polar solvents immiscible with fat to extract from fatty foods. Styrene shows a very good response by fluorescence and ultraviolet and can be determined by HPLC using those detectors.

For all migrants and depending of each combination analyte/food matrix, more purification steps could be necessary, usually using SPE or liquid-liquid extraction, again. But obviously this step only can be discussed with specific problems and it is out of this compilation.

ANNEX I

EXPERIMENTAL PROCEDURE

In order to get more information about interesting properties of model migrants migrants, different test were performed.

Standard solutions

Stock solutions of all migrants were prepared in ethanol at a concentration of 1000 mg L^{-1}. Intermediate dissolutions were prepared at levels of 10, 1 and 0.1 mg L^{-1}.All these solutions were stored in the darkness at 4ºC.

Apparatus and Conditions

Fluorescence Spectrometer:

Spectrofluorimetric measurements were performed with a Perkin Elmer LS 50 spectrometer fitted with a xenon flash lamp, Monk Gillieson monochromators and 1 cm quartz cuvettes. Spectral data acquisition and processing were carried out by means of the program FL Winlab on a PC serially interfaced to the LS 50.

Firstly, pre-scan was achieved for all migrants. Scans were recorded between 200 and 800 nm for excitation and 200 and 900 nm for emission. The scan speed was 240 nm/min.

Subsequently maximum values for excitation wavelengths were selected, only for those compounds that showed fluorescence, and emission scans were completed. In the same way, maximum values for emission wavelengths were selected and excitation scans were completed.

For all compounds solutions of 10 mg/L were used exceptf for diphenylbutadiene, styrene and bisphenol A, where solutions of 0.1 mg/L were used. Otherwise, for Uvitex OB a solution of 0.01 mg/L was used.

UV-Visible Spectrophotometer:

A Cary 3E UV-Visible double-beam spectrophotometer was used to perform single scans of all solutions from 200 to 400nm. Software Cary WinUV was used for the acquisition of the data. For all compounds solutions of 10 mg/L were used except for diphenylbutadiene where a solution of 1 mg/L was utilized.

MS Detector:

APcI (negative and positive mode) spectra were recorded on a VG Platform (Fisons Instruments) single-quadrupole spectrometer, which was coupled via its APcI interface to a Spectra Physics model P200 HPLC gradient pump. The Masslynx ^{TM} data system was used to control the system.

Total ion chromatograms and selected-ion chromatograms of the analytes were obtained by direct injection in flow, using a 50 μL loop, the mobile phase was 70:30 acetonitrile:water at a flow rate: 1mL min^{-1}. Injections were monitored using real time chromatogram updates. Full scan spectra (m/z 70-700) were acquired every second, with a scan delay of 0.10 sec. In SIR mode, the dwell time of each ion was set at 0.10 sec.

The following instrument parameters were applied: probe temperature: 450 ºC and 250 ºC, ionisation source temperature: 130 ºC, cone voltage: ±30 V, electron multiplier voltage: 700 V, sheath gas nitrogen at 200 L/h and drying gas nitrogen at 250 L/h.

GC-FID:

Initial experiments were performed with a Fisons 8000 series gas chromatograph equipped with a flame ion detector (FID; 260ºC), an auto sampler (AS 800) and a split-splitless injector. The column used was a 30m × 0.32 mm i.d. × 0.25 μm film thickness Supelcowax 10 from Supelco. To improve the column life a 50 cm pre-column (deactivated

fused silica 0.32mm i.d.) was used. Helium was used as carrier gas flowing at 1 mL min^{-1}. The injector temperature used was 280 ºC and the split ratio selected was 1:30.

In a first test the column temperature was held at 40ºC for 2 min. during injection, and then increased at 7ºC/min. to 260 ºC, which was held for 10 min. In a second test the column temperature was held at 150ºC for 2 min., and then increased at 5ºC/min. to 260ºC, which was held for 20 min.

Solutions of 1000 mg/L were injected using these conditions.

HPLC-UV-Fluorescence:

Standard solutions of 10 mg/L of each migrant were prepared in acetonitrile. Then to 10 g of olive oil 10 mL of each standard solution were added and shaken vigorously for three minutes in a centrifuge tube of 40 mL. After phase separation, 0.1 mL of acetonitrile phase was filtered and injected. Areas obtained were compared with the areas obtained for the individual standards.

Chromatographic conditions were:

Column: A Kromasil 100 C18 column (5µm i.d., 150mm x 40mm).
The mobile phase consisted of acetonitrile/water 75:35 (v/v) in an isocratic mode for two minutes, followed by a gradient to 100% acetonitrile for eight min, and finally an isocratic elution during twenty min. The flow rate was 1 mL min^{-1}. The injection volume was 50 µL. The column oven temperature was kept at room temperature. UV detection was performed with 205, 225 and 255 nm, and fluorescence detection was performed with excitation and emission wavelengths of 250 nm and 306 nm, respectively.

Bibliographic references

Andrés A. I., Cava R., Ruiz J., 2002, Monitoring volatile compounds during dry-cured ham ripening by solid-phase microextraction coupled to a new direct-extraction device. J. Chromatogr. A, 963, 83-88.

Avison S. J., Gray D. A., Davison G. M., Taylor A. J., 2001, Infusion of volatile flavour compounds in to low-density polyethylene. J. Agric. Food Chem., 49, 270-275.

Barky C. T., Lawson G., 1993, Analysis of migrants from nylon 6 packaging films into boiling water. Food Addit.Contam. 10, 5, 541-553.

Bentivenga G., D´Auria M., De Luca E., De Bona A., Mauriello G., 2001, The use of SPME-GC-MS in the analysis of flavour of virgin olive oil. La Rivista Italiana delle Sostanze Grasse, 78, 157-162.

Bonifaci L., Frezzotti D. Cavalca G., Malaguti E., Ravanetti G. P., 1991, Analysis of caprolactam and its cyclic oligomers by high –performance liquid chromatographic. J. Chromatogr. 585, 333-336.

Boccacci, M., Chiaccherini E., Gesumundo C., 1999, Potential migration of diisopropyl naphthalenes from recycled paperboard packaging into dry foods. Food Addit.Contam, 16, 5, 207-213.

Cano J. M., Marín M. L., Sánchez A., Hernandis V., 2002, Determination of adipate plasticizers in poly (vinyl chloride) by microwave-assisted extraction. J. Chromatogr. A, 963, 401-409.

Castle L., Gilbert J., Jickells S. M., Gramshaw J. W., 1988, Analysis of the plasticiser acetyltributyl citrate in foods by stable isotope dilution gas chromatography-mass spectrometry. J. Chromatogr, 437, 281-286.

Dieffenbacher A., 1998, Determination of phenolic antioxidants in foods. Deut Lebensm-Rundsch., 11, 381-385.

Doehl J., Farbrot A., Greibrokk T., Iversen B., 1987, Supercritical fluid chromatographic separation of low to medium-polarity compounds that are difficult to elute from packed columns. J. Chromatogr, 392, 175-184.

Feigenbaum A., Scholler D., Bouquant J., Brigot G., Ferrier D., Franz R., Lillemark R., Riquet A.M., Petersen J.H., Van Lierop B., Yagoubi N., 2002, Safety and quality of food contact materials. Part I: Evaluation of analytical strategies to introduce migration testing into good manufacturing practice. Food Addit.Contam, 19, 2, 184-201.

Garde J., Catalá R., Gavara R., 1998, Analysis f antioxidants extracted from polypropylene by supercritical fluid extraction. Food Addit.Contam., 15, 6, 701-708.

Garde J., Catalá R., Gavara R., Hernadez R. J., 2001, Characterizing the migration of antioxidants from polypropylenene into fatty foods simulants. Food Addit.Contam., 18, 8, 750-762.

Helmroth I.E., Dekker M., Hankemeier T., 2002, Influence of solvent absorption on the migration of Irganox 1076 from LDPE. Food Addit.Contam., 19, 2, 176-183.

Health Canada. Tobacco control programme. 1999, Determination of triacetin in whole tobacco (http://www.hc-sc.gc.ca/hecs-sesc/tabac/pdf/T-311e4.PDF)

Horiuchi M., Umano K., Shibamoto T., 1998, Analysis of volatile compounds formed from fish oil heated with cysteine and trimethylamine oxide. J. Agric. Food Chem., 46, 5232-5237.

Howe S. R., Borodinsky L., 1998, Potential exposure to bisphenol A from food-contact use of polycarbonate resins. Food Addit.Contam, 15, 3, 370-375.

Jickells Sue M., Gancedo P., Nerin C., Castle L., Gilbert J., 1993, Migration of styrene monomer from thermoset polyester cookware into foods during high temperature applications. Food Addit.Contam, 10, 5, 567-573.

Jordan M. J., Margaría C. A., Shaw P. E., Goodner K. L., 2002, Aroma actives components in aqueous kiwi fruit essence and kiwi fruit puree by GC-MS and multidimensional GC/GC-O. J. Agric. Food Chem., 50, 5386-5390.

Kang J. H., Kondo F., 2002, Bisphenol A migration from cans containing coffee and caffeine. Food Addit.Contam., V19, 9, 886-890.

Lawrence A. H., Ducharme D., 1980, Simple rapid thin-layer chromatographic method for the detection of optical brighteners in polymers. J. Chromatogr, 194, 434-436.

Linssen J. P. H., Janssens J. L. G., Roozen J. P., Posthumus M. A., 1993, Combined gas chromatography and sniffing port analysis of volatile compounds of mineral water packed in polyethylene laminated packages. Food Chem., 46, 367-371.

Marque D., Feigenbaum A., Dainelli D., Riquet A. M., 1998, Safety evaluation of an ionized multilayer plastic film used for cooking and meet preservation. Food Addit.Contam., 15, 7, 861-841.

Monteiro M., Nerín C., Rubio C., Reyes F. G. R., 1998, A GC /MS method for determining UV stabilizers in polyethyleneterephthalate bottles. J. High Resol. Chromatogr., V21, 317-320.

Mountfort K. A., Kelly J., Jickells S. M., Castle L., 1997, Investigations into the potential degradation of polycarbonate baby bottles during sterilization with consequent release f bisphenol A. Food Addit.Contam, V14, 6-7, 737-740.

Munguia-López E. M., Soto-Valdez H., 2001, Effect of heat processing and storage time on migration on Bisphenol A (BPA) and bisphenol A-Diglycidyl ether (BADGE) to aqueous food simulant from mexican can coatings. J. Agric. Food Chem., 49, 3666-3671.

Nerín C., Salafranca J., Cacho J., 1996, Behaviour of Chimassorb 81 in the recycling process of agricultural films used as oil covers. Food Addit.Contam, 13, 2, 243-250.

O´Brien A. P., Cooper I., Tice P. A., 1997, Correlation of specific migration (Cf) of plastics additives with their initial concentration in the polymer (Cp). Food Addit.Contam., 14, 6, 705-719.

Oi-Wah L., Siu-Kay W., 1996, Determination of plasticisers in food by gas chromatography-mass spectrometry with ion-trap mass detection. J. Chromatogr. A, 737, 338-342.

Oliver J., Palou A., 2000, Chromatographic determination of carotenoids in foods. J. Chromatogr. A, 881, 543-555.

Page, B. D., Lacroix G. M., 1995, The occurrence of phthalathe ester and di-2-ethylhexyl adipate plasticizers in Canadian packaging and food sampled in 1985-1989: a survey. Food Addit.Contam., 12, 1, 129-151.

Papilloud S., Baudraz D., 2002, Analysis of food packaging UV inks for chemicals with potential to migrate into food simulants. Food Addit.Contam., 19, 2, 168-175.

Pérez C., Denoyer C., Tournayre P., Berdagué J. L., 2002, Fast characterization of cheeses by dynamic headspace-mass spectrometry. Anal. Chem., 74, 6, 1386-1392.

Pérez C., Viallon C., Berdagué J. L., 2001, Solid-phase microextration-mass spectrometry: A new approach to the rapid characterization of cheeses. Anal. Chem., 73, 6, 1030-1036.

Salafranca J., Cacho J., Nerín C., 1999, Supercritical fluid extraction (SFE) optimization by full-factorial design for the determination of Irganox 1076, Irgafos 168, and Chimassorb 81 in virgin and recycled polyolefins. J. High Resol. Chromatogr., 22, 553-558.

Silva C. F., De Carvalho R. C., Cardeal Z., 2000, Solid-phase microextraction method the quantitative analysis of styrene in water. J. Chromatogr. Sci., 38, 315-318.

Simal Gándara J., Paz Abuín S., Paseiro Losada P., Simal Lozano J., 1993, Determination of bisphenols A and F in noncured epoxy resins by RP-HPLC-Fluorescence Techniques. J. Chromatogr Sci, 31, 450-454.

Simoneau C., Hannaert P., Stabilising testing of selected plastics additives for food contact in EU aqueous, fatty and alternative simulants. Food Addit.Contam, 16, 5, 197-206.

Spyropoulos D. V., 1998, Stabilising testing of the plastics additives 2,4-dihydroxy-benzophenone, 2,2´-dihidroxy-4-methoxybenzophenone, 2-hidroxy-4-n-octyloxybenzophenone, 2-ethoxy-2- ethyloxanilide (tinuvin312) and 2,2´-methylenebis (4-methyl-t-tert-butyl phenol) in aqueous and fatty food simulants. Food Addit.Contam., 15, 3, 362-369.

Startin J. R., Parker I., Sharman R., Gilbert J., 1987, Analysis of di-(2-ethylhexil) adipate platiciser in foods by stable isotope dilution gas chromatography-mass spectrometry. J. Chromatogr, 387, 509-514.

Stoffers N. H., Brandl F., Linssen J. P. H., Franz R., 2003, Development and validation of analytical methods for monomeric and oligomeric migrants from nylon 12 packaging materials. Food Addit.Contam., 20, 4, 410-416.

Summerfield W., Cooper I., 2001, Investigation of migration from paper and board into food-development of methods for rapid testing. Food Addit.Contam., 18, 1, 77-88.

Tombesi N. B., Freije H., 2002, Application of solid-phase microextraction combined with gas chromatography-mass spectrometry to the determination of butylated hydroxytoluene in bottled drinking water. J. Chromatogr. A, 963, 179-183.

Triantafyllou V. I., Akrida-Demertzi K., Demerzis P. G., 2002, Migration studies from recycled paper packaging materials: development of an analytical method for rapid testing. Anal. Chim. Acta, 467, 253-260.

Ulsaker G. A., Teien G., 1992, Identification of caprolactam as a potential contaminant in parenteral solutions stored in over wrapped PVC bags. J. Pharmaceut. Biomed. 10, 1, 77-80.

Umano K., Shibamoto T., 1987, Analysis of headspace volatiles from overheated beef fat. J. Agric. Food Chem., 35, 1, 14-18.

Valero E., Sanz J., Martínez-Castro I., 2001, Direct thermal desorption in the analysis of cheese volatiles by gas chromatography and gas chromatography-mass spectrometry: comparison with simultaneous distillation-extraction and dynamic headspace. J. Chromatogr. Sci, V39, 222-228.

Van Lierop J. B. H., Van Veen R. M., 1988, Determination of plasticizers in fat by gas chromatography-mass spectrometry. J. Chromatogr, 447, 230-233.

Vargo J. D., Olson K. L., 1985, Identification of antioxidant and ultraviolet light stabilizing additives in plastics by liquid chromatography/mass spectrometry. Anal. Chem., 57, 672-675.

Wessling C., Nielsen T., Leufvén A., Jägerstad M., 1998, Mobility of alfa-tocopherol and BHT in LDPE in contact with fatty food simulants. Food Addit.Contam., 15, 6, 709-715.

Yankah V. Y., Ushio H., Ohshima T., Koizumi C., 1998, Quantitative determination of butylated hydroxyanisole, butylated hydroxytoluene, and tert-butyl hydroquinone in oils, foods, and biological fluids by high-performance liquid chromatography with fluorometric detection. Lipids, 33, 11, 1139-1145.

Yoshida T., Horie M., Hoshino Y., Nakazawa H., 2001, Determination of bisphenol A in canned vegetables and fruit by high performance liquid chromatography. Food Addit.Contam., 18, 1, 69-75.

Certified Reference Materials for the Specific Migration testing of plastics for food packaging needed by industry and enforcement laboratories (Specific Migration); Project No: G6RD-CT200-00411; Project Coordination: Angela Stormer (Fraunhofer IVV, Freising, Germany)

Development of methods of Analysis for Monomers and other starting substances with SML and/or QM Limits in Directives 90/128/EEC and 92/39/EEC (Monomers); Project No: MAT1-CT92-0006; Project Coordination: R. Franz (Franhofer-Institut, Freising, Germany) and R. Rijk (TNO, Zeist,)

Table 1: Chemical and physical information of the model migrants

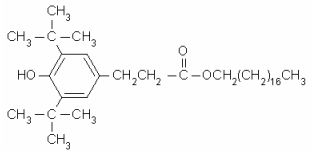
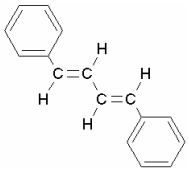
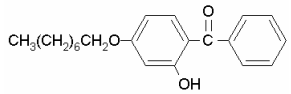
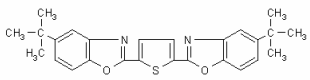
No	MIGRANTS	Ref. Nº/ CAS Nº	Formula	Chemical Name	MW	mp (ºC)	bp/Fp (ºC)	Water solubility mg/L	Solubility	Application	Structure	Spectral Data
1	**Irganox 1076** SML = 6 mg/kg	68320 2082-79-3	C35H62O3	Benzenepropanoic acid-3,5-bis(1,1-dimethylethyl)-4-hydroxyoctadecyl ester	531	50-55	-/273	< 0.03	g/100g solution: Acetone: 19 Benzene: 57 Chloroform: 57 Ciclohexane: 40 Ethanol: 1.5 Ethylacetate: 38 n-hexane: 32 Methanol: 0.6 Toluene: 50 Water: <0.01	Antioxidant and thermal stabilizer blend		
2	**DPBD**	- 538-81-8	C16H14	Diphenylbutadiene	206	153	350/-	0.5-1.2	Very soluble in: Benzene, ethanol and ether	Fluorescent additive		**UV** max: 319 nm (cyhex) FL, Ex: 320, Em max: 375 (hex)
3	**Chimassorb 81** SML = 6 mg/kg	61600 1843-05-6	C21H26O3	2-Hydroxy-4-n-octyloxy benzophenone	326	48-49	>400/>200	< 0.03	g/100mL solution: Acetone: 43 Benzene: 72 Chloroform: 61 Ethanol: 3.5 Ethyl acetate: 44 n-Hexane: 12 Methanol: 1.7 MEK: 65 Methylene chloride 67 Toluene: >50 Water: <0.01	UV absorber /stabiliser		**UV** max: 290, 243 nm (ethanol) **MS** peaks: 213(100) 214(39) 137(38) 105(24) 326(19) 325(14) 43(11) 77(10) 41(10) 215(7)
4	**Uvitex OB** SML = 0.6 mg/kg	38560 7128-64-5	C26H26N2O2S	2,5-Bis(5-tert-butyl-2-benzoxazolyl) thiophene	431	196-203	-/ >350	< 0.03	g/100mL solution: Acetone: 0.2 Dioxane: 2 DMF: 0.8 Ethanol: 0.1 Ethyl acetate: 1 n-Hexane: 12 Methanol: 0.05 MEK: 1 Toluene: 5 THF: 5 Xylene: 5	UV stabiliser Optical brightener, fluorescent white agent		**FL**, Ex max: 375, Em max: 435 nm

Table 1: Chemical and physical information of the model migrants

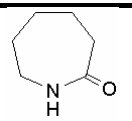
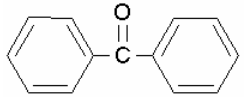
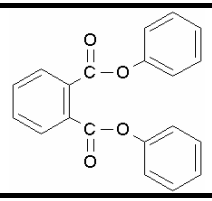
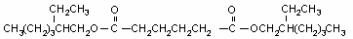
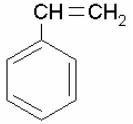
No	MIGRANTS	Ref. Nº/ CAS Nº	Formula	Chemical Name	MW	mp (ºC)	bp/Fp (ºC)	Water solubility mg/L	Solubility	Application	Structure	Spectral Data
									Water: <0.01			
5	**ε-Caprolactam** SML = 15 mg/kg	14200 105-60-2	C6H11NO	Aza-2-cycloheptanone	113	70-72	267/125	>700000	Soluble in H_2O, ethanol, benzene, chloroform, cyclohexane, acetone and DMSO.	Monomer		**MS** Peaks: 55(100) 113(87) 30(81) 56(66) 84(60) 85(57) 42(51) 41(33) **UV** max: 198 nm (H_2O)
6	**Benzophenone** SML = 0.6 mg/kg	38240 119-61-9	C13H10O	Diphenylmetanone	182	48	305/-	137	H_2O: 1; EtOH: 4; eth: 4; ace: 4; bz:3; chl: 4; HOAC:4; MeOH: 3; CS_2: 4	Light stabiliser		**MS** Peaks: 105(100) 77(49) 182(32) **UV** max: 33, 252nm (MeOH)
7	**Diphenyl Phthalate**	84-62-8	C20H14O4	1,2-Benzenedicarbosylic acid, dipheyl ester	318	73	405/224	0.08	H_2O: 1; EtOH: 2; eth: 2; ctc:2	Plasticizer		**MS** Peaks: 94(100) 28(83) 225(32) **UV** max: 225 nm (MeOH)
8	**DEHA** SML = 18 mg/kg	31920 103-23-1	C22H42O4	Adipic acid, bis(2-ethylhexyl) ester	371	< -70	417/-	0.78	Immiscible with water	Plasticizer		**MS** peaks: 129, 57, 112, 147
9	**Styrene**	24610 100-42-5	C8H8	Ethenylbenzene	104	-31	145-146/32	310	Slightly soluble in water. Soluble in alcohol, ether, acetone, and carbon disulfide.	Monomer for HIPS and GPPS		**UV** max: 289 281 272 246 nm (cyhex) **MS** Peaks: 104(100) 103(41) 78(32) 51(28) 77(23) 105(12) 50(12) 52(11)

Table 1: Chemical and physical information of the model migrants

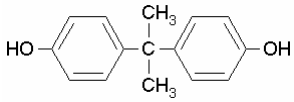
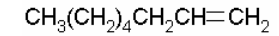
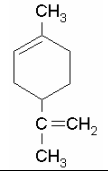
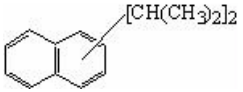
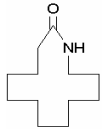
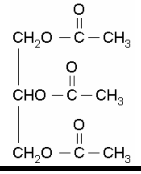
No	MIGRANTS	Ref. Nº/ CAS Nº	Formula	Chemical Name	MW	mp (ºC)	bp/Fp (ºC)	Water solubility mg/L	Solubility	Application	Structure	Spectral Data
10	**Bisphenol A** SML = 3 mg/kg	13480 80-05-7	C15H16O2	2,2'-Bis(4-hydroxyphenyl)propane	228	153	250/-	120	H_2O: 1; EtOH: 4; eth: 4; bz:4; alk: 4; HOAC:3	Monomers		**MS** Peaks: 213(100) 228(26) 119(25) **UV** max: 279, 227 nm (MeOH)
11	**1-Octene** SML = 15 mg/kg	22660 111-66-0	C8H16	1-octene	112	-101	121/-	4.1	H_2O: 1; EtOH: 5; eth: 3; ace: 3; bz:3; ctc: 2; chl: 4; os: 4	Monomers		**MS** Peaks: 43(100) 41(82) 55(80) 56(67) 70(54) 29(44) **UV** max: 177 nm (hp)
12	**Limonene**	63970 138-86-3	C10H16	1-methyl-4-(1-methylethenyl)cyclohexene	136	-95	178/-	7.6	H_2O: 1; EtOH: 5; eth: 5; ctc: 3			**MS** Peaks: 68(100) 67(64) 93(60) 39(58) 41(46) 79(41) 43(41) 27(40)
13	**DIPN**	38640-62-9	C16H20	Diisopropylnaphthlene	212			0.11		Employed in paper and board		
14	**Laurolactam** SML = 5 mg/kg	19490 947-04-6	C12H23NO	12-Aminododecanoic acid lactam Aza-2-cyclotridecanone	167	149-153		290	Water solubility: 290 mg/L	Monomer		**MS** Peaks: 30(100) 55(98) 41(96) 100(64) 98(64) 73(53) 86(51) 43(46) 72(45) 44(44)
15	**Triacetin**	57760 102-76-1	C9H14O6	1,2,3-Propanediol triacetate	218	-78	259//138	58000	H_2O: 2; EtOH: 5; eth: 5; ace: 4; bz:5; chl: 5; lig: 2; CS_2: 2	Antifungal agent		**MS** Peaks: 43(100) 103(44) 145(34)

Table 1: Chemical and physical information of the model migrants

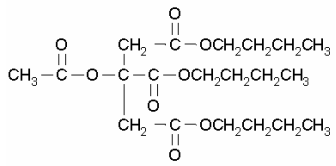
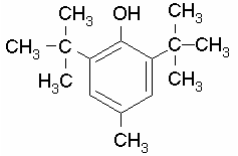
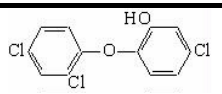
No	MIGRANTS	Ref. Nº/ CAS Nº	Formula	Chemical Name	MW	mp (ºC)	bp/Fp (ºC)	Water solubility mg/L	Solubility	Application	Structure	Spectral Data
16	**ATBC**	93760 77-90-7	C20H34O8	Tri-n-butylacetyl Citrate	402	>330	170/-	5	Soluble in ethanol, acetone, DMSO and Toluene Water solubility: 20 mg/L	Plasticiser		**MS** Peaks: 185(100) 129(57) 259(54) 43(54)
17	**BHT**	46640 128-37-0	C15H24O	2,6-Bis(1,1-dimethylethyl)-4-methylphenol	220	71	265/	0.6	H_2O: 1; EtOH: 3; ace: 3; bz:3; alk: 1; peth: 3	Antioxidant		**MS** Peaks: 205(100) 220(27) 57(27) **UV** max: 283, 277, 227 nm (Iso)
18	**Triclosan**	93930 3380-34-5	C12H7Cl3O2	2,4,4'-Trichloro-2'-hydroxydiphenyl ether	289	56-58	-/223	10	Soluble in acetone, methanol, benzene and isopropanol Water solubility: 17 mg/L	Bacteriostactic agent		

Table 2. Abstract of the revised literature on analytical methods of the model migrants

Substance	Sample preparation	Analysis conditions	References
Irganox 1076 Antioxidant	**Migration:** **1-** Polymer film (38.5 cm^2) was put into contact with 20 mL of solvent in a migration cell. **2-** Cells were incubated under gentle shaking (55 rpm) in a water-bath of 40ºC. Solvents used: - ethanol - isooctane - ethyl acetate - 2-propanol - ciclohexane - dichloromethane - olive oil - tricaprylin (octanoic acid, 1,2,3-propanetriyl ester) - tributyrin (butanoic acid, 1,2,3-propanetriyl ester) **3-a)** 40µL of sample were added to 5µL of internal standard solution (1mg/mL in 2-propanol). And then analysed by **GC-FID** **b)** With triglycerides (tributyrin, tricaprylin and olive oil), 40µL of sample was added to 5µL of internal standard and then diluted with 160µL of acetone and analysed by **HPLC.** **Analysis of aqueous simulants:** Simulants (3% w/v acetic acid; 15% v/v ethanol) were analysed directly. Calibration solutions range from 0.5 to 20 µg/ mL	**GC-FID** **Equipment:** Fisons GC 8000 series **Column:** DB5-MS, 15 m x 0.25 mm i.d., 0.1µm thickness **Retention gap:** 0.5m x 0.53 mm i.d. deactivated with a thin film of OV-1701-OH. **Carrier gas:** helium **Flow rate:** 1.8 mL/min **Injection mode:** on column **Injected volume**: 1µL **Oven program:** started 1ªC under the boiling point of the solvent, 15ºC/min to 150ºC, 10ºC/min to 310ºC, 1min at 310ºC. **Detector:** FID **Detector temperature:** 315ºC **HPLC-F** (for triglycerides analysis) **Equipment:** Waters Alliance 2690 with a Waters 474 fluorescence detector **Column:** Xterra RP18, 150mm x 4.6 mm i.d (particle size, 5µm) $\lambda_{excitation}$= 282 nm; $\lambda_{emisson}$= 308 nm **Volume injected:** 10µL **Mobile phase:** Linear concentration gradient water/acetonitrile (2/8 v/v) to water/acetonitrile (1/19 v/v); after 5 min, 100% acetonitrile. **HPLC** **Column:** Hypersil 5 ODS **Mobile phase:** tetrahydrofuran/ acetonitrile (20:80) **Flow rate:** 1.5 mL/min **Detector:** ultraviolet **Sample volume:** 20µL λ = 275 nm **Internal standard:** Irganox 1010	Helmroth, I.E**.** et al**,** **Food Additives and Contaminants**, 2002, 19 (2), 176-183. O'Brien, A.P. et al, **Food Additives and Contaminants,** 1997, 14 (6-7), 705-719.

Table 2. Abstract of the revised literature on analytical methods of the model migrants

	Analysis of olive oil: 2g of olive oil from migration study were diluted with acetone in a 10 mL flask. **Note**: **1-**Many means of extracting the additive from oil were investigated, including solvent extraction and solid phase extraction processes. None of these was found to be effective. **2-** Fluorescence was used because is more selective for the additive. **3-** The response for olive oil was considerably less in the fluorescence mode than for UV detection. **4-** It was not possible to quantify (adding an internal standard) because olive oil has a complex trace.	**HPLC** **Column:** Hypersil 5 ODS **Mobile phase:** 0 to 8 min- Prop-2-ol/Acetonitrile (25:75) 9 to 14 min- THF/Acetonitrile (50:50)- to clean up 15-20 min- Propan-2-ol/Acetonitrile (25:75)- column regeneration **Flow rate:** 1.5 mL/min **Detector:** fluorescence **Sample volume:** 20µL $\lambda_{excitation}$= 282 nm $\lambda_{emission}$= 308 nm **Sample dilution:** 20% in acetone	
	Specific migration in sunflower oil: **1-** Weigh 2g of sunflower oil in a 10mL volumetric flask; **2-** Add 100µL of internal standard (Irganox 1330- 1mg/mL); **3-** Make up to the mark with acetone and mix thoroughly. Inject in the HPLC.	**HPLC-FL** Column: **Phenomenex Sphereclone ODS2, 250 x 4.6 mm** **Mobile phase:** 100% propan-2-ol/acetonitrile (5:95), till 20 min; gradient program within 5 min to 100% acetonitrile/tetrahydrofuran (50:50); 5min with 100% acetonitrile/tetrahydrofuran (50:50); gradient program within 5 min to 100% propan-2-ol/acetonitrile (5:95); 5min with 100% propan-2-ol/acetonitrile (5:95). **Flow rate:** 1.5mL/min **Injected volume:** 20 µL $\lambda_{excitation}$ = 282 nm $\lambda_{excitation}$ = 342 nm **Repeatability** was 0.56 mg/dm^2 for a specific migration of 3.69 mg/dm^2. **Detection limit** = 6.7 mg/Kg	EU project- Specific Migration **G6RD-CT200-00411**

Table 2. Abstract of the revised literature on analytical methods of the model migrants

Irganox 1076 and others additives	Additives: -Irganox 1076 -Irgafos 168 Use 2 extraction procedures: 1-Ultrasonic extraction: (recommended for more volatile compounds) -**GC**: 300mg of polymer with 100mL CH_2CL_2 for 1 h -**HPLC**: 3.7 g of polymer with 50 mL CH_2CL_2 for up to 9 h. 2- Soxhlet extraction: 7.5g polymer with 100mL CH_2CL_2 for 9 h.	**HPLC** **Equipment:** Liquid Chromatograph 5000, Varian **Pre-Column:** C-18, 1cm Column: **Spherisorb ODS1, 350 mm x 4.6 mm, 5µm** **Mobile phase:** acetonitrile/water (75:25) **Flow rate:** 1 mL/min **Injected volume:** 10 µL λ = 275 nm **Quantification** by external standard **^{1}H-NMR** Extracts were evaporated to dryness and residues dissolved in deuterated chloroform ($CDCL_3$) containing tetramethylsilane (TMS) as internal standard **Equipment:** spectrometer AC250	Marque, D. et al, **Food Additives and Contaminants**, 1998, 15(7), 831-841
	Migration: **1-**Film samples (50 cm^2) were immersed in 50 mL of simulant in a cylindrical glass cells; **2-** After different contact periods the simulant was removed from the cells and evaporated to dryness under nitrogen; **3-** Residues were dissolved in 1mL of chloroform; Solvents used as fatty food simulants: - 95% ethanol - n-heptane	**GC** **Equipment.** Fisons 8340 **Column:** SPB5, 30 m x 0.25 mm x 0.25 µm thickness. **Injector temperature:** 320ºC **Oven program:** 1 min at 300ºC, ramp at 5ºC/min to 350ºC, 10 min at 350ºC **Detector:** FID **Detector temperature:** 350ºC **Quantification** by external standard **Note:** Irgafos 168 chromatograms showed two peaks, one corresponding to the product and the other to a degradation product. Fully degraded Irgafos 168 was obtained after 24h of dissolution in tetrahydrofuran (THF). In stabilized THF the reaction was very slow, suggesting an oxidation process.	Garde, J. A. et al, **Food Additives and Contaminants**, 2001, 18 (8), 750-762
	Determination in polyolefins: **1-** Cut the polymer into pieces (~0,2 cm^2). Weigh 0.25g into 40 mL vials.	**HPLC-UV** Column: **Hypersil ODS-2 C18 25 cm x 4.6 mm, 5µm** **Mobile phase:** ethanol/water (90:10)	EU project- Specific Migration **G6RD-CT200-00411**

Table 2. Abstract of the revised literature on analytical methods of the model migrants

	2- Add 0.1mL of a 500 mg/L Tinuvin 234 (internal standard) solution and 10mL of toluene to each vial. **3-** Heat the vials until the solvent is boiling vigorously and the polymer dissolves. **4-** Cool to about 60ºC and add 10mL MeOH, shake and let polymer reprecipitate **5-** Filter the liquid using a 0,2µm HPLC syringe filter to a vial (or) and inject in the HPLC. If solution is difficult to filter centrifuge for 2 min at 4000rpm and then filter.	**Flow rate:** 1 mL/min **Injected volume:** 10 µL **λ** = 230 nm $t_{r\ Irganox}$ **1076** = 9.3 min; $t_{r\ Irgafos}$ **168** = 10.5 min; $t_{r\ oxidized}$ **Irgafos phosphate 168** = 6.0 min; $t_{r\ Tinuvin}$ **234** = 5.3 min; Polypropylene: $LOD_{Irganox\ 1076}$ = 102 mg/Kg $LOD_{Irgafos}$ 168 = 32 mg/Kg $RSD_{\ Irganox\ 1076}$ = 1.2% $RSD_{\ Irgafos}$ 168 = 12% **LDPE:** $LOD_{Irganox\ 1076}$ = 232 mg/Kg $LOD_{Irgafos}$ 168 = 193 mg/Kg $RSD_{\ Irganox\ 1076}$ = 3% $RSD_{\ Irgafos}$ 168 = 5% HDPE: $LOD_{Irganox\ 1076}$ = 236 mg/Kg $LOD_{Irgafos}$ 168 = 156 mg/Kg $RSD_{\ Irganox\ 1076}$ = 6% $RSD_{\ Irgafos}$ 168 = 13%	
	Determination of the initial concentration of Irganox 1076, Irgafos 168, Chimassorb 81 and Uvitex OB in polyolefins **1-** Weigh 0.1g of the material (cut in small pieces) into a 40mL vial; **2-** Add 3mL toluene and a magnetic stirrer; **3-** Heat (100ºC) and stir the closed vial until the polymer is solved; **4-** Cool down (room temperature) and add 15mL methanol; **5-** After 15 min the precipitate is removed by filtration;	**HPLC-UV** Column: **EC 25/3 Nucleosil 100-5 C18** **Column temperature:** 30ºC **Mobile phase:** -Irganox 1076 and Irgafos 168 ethanol 95%/water (97:3), 0 min; gradient program within 10min to 100% ethanol 95%; 5min with 100% ethanol 95%; -Chimassorb 81 and Uvitex OB ethanol 95%/water (93:7), 0 min; gradient program within 6.5min to 100% ethanol 95%; till 10min with 100% ethanol 95%; **Flow rate:** 0.6 mL/min **Injected volume:** 20 µL $\lambda_{\ Irganox\ 1076\ and\ Irgafos\ 168}$ = 230 nm $\lambda_{\ Chimassorb}$ = 290 nm $\lambda_{\ Uvitex\ OB}$ = 374 nm (after Chimassorb peak is detected the wavelength is switched- 3.8-6.0min)	EU project- Specific Migration **G6RD-CT200-00411**

Table 2. Abstract of the revised literature on analytical methods of the model migrants

	6- Evaporate the solution to dryness on the rotatory evaporator and resolve the residue with 5mL methanol; **7-** Fill a vial with this solution and analyse by HPLC. **Migration** Additives 1: - Irganox 1076 - Irgafos 168 Additives 2: - Chimassorb 81 - Uvitex OB **Simulant:** - 95% ethanol Internal standards: -Tinuvin 234 (for Irganox 1076 and Irgafos 168)- 30µg/mL -Tinuvin 327 (for Chimassorb 81 and Uvitex OB)-30µg/mL **1-** Place a circular sample (d=10cm) of the polymer into the migration cell and close it with the clamp (contact area= 0.48dm^2; **2-** Add 50mL ethanol 95% through the screw closure in the migration cell. **3-** Store the cell at the defined time and temperature conditions; **4-** At each defined time fill 250µL of this solution into a 2mL vial and cool it down to room temperature; **5-** Prepare another 2 ml vial: fill with 50µL internal standard solution and 100µL of migration solution and mix carefully; **6-** Analyse by HPLC.	**HPLC-UV** Column: **EC 25/3 Nucleosil 100-5 C18** **Column temperature:** 30ºC **Mobile phase:** -Irganox 1076 and Irgafos 168 ethanol 95%/water (97:3), 0 min; gradient program within 10min to 100% ethanol 95%; 2min with 100% ethanol 95%; -Chimassorb 81 and Uvitex OB ethanol 95%/water (90:10), 0 min; gradient program within 10min to 100% ethanol 95%; till 12min with 100% ethanol 95%; **Flow rate:** 0.6 mL/min **Injected volume:** 20 µL $\lambda_{\text{Irganox 1076 and Irgafos 168}}$ = 230 nm $\lambda_{\text{Chimassorb and Uvitex OB}}$ = 340 nm $t_{r\ \text{Irganox 1076}}$= 7.0 min; $t_{r\ \text{Irgafos 168}}$= 9.0 min $t_{r\ \text{Chimassorb 81}}$= 3.8 min $t_{r\ \text{Uvitex OB}}$= 5.6 min	EU project- Specific Migration **G6RD-CT200-00411**

Table 2. Abstract of the revised literature on analytical methods of the model migrants

	Additives: - DEHA - DEHP (bis (2-ethylhexyl)phthalate) - Irganox 1076 Simulants: - 15% ethanol - 95% ethanol - 3% acetic acid Test conditions: Aqueous simulants: - 10 d at 40ºC - 1 h at reflux temperature (using condensers for reflux) Oil simulant**:** - 10 d at 40ºC - 1h at 175ºC Iso-octane simulant: - 2 d at 20ºC - 3 h at 60ºC **1- 95% ethanol** was extracted with hexane (1:1) and 10% of distilled water; **15% ethanol and 3% acetic acid** were extracted with hexane (1:1); **olive oil** was extracted equal volume of acetonitrile **2-** Mixtures were shaken manually for 1 min and sonicated for 15 min. **3-** Phases were left to separate (1h) **4-** Organic phase was taken to a GC vial for analysis. Samples exposed to isooctane were analysed directly.	**GC** **Equipment:** Hewlett Packard HP 6890 **Injection volume:** 1µL **Carrier gas:** Helium **Flow rate:** 33 cm/s Aqueous simulants: **Column:** HP5, 25 m x 0.25 mm x 0.25 µm thickness. **Injector temperature:** 250ºC **Injection mode:** split (1:1) for DEHA and DEHP splitless for Irganox 1076 **Oven program:** ramp at 30ºC/min from 100ºCto 350ºC, 5min of holding time for DEHA and DEHP and 10 min for Irganox 1076. **Detector:** FID **Detector temperature:** 300ºC Oil simulant**:** **Column:** DB5 HT, 25 m x 0.32 mm x 0.15 µm thickness. **Injector temperature:** 300ºC **Injection mode:** split (1:1) for DEHA and DEHP splitless for DEHP **Oven program:** ramp at 30ºC/min from 100ºC to 365ºC, 25min at 365ºC. **Detector:** FID **Detector temperature:** 365ºC	Simoneau, C.; Hannaert, P. **Food Additives and Contaminants**, 1999, 16 (5), 197-206

Table 2. Abstract of the revised literature on analytical methods of the model migrants

	Determination in polyolefins: <u>Additives:</u> - Irgafos 168 - Irganox 1076 - Chimassorb 81 <u>Polyolefins:</u> - LDPE - HDPE **Supercritical Fluid extraction (SFE)** **1-** About 2.5 g of ground/cut polymer were weighted in a 250mL flask; **2-** 100mL of toluene and 10µL of internal standard were added (Irganox 1010- 2mg/g in toluene); **3-** A Vigreux column was fitted, the flask covered with an aluminium foil, and heated under reflux with magnetic stirring (to avoid adhesion of polymer to the flask) until complete dissolution; **4-** Solution is cooled to about 50ºC and 125 mL of methanol added carefully via the upper part of the Vigreux column; **5-** Solution was hand shaken (vigorously) by 1 min, cooled in a ice bath and filtered under vacuum; **6-** After washed with 3 x 15 mL of methanol it was concentrated in a rotatory evaporator to about 1 ml;	**HPLC-UV** **Equipment:** Kontron Instruments Column: **Spherisorb ODS-2.5µm, 25 cm x 4.6 mm i.d.** Column temperature: **25ºC** **Mobile phase:** Ethyl acetate/methanol/water 50/38/12 (v/v/v) **Flow rate:** 1.5 mL/min **Injected volume:** 20 µL **System pressure:** 232 bar λ = 280 nm **Analysis time:** 15 min	Salafranca, J., Cacho, J. Nerín, C**., J. High Resol. Chromatogr.,** 1999, 22 (10), 553-558

Table 2. Abstract of the revised literature on analytical methods of the model migrants

	7- 5ml of dichloromethane were added and solution filtered by a PTFE syringe filter (0.2µm pore size); **8-** Solution was analyse by HPLC after being evaporated in a nitrogen stream. <u>Additives (6):</u> - Erucamide - Irganox 1076 - Tinuvin 327	**SFC(supercritical fluid chromatography)-FID** **Equipment:** HP 5790A **Column:** DB-1, methylsilicone, 20 m x 100µm i.d. x 0.4 µm thickness **Injector system:** Rheodyne 7520 injector **Detector:** FID **FID block:** 350ºC **Carrier gas**: a-carbon dioxide (present an increased capacity factor and the peak shape improved); b- nitrous oxide **Injection volume:** 0.2 µL **Injection mode:** split 1:32 **Pressure:** 207 bar **Oven temperature:** 80ºC **Note: 1-**Erucamide can not be detected by UV absorption; **2-**Erucamide was not eluted from two bonded phase columns: **Novapak** (150mm x 1.2 mm i.d., 4µm C_{18} silica) and **Brownlee** (30mm x 2.1 mm i.d., 5 µm cyano silica) with carbon dioxide or nitrous oxide as the mobile phases. While aromatic compounds like **Irganox 1076** and Tinuvin 327 were readily eluted. Since **amide, carboxy and amino** groups are the 3 substituents that most contribute to retention on silica adsorption systems. The retention is caused by active, unreacted silanol groups in the bonded phase material.	Doehl, J. et al **J. of Cromatography**, 1987, 392, 175-184
Diphenylbutadiene (DPBD)	***Migration:*** Simulant: - 95% ethanol	**HPLC-FLD** **Column:** Hypersil Phe-3 12,5 cm x 3 mm id 3µm particle **Mobile phase:** 100% Acetonitrile /water (50:50),0-0,1 min;	EU project- Specific Migration **G6RD-CT200-00411**

Table 2. Abstract of the revised literature on analytical methods of the model migrants

	1- 40 mL of 95% ethanol were put in contact with a 10 cm diameter circle of the film (LDPE) in a migration cell and stored at the relevant temperature; **2-** At 0, 2, 4, 8, 24 and 48 h was taken (after shaking the migration cell) a 250µL sample to a HPLC vial and added 50µL of internal standard stock solution (DPHT- trans-,trans-1,6-diphenyl1,3,5-hexatriene-100mg/L). Inject 20µL. **Determination of DPBD in LDPE:** **1-** Cut the polymer into pieces (~0,5cm^2). Weigh 1,0 g into three 40 mL vials; **2-** Add 1mL of a 2000 mg/L DPHT (1,6-diphenyl-1,3,5-hexatriene- internal standard) solution and 20mL of hexane to each vial. Store for 3h at 60ºC; **3-** Dilute 100µL of the extract to 10 mL with hexane and inject in the HPLC; Repeat extraction with the same polymer pieces.	50% Acetonitrile / water (50:50)/ 50% Acetonitrile, 5,0-5,1 min; 100% Acetonitrile / water (50:50), 5,1-10 min **Flow rate:** 1,0 mL/min **Injection volume:** 20µL **$\lambda_{excitation}$**= 347 nm**; $\lambda_{emission}$**=375 nm Quantification by internal standard method **t_r**= 3.655 min **Recovery**= 94% **Repeatability**= 1,7% (n=6) **Detection limit**= 0,6 mg/L ethanol **HPLC-FLD** **Column:** Sphereclone-Silica25cm x 4,6 mm id 5µm particle **Column temperature**:25º± 5ºC **Mobile phase:** 100% Hexane **Flow rate:** 0,5 mL/min **Injection volume:** 20µL **$\lambda_{excitation}$**= 346 nm**; $\lambda_{emission}$**=374 nm **$t_{r\ DPBD}$**= 10.6 min; **$t_{r\ DPHT}$**= 12.2 min **Detection limit** = 0.2 mg/kg polymer	EU project- Specific Migration **G6RD-CT200-00411**
Chimassorb 81 UV stabiliser	***Migration:*** *Simulants:* - 15% aq ethanol - 95% aq ethanol - 3% aq acetic acid - olive oil - isooctane Exposure conditions: - 10 d/40ºC - 1 h/100ºC - 30 min/150ºC - 1 h/175ºC - 2 h/20ºC - 3 h/60ºC	**HPLC-UV** **Equipment:** HP 1050, Ti series **Pre-column.** Hichrom **Column:** Nucleosil 120, C18, 250mm x 4.6 mm, 5µm **Injection volume:** 20µL **Mobile phase**: (1) Methanol /water (97:3), (2) Acetonitrile / water (95:5) or (3) Methanol / Water (90:10) **Flow rate.** (1) 1.2 mL/min; (2) 1.5 mL/min; (3) 1.2 mL/min **λ**= 330 nm tr (1)= 5.5 min tr(2)=5.4 min tr(3)=7.6 min	Spyropoulos, D.V. et al, **Food Additives and Contaminants**, 1998, 15 (3), 362-369

Table 2. Abstract of the revised literature on analytical methods of the model migrants

	1- Standard solutions were prepared in ethanol and spiked into simulants at the specific migration limit (6mg/kg for Chimassorb 81). Typical spiking level is 50 µL/25mL of simulant; **2-** Samples were subject to exposure conditions and were diluted 1.1 with THF before HPLC analysis. <u>For olive samples:</u> **1-** 5g of olive oil were weighed in 10 mL vials. 10 µL of standard solution was added; **2 a)-** samples were added to 5mL of methanol (for polar or semi-polar additives), shaken and methanol phase injected, but recovery was very poor; **b)-** samples diluted in 10mL of THF (for non-polar additives), and injected.	**Note:** The tests in 15% ethanol and 3% acetic acid at 10 d/40ºC were not conducted due to the insolubility of the substance. <u>For olive oil samples:</u> **Mobile phase:** Acetonitrile/water (85:15) , 0 to 6 min Acetonitrile/water (95:5), 7 to 10 min Acetonitrile 100%, 12 to 28 min Acetonitrile/water (85:15), 29 to 31 min **Flow rate:** 1.2 mL/min, 0 to 12 min; 2 mL/min, 2 mL/min, 12 to 28 min; 1.2 mL/min, 29 to 31 min. **λ**= 330 nm tr= 11.7 min	
	Extraction: **1-**1g of PET was added to 10 mL of internal standard (Tinuvin 327 30µg/mL) and 20mL of CH_2Cl_2; **2** After maceration (6h) and 1h in ultrasonic bath it was filtered and concentrated in a rotary evaporator; **3-** After filtration, a 5mL aliquot was added to the second internal standard (benzophenone) and adjusted to 10mL with dichloromethane; **4-** Analysis by GC/MS	**GC/MS** **Equipment:** H-P 5890 series II with a 5971-A mass selective detector **Column:** DB 1701 capillary column, 60 m x 0.25 mm i.d. x 0.25 µm thickness **Pre-column:** 2 m x 0.32 mm i.d. **Oven program.** 2 min at 100ºC, at 10ºC/min to 280ºC, 30 min at 280ºC **Carrier gas:** helium **Pressure:** 110kPa **Injection volume:** 1µL **Injector temperature:** 280ºC **Injection mode.** splitless, 0.5 min **Detector:** MS **Detector temperature:** 280ºC **Detector mode:** SIM **Solvent delay:** 9 min	Monteiro, M. et al**, J. High Resol. Chromatogr.,** 1998, 21, 317-320

Table 2. Abstract of the revised literature on analytical methods of the model migrants

		t_r = 38.24 ± 0.06 min **LOD** = 2.6 x 10^2 pg **RSD** =5.46% **Linear range.** 2.3-96.7 pg/ mL **Recovery** = 78.6%	
	Extraction from polymer: **1**-Polymer (0.25g) were added to 3mL of dichloromethane and 400µL of internal standard solution (2mg/mL Irganox 1010 in dichloromethane) in a glass tube; **2-** The tube was closed, shaken by hand for 30s and held in the dark for 6h (to avoid degradation by light); **3-** The tube was placed in the ultrasonic bath for 15 min. The extracted obtained was separated and ultrasonic separation repeated 3 times with 3 mL of dichloromethane; **4-** Extracts were combined and evaporated up to 1 mL Migration: <u>Simulants:</u> - water - acetic acid 3% - ethyl alcohol 15% - rectified olive oil <u>Exposure conditions:</u> 40ºC ±0.6ºC, 10 d on the dark **1-** 0.23g of polymer were immersed in 18 mL of simulant (ratio area/ volume was 6:1); **2a)-**Aqueous simulants were extracted 3 times with dichloromethane. After filtration they were concentrated under nitrogen stream up to 1 mL and analysed;	**HPLC** **Equipment:** Kontron Instruments liquid chromatograph **Column:** PL- Gel 50A, 300 mm x 7.5 mm i.d. **Pre-column:** 50 mm x 7 mm i.d. **Mobile phase:** Dichloromethane /hexane (45:55) **Flow rate:** 1.2 mL/min **Pressure:** 36 bar **Column temperature:** 35ºC **Injected volume:** 20 µL **λ**= 280 nm **Quantification** by internal standard	Nerin, C. et al, **Food Additives and Contaminants**, 1996, 13 (2), 243-250

Table 2. Abstract of the revised literature on analytical methods of the model migrants

	b)-Olive oil was diluted with dichloromethane and injected directly.		
Chimassorb and Uvitex OB	**Specific migration in sunflower oil:** **1-** Weigh 2g of sunflower oil in a 10mL volumetric flask; **2-** Add 100µL of internal standard (Tinuvin 326- 1mg/mL) **3-** Make up to the mark with acetone and mix thoroughly. Inject in the HPLC.	**HPLC-UV** Column **ODS, 250 x 4.6 mm** **Mobile phase:** 100% acetonitrile/water (95:5) then programmed to 100% tetrahydrofuran and held for 5 min. **Flow rate:** 1.5mL/min **Injected volume:** 20 µL **λ** = 330 nm **Repeatability** was 0.56 mg/dm^2 for a specific migration of 3.69 mg/dm^2. **Detection limit** = 6.7 mg/Kg	EU project- Specific Migration **G6RD-CT200-00411**
	Determination in HDPE and PP **1-** 0.5g of polymer (cut into 0.5cm^2) were weighted in 40mL vials; **2-** 0.5 mL of the internal standard (Tinuvin 326 – 500mg/L) and 10 mL of tetrahydrofuran were added ; **3-** After having vials caped they were stored for 24h at 60ºC; **4-** Liquid was put in a vial and a small quantity filtered using a 0.2µm HPLC syringe filter for HPLC analysis. Repeat the extraction with the same polymer pieces.	**HPLC-UV** Column: **Hypersil ODS-2 C18 25 cm x 4.6 mm id 5µm** **Column temperature:** 25ºC **Mobile phase:** acetonitrile containing 1% v/v acetic acid and water (95:5), held for 8 min, then programmed to 100% tetrahydrofuran, held for 5 min. **Flow rate:** 1.5 mL/min **Injected volume:** 20 µL **λ** = 330 nm $t_{r\ Chimassorb\ 81}$= 4.8 min $t_{r\ Tinuvin\ 326}$= 6.5 min $t_{r\ Uvitex\ OB}$= 8.0 min HDPE $RSD_{Chimassorb\ 81}$= 1.4% RSD_{Uvitex} OB= 0.6% PP $RSD_{Chimassorb\ 81}$= 3.2% $LOD_{Chimassorb\ 81}$ = 47 mg/kg LOD_{Uvitex} OB = 42 mg/kg	EU project- Specific Migration **G6RD-CT200-00411**

Table 2. Abstract of the revised literature on analytical methods of the model migrants

	Determination in polymers: Additives (9): - Chimassorb 81 - BHT - Irganox 1076 - Others **1-** 1g of plastic shavings was extracted overnight with 5 mL of acetonitrile (at room temperature and with constant stirring); **2-** Extracts were filtered prior to analysis.	**LC-UV-MS** **Equipment:** HPLC: Waters 6000a pumps, model 680 LC gradient controller; MS: Finnigan-MAT model 4615 quadrupole mass spectrometer; Column: **1/8 in. o.d. x 2.1 mm i.d. x 25 cm, 5µm ODS** **Mobile phase:** acetonitrile **Flow rate:** 0.2 mL/min **Injected volume:** 20 µL λ = 280 nm **Ion source pressure:** 0.3 torr **Ionisation:** methane chemical ionisation **Ionisation:** 70eV electrons **Scan range:** 50-800 **Scan speed:** 3s The absorbance detector outlet was connected with the LC/MS interface by a 20 cm length of 0.01 in. i.d. stainless steel tubing	Vargo, J.D., Olson, K. **Anal. Chem.** 1985, 57, 672-675
Uvitex OB UV stabilizer- improve the whiteness and impart excellent brightness to plastics (Lawrence, A. H., Ducharme, D., **Journal of Chromatography**, 194 (1980) 434-436)	Migration: Simulants. - water - 3% acetic acid - 10% ethanol Exposure conditions: - 48h at room temperature - 30 min at 120ºC - 10 d at 45ºC *The plastic contained 0.01-0.06% of Uvitex OV.* Extraction: Water, 2% Acetic acid or 5%NaCl does not extract Uvitex OB from polystyrene during 1 d at 20ºC. Uvitex OB was extracted with chloroform.		Ganesa, M., et al, **Khigiena i Zdraveopazvane,** 1980, 23 (5), 445-450.

Table 2. Abstract of the revised literature on analytical methods of the model migrants

		TLC **1-** One drop (1-2µL) of a solution of plastic films in chloroform (for Uvitex OB) was applied to the thin layer. **2-** Plates were dried in a 110ºC oven and developed in a cylindrical tank for a distance of 10 cm past the origin. Uvitex OB developer: chloroform **3-** after elution and drying, the optical brighteners were located under UV light (λ = 365 nm)	Lawrence, A. H., Ducharme, D., **Journal of Chromatography**, 194 (1980) 434-436
ε-Caprolactam Monomer of polyamide 6 (nylon)		**HPLC-UV-RI (refractive index)** $t_{r\ Caprolactam}$= 11.75 min **Equipment:** GPC-ALC 150C Waters **Column:** Spherisorb RP-18 Phase-Sep, 25 cm x 4.6 mm i.d. **Mobile phase:** methanol-water (40:60) **Temperature:** 25ºC **Flow rate:** 0.5 mL/min **Injected volume:** 20µL **Sample concentration:** 0.01% (w/v) λ =210 nm	Bonifaci, L. et al, **J. of Chromatography,** 1991, 585, 333-336
	Migration: **1-** 200 cm^2 polymer (Nylon 6) were placed in a flask with 100 mL of boiling water; **2-**Nylon was boiled under reflux for 1h and the aqueous phase removed for analysis; **3-**After cooled, diluted 20 times. For **HPLC** analysis the aqueous extract was rotary evaporated to 6 mL and then made up to a final volume of 10 mL with acetonitrile/water (90:10) solution.	**LC-MS** (used just as qualitative method) **Equipment:** MS: JEOL AX_505WA; HPLC: HP-1090L **Accel voltage:** 3kV **Ionisation:** FAB **Polarity:** positive **Scan range:** 50-1500 **Scan speed:** 5s **Column:** Capcellpak ODS, 4.6 mm i.d. x 150mm) **Mobile phase:** methanol / water (20:80) at 0 min to methanol/water (70:30) at 25 min (linear gradient) **Flow rate:** 1 mL /min **Temperature:** 40ºC λ =230 nm **Injected volume:** 100µL	Barky, C.T. et al, **Food Additives and Contaminants**, 1993, 10(5), 541-553.

Table 2. Abstract of the revised literature on analytical methods of the model migrants

		HPLC **Equipment:** Pye Unicam SP 800 UV/VIS Normal phase: LOD= 50 µg/mL **Column:** Partisil 5µ silica, 250 mm x 4.6 mm i.d. **Mobile phase:** acetonitrile/water (82:18) **Volume injected:** 100µL λ = 220 nm **Quantification** by external standard Reverse phase: **Column:** Alphasil 5 µ ODS, 250 x 4.6 mm i.d. **Mobile phase:** acetonitrile/water (45:55) **Volume injected:** 100 µL λ =230 nm **Quantification** by external standard	
	Determination in isooctane: **1-** A 10cm diameter circle was cut and fitted into the migration cell; **2-** 40mL of preheated isooctane was put into the migration cell and cells stored at the relevant temperature; **3-** After 0, 1, 2, 4, 6, 8 and 10 days, migration cell was shaken and a 250 µL sample put into a GC vial; **4-** 50µL of internal standard solution were added (capryllactam- 100mg/L); **5-** Solutions are ready to GC analysis.	**GC-FID** **Column:** DB624, 30 m x 0.32 mm, 1.8 µm thickness **Injection mode**: split 1:10 **Injector temperature:** 220ºC **Carrier gas:** hydrogen **Detector:** FID **Detector temperature:** 240 ºC **Oven program:** 3 min at 130ºC, 10ºC/min to 240ºC, 5 min at 240ºC. **Injected volume**: 1 µL $t_{r\ caprolactam}$= 12.7 min LOD = 1.0 mg/L isooctane	EU project- Specific migration **G6RD-CT200-00411**
	Determination in water: **1-** 4.0 ml of water from migration experiment are put into a 5 ml flask; **2-** 100µL internal standard solution (capryllactam- 1mg/ml) are added. **3-** After made up to the mark with methanol, solution is mixed thoroughly. **4-** Solution is ready for HPLC analysis.	**HPLC-UV** **Column:** Phenomenex Sphereclone ODS2 5µm, 250 x 3.2 mm **Flow rate:** 1mL/min **Mobile phase:** 60% distilled water / 40% acetonitrile at 0min, gradient to 20% distilled water/80% methanol in 10 min, returning to 60% distilled water/ 40%methanol in 3 min, which was held 2 min. **Volume injected:** 20µL λ= 210nm	EU project- Specific migration **G6RD-CT200-00411**

Table 2. Abstract of the revised literature on analytical methods of the model migrants

	Determination in a polyamide film: **1-** About 1g of the polymer (pieces of 0.5cm^2) was weighted into 40mL vials; **2-** After adding 1mL of internal standard (capryllactam-4000 mg/mL) and 25 mL of 95% ethanol in water, vials were stored at 60ºC for 24h; **3-** Solvent was drained off and analysed by GC. Repeat the extraction with the same polymer pieces.	**GC-FID** **Equipment:** Fisons 8000 **Column:** SGE BPX-5, 25 m x 0.32 mm, 3.0 µm thickness **Injection mode**: on column **Carrier gas:** helium **Column head pressure:** 70 kPa **Detector:** FID **Detector temperature:** 290 ºC **Oven program:** 1 min at 80ºC, 30ºC/min to 280ºC, 5 min at 280ºC, 20ºC/min to 260ºC, 2 min at 260ºC **Injected volume**: 1 µL $t_{r\ caprolactam}$= 7.1 min $t_{r\ capryllactam}$= 9.1 min RSD = 4.2% LOD = 12.5 mg/kg	EU project- Specific migration **G6RD-CT200-00411**
	Determination in parenteral solutions stored in PVC bags: <u>Semi preparative LC- compounds isolation</u> **1-** 400 ml of intravenous infusions with added sodium hydrogen carbonate (1.0g) were extracted with chloroform (4 x 80 mL); **2-** After drying (over $MgSO_4$) and evaporation, the residue was dissolved in 1 ml of 2- propanol before LC. <u>Reduction of caprolactam for GC-MS:</u> **1-** 50 mg of lithium aluminium hydride were added to an extract of intravenous solution dissolved in diethyl ether (10mL); **2-** After an overnight 10 mL water were slowly added before extraction with ether (2 x 5 mL)	**Semi preparative LC-** compounds isolation **Equipment:** Waters System 600 **Column:** 250 x 8 mm i.d. packed with 15-25 µm Lichroprep silica **Mobile phase:** n-hexane-2-propanol (3:1, v/v) **Flow rate:** 2.5 mL/min **Injection volume:** 150µL t_r = 17-20 min **GC-MS-** compounds identification **Equipment:** LKB 2091 **Column:** SPB-1, 30 m x 0.32 mm i.d., 0.25µm **Detector mode:** electron ionisation, 70eV when scanning the mass spectra and at 14 eV for low energy spectra **Carrier gas**: Helium **Flow rate:** 1.4 mL/min **Flow rate for make-up gas**: 20 mL/min **Injection volume:** 2 µL **Injection mode:** split **Valves:** open 30s after injection **Injector, separator, and ion source temperature:** 180ºC **Oven temperature:** 4ºC/min from 40 to 150ºC	Ulsaker, G.A. et al **Journal of Pharmaceutical and Biomedical Analysis**, 1992, 10 (1), 77-80

Table 2. Abstract of the revised literature on analytical methods of the model migrants

	3- Extract was dried (over MgSO4) prior to GC-MS Extraction of plastic: **1-** 10 g of plastic material (cut into small pieces) were added to 250mL of 2-propanol; **2-** After extraction (10d, room temperature), the solvent was decanted and evaporated; **3-** Residue was dissolved in n-hexane-2-propanol (1:1, v/v) to a total volume of 5 mL prior to LC analysis. Extraction from intravenous infusion: **1-** Sodium hydrogen carbonate (0.1 g) and 1 ml of internal standard (octahydro-1H-azonin-2-one dissolved in methanol-water -1:1, v/v) were added to 50 mL of intravenous infusion; **2-** After mixing, sample was extracted with chloroform (3 x 25 mL); **3-** After drying (over $MgSO_4$) and evaporation, the residue was dissolved in 2-propanol (5mL) before LC analysis.	**Normal phase LC-** compounds quantification **Equipment:** Waters System 600 **Column:** Supelcosil /LC-SI, 250 x 4.6 mm i.d. column packed with 5µm **Mobile phase:** n-hexane/2-propanol (9:1, v/v) **Flow rate:** 1.5 mL/min **Injection volume:** 25µL **λ=** 210 nm t_r = 17.33 min Recovery: 93% LOD = 0.02 mg/L	
Benzophenone UV ink	**Migrants**: - acrylates (9) - photo-initiators (6): benzophenone **Simulants**: -Water -Acetic acid aq 3% -Ethanol aq 15% -Rectified olive oil **Specific migration:** 1- Two discs (1dm^2) from the printed substrates were placed in the extraction cell;	**GC-MS** **Column:** Optima Delta 6, 30 m x 0.25 mm i.d. **Oven temperature**: ramp from 50ºC to 250ºC at 10ºC/min **Detector mode:** full scan RSD = 8.7% in isooctane RSD = 10.5% in ethanol *Recovery was close to 100% and >70% in all the cases.* Recovery can be improved using an HPLC method. Isooctane seems to be a better simulant once presents better recovery results.	Papillound, S. and Baudraz, D., **Food Additives and Contaminants**, 2002, 19 (2), 168-175.

Table 2. Abstract of the revised literature on analytical methods of the model migrants

	2- The cell was filled with 200mL of solvent and left 3 days in the dark at room temperature; 3. a) aqueous solution 1- The solution was spiked with internal standard (200µL methanol solution of dimethylphthalate at1000µg/L) and stabilizer (200µL methanol solution of HQMME at 1000µg/L); 2- 200ml of the solution was poured through the activated silica-bound-phase cartridge; 3- Cartridges were eluted with methanolic solution and solution filtered and analysed. b) organic solution (ethanol 95% and isooctane) 1- The solution was spiked with internal standard (200µL methanol solution of dimethylphthalate at 1000µg/L) and stabilizer (200µL methanol solution of HQMME at 1000µg/L); 2 -Solutions were evaporated to dryness under mild vacuum conditions at 55ºC and 100 mbar on a rotatory evaporator; 3- Solutions were diluted with 2 mL acetonitrile and analysed after filtration.		
Diphenyl phthalate plasticiser			
DEHA	**To determine DEHA in fat:** **1-** 10 mL of fat were added in a vessel to the internal standard (prepared in synthetic mixture of triglycerides) and an antifoam solution; **2-** The vessel was connected to a Tenax absorption column and heated at 210ºC in an oil-bath. At the same time, nitrogen was passed through the fat at a	**GC-MS** **Equipment:** Finnigan 4000 GS-MS **Column:** DB-5 fused-silica column, 30m x 0.32 mm i.d. **Detector:** MS **Detector mode:** electron impact **Carrier gas**: Helium **Injection volume:** 1µL **Injection mode:** split **Oven program:** 3 min at 60ºC, to 300ºC at 40ºC/min, at 300ºC for 15 min.	Van Lierop, J. B. H**.** et al**, J. of Chromatography**, 1998, 447, 230-233

Table 2. Abstract of the revised literature on analytical methods of the model migrants

	rate of about 170 mL /min for 30 min; **3-** Tenax (15mg) was extracted with 1-3 mL hexane. **To determine DEHA in fatty food**: **1-** The fat content of the food were determined; **2-** A sample of food was taken so as to obtain 10 mL of fat; **3-** Sample was mixed with twice its weight of dry sodium sulphate; **4-** The mixture was extracted with diethyl ether in a Soxhlet apparatus for 6-7 h; **5-** The ether was removed by evaporation. Continue with the above description.	**Quantification:** by internal standard	
	To determine DEHA in food: **1**- 30-50g of homogenized food were blended with acetone-hexane (150 mL, 1:1); **2-** 0.5-3 mg of an internal standard [$^2H_4$]DEHA (in hexane) were equilibrated with analyte during a night; **3-** Supernatant was decanted and the residue was re-extracted with 2 portions of solvent (75 mL each); **4-** The combined extracts were dried over sodium sulphate, evaporated to dryness, and the residue re-dissolved in 20 mL of dichloromethane- ciclohexane (1:1); **5-** Centrifugation at low speed to achieve a clear solution..	**SEC (Size exclusion chromatography)**- to clean-up ***Column:*** *glass, 1 m x 25 mm i.d. containing an 80 cm bed of Biobeads S-X3* **Flow rate:** 3.0 mL/min **Mobile phase:** dichloromethane-cyclohexane (1:1) **Injection volume:** 1.5 mL λ = 254 nm Fraction containing DEHA was evaporated to dryness and transferred to a small vial using acetone. Sample was dried under nitrogen and stored at 5ºC prior to GC-MS analysis. **GC-MS** **Equipment:** Carlo Erba4160 coupled with a VG 7070H mass spectrometer **Column:** CPSIL 5 CB fused silica column, 25m x 0.23 mm i.d. (Chrompack, U.K.) **Detector mode:** electron ionisation, 70eV with 200 μA trap current **Carrier gas**: Helium	Startin, J.R. et al, **J. of Chromatography,** 1987, 387, 509-514

Table 2. Abstract of the revised literature on analytical methods of the model migrants

	DEHA analysis in polymers: -<u>by Soxhlet extraction:</u> **1-** 1-5g of polymer was extracted with 15 mL of dichloromethane for 16 h; **2-** Extraction was repeated with fresh dichloromethane for further 16 h; **3-** Extracts were concentrated by evaporation and transferred to a 10mL flash. Internal standard was added (10mg/mL in dichloromethane) and solutions made up to the mark; -<u>by total dissolution</u>. **1-** 0.5g of polymer was added to 2 mL of internal standard; **2-**After evaporation to dryness, 5 mL of chloroform was added (polymer dissolved); **3-** 15 mL of methanol were added to precipitate the polymer; **4--**Vials were centrifuged and supernatant solution analysed. **DEHA analysis in aqueous simulants:** **1-** 4g of sodium sulphate and 2mL of intermediate standard (2mL of an 2000ppm solution of dinonyl adipate in propan-2-ol to 50 mL) was added to 50 mL of exposed simulant. **2-** Extraction was done with 10 mL of heptane.	**Injection volume:** 1.5µL **Injection mode:** split, 1:30 **Oven temperature:** 230ºC Quantification: by internal standard [$^2H_4$]DEHA m/z (DEHA) = 129; m/z (internal standard) = 133 **GC-FID** **Column:** BPX5, 12 m x 0.32 mm, 0.25 µm **Carrier gas:** helium 15 psi **Injector mode:** split **Injector volume.** 1 µL **Flow rate.** 100 mL/min **Detector:** FID **Detector temperature:** 370ºC **Oven temperature:** 1 min at 240ºC, 12ºC/min to 300ºC, 1min at 300ºC **GC-FID** **Column:** BPX5, 12 m x 0.32 mm, 0.25 µm **Carrier gas:** helium 50kPa **Injector mode:** on column **Detector:** FID **Detector temperature:** 370ºC **Oven temperature:** 1 min at 100ºC, 30ºC/min to 210ºC, 10ºC/min to 240ºC, 30ºC/min to 360ºC, 10 min at 360ºC	O'Brien, A. P., **Food Additives and Contaminants**, 1997, 14 (6-7), 705-719.

Table 2. Abstract of the revised literature on analytical methods of the model migrants

	DEHA analysis in olive oil samples: 0.5g of oil were added to 2 mL of internal standard (dinonyl adipate 10μg/mL in heptane) and heptane until get a total volume of 25mL. **Extraction:** To the mixed plasticisers standard (containing 11.2 mg/L DEHA)was added the internal standard (DIBP-diisobutyl phthalate 10 mg/L). Food samples: - gummy candy - egg custard roll - bacon biscuits Recovery studies: Food: - jelly - candy - bacon - biscuit - cheese <u>For non fatty foods</u> (jelly and candy): **1-** 30g of homogenized sample (which has been already in contact with the film) was added to 1 mL of internal standard and distilled water (to a total of 100 mL); **2-**Mixture was shaken with 20 mL of cyclohexane- dichloromethane (1:1) for 2h with an automatic shaker (150 rpm); **3-**Extract was dried over anhydrous sodium sulfate and analysed. <u>For fatty food</u> (bacon and cheese) **1-** 30g of homogenized sample was added to 1 mL of internal standard and	**GC-MS** **Equipment:** Varian 3400 **Column:** DB-5 capillary column, 30 m x 0.23 mm i.d. **Oven program:** 5 min at 100ºC, 15ºC/min to 300ºC, 10 min at 300ºC **Carrier gas:** helium **Pressure:** 9 psi **Detector:** ion-trap mass detector **Detector mode:** SIM, m/z (adipates) = 129; m/z (ATBC) = 185 **Gel-Permeation Chromatography** (clean-up) Column: Bio-Beads SX3, 50 cm x 2.5 cm, 60-120 μm diameter	Oi-Wah, L. et al, **J. of Chromatography,** 1996, 737, 338-342.

Table 2. Abstract of the revised literature on analytical methods of the model migrants

	50 mL of cyclohexane-dichloromethane (1:1); **2-** Mixture was shaken for 2 h by an automatic shaker (150 rpm); **3-** 5 mL of the extract were taken and dried with anhydrous sodium sulfate; **4-** Solvent was removed by heating and residue redissolved in 2mL of dichloromethane-cyclohexane (1:1); **5-**Then a **gel-permeation chromatography** was made to clean-up. First 90 mL of the eluent were discarded and the following 40 mL of eluent collected; **6-**Eluent was evaporated to 2 mL by heating and analysed. **Standards:** **GC:** 1mg/mL DEHA in hexane. This solution was dilutes to 10, 1 and 0.25 µg/mL in ethyl acetate or 6% ethyl acetate in hexane. **LC:** 1 mg/mL in acetonitrile. This solution was diluted to 100 and 40 µg/mL in acetonitrile. **Extraction:** <u>A- Acetonitrile-hexane partition for non-fatty foods:</u> Food: - fresh, frozen, canned and dehydrated frits and vegetables - fruit juices - wines - beers - maple syrups - cereal grains - whole meals	**GC-FID** **Equipment:** Varian 3400 **Column:** DB-5, 15 m x 0.30 mm, 0.25 µm thickness **Injection mode**: on column **Carrier gas:** helium **Flow rate:** 2.5 mL/min **Detector:** FID **Detector temperature:** 340 ºC **Oven program:** 1 min at 60ºC, 30ºC/min to 120ºC, 10ºC7min to 250ºC, 3 min at 250ºC, 50ºC/min to 295ºC, 12 min at 295ºC **Injected volume**: 1 µL **Linear response**: 0.25 to 10 µg/mL **LC** (used to confirm) **Equipment:** LKB 2140 **Detector:** diode array and absorbance **Column:** Supelcosil LC-18, 150 mm x 4.6 mm **Mobile phase:** acetonitrile/ water (50:50) to acetonitrile (100%) in 10 min , 3 min at 100% acetonitrile (to separate the phthalate esters. $\lambda_{max\ DEHA}$= 209-211 nm	Page, B. D., et al **Food Additives and Contaminants**, 1995, 12 (1), 129-151.

Table 2. Abstract of the revised literature on analytical methods of the model migrants

	1- Food was blended with acetonitrile and, if required, water; **2-** Mixture was filtered, diluted with water and extracted with hexane and dichloromethane (10 + 1); **3-** Sodium sulphate was added (to minimize emulsion formation); **4-** Hexane extract was washed with water, dried with anhydrous sodium sulphate, evaporated to dryness and made to volume with hexane; **5-** Samples were analysed by capillary GC. When other peaks eluting with or near plasticizers of interest the extract were: -passed through Florisil column or - or distillated (sweep co-distillation) <u>B- Dichloromethane lipid extraction for fatty food:</u> Food: - animal tissues - fats - high fat content cheese **1-** Food was blended with sodium sulphate and dichloromethane; **2-** Dichloromethane was filtered and removed by rotary evaporation; **3-** Fat was weighted. When less than 1g was present, the lipid was transferred quantitatively in a small quantity of hexane and made to 5 mL; **4-** Plasticizers where isolated by: -sweep co-distillation -florisil trapping or		

Table 2. Abstract of the revised literature on analytical methods of the model migrants

	-selective elution **5-** Samples were analysed by capillary GC. <u>C- Acetone-hexane lipid extraction</u> Food: - milk - cream **1-** Food, acetone and hexane were blended and centrifuged; **2-** Hexane was removed and extraction repeated; **3-** Hexane extract was dried and lipid material recovered after hexane evaporation; **4-** Extracted fat was weighted. When less than 1g , the lipid was quantitatively transferred in a small quantity of hexane and made up to 5 mL; **5-** Plasticizers where isolated by sweep co-distillation and analysed by GC. **D-** Food: - butter - margarine **1-**Samples were liquefied (in an oven, 70ºC); **2-** Supernatant oil was mixed, decanted, filtered, centrifuged and stored under refrigeration until analysis.		
Styrene	**Migration into food:** Recipes. - sliced potatoes with grated cheese (35 min for 170ºC)	**Headspace GC/MS** **Equipment:** HP 5971 series **Column:** CPSIL 5CB, 17 m x 0.25 mm i.d., 0.25µm thickness **Temperature:** 35ºC **Carrier gas.** Helium	Jickells, S. M**., Food Additives and Contaminants**, 1993, 10 (5), 567-573

Table 2. Abstract of the revised literature on analytical methods of the model migrants

	- minced beef fried with tomato sauce (90 min for 140ºC) **1-** A measured amount of water was added to obtain a slurry; **2-** To 6g sub-samples was added internal standard (10µL/200µL cyclohexane of a d8-styrene solution - 20µg/mL in ethyl acetate); **3-** Samples were stored at –20ºC before analysis; **Migration into olive oil:** Exposure conditions: -**sealed system**- dishes with olive oil were heated (2h/175ºC). Afterwards, they were removed and cooled in water. The oil was transferred to headspace vials and stored at –20ºC until distillation; -**open system**- olive oil was heated at 175ºC/2h in trays. Then was transferred to glass vials and stored at –20ºC; **- immersion**-polymer (6-10g) was immersed in sufficient olive oil and heated at 175ºC/2h. After cooled at room temperature for 5 min was again cooled for 10-15 min in a freezer at -20ºC. Then, polymer was removed and olive oil analysed; **1-** Olive oil was removed from storage at –20ºC; **2-** The oil (8-10g) was mixed and added to a few anti-bumping granules, distilled water (100mL) and internal standard (20 µL d8-styrene, 5 µg/mL in hexane);	**Flow rate:** 0.5 mL/min **Injector temperature:** 250ºC **Detector mode: SIR** (m/z =104/112) **Detector temperature:** 180ºC **Injection mode:** splitless, 3s **Vials pressurization:** 10s / **Vent to the sample loop:** 5s **Headspace sample carousel temperature:** 90ºC **Equilibrium period:** 30 min **GC/MS for olive oil** **Equipment:** HP 5991 series **Column:** CPSIL 5 CB, 25 m x 0.25 mm I:D: x 0.12 µm thickness **Oven program:** 2min at 35ºC, 5ºC/min to 65ºC, 40ºC/min to 200ºC and 1min at 200ºC **Injection volume.** 1µL **Injector temperature:** 250ºC	

Table 2. Abstract of the revised literature on analytical methods of the model migrants

	3-Sample was distilled in a continuous steam distillation apparatus of Likens-Nikerson type (90min) with hexane (2mL); **4-** Hexane was analysed directly. **SPME extraction:** Fiber: 85 µm polyacrylate coating Conditionation: 3h in the port injector at 280ºC Adsorption: 10mL styrene solution (30.0 µg/L) was introduced in 22 mL Pyrex vials, fiber was exposed to the sample headspace during 10 min at 55ºC. Desorption**:** into the injection port at 220ºC for 2 min. Liner: splitless, 0.75mm i.d.: **Headspace extraction:** 250 µL styrene solution (with 20% NaCl) was placed in 6 mL Pyrex vials. Vials were heated at 80ºC for 20 min. 250µL of the sample headspace were injected into the GC.	**GC** **GC-MS (identification)** **Equipment:** HP 5890 series II GCS equipped with an HP 5972 MS **Ionisation mode:** electron impact, 70eV, full-scan, mass range: 50-150u The other conditions are the same as for GC-FID. **GC-FID (quantification)** **Equipment:** HP 5890 series II GC **Column:** HP-5 capillary column, cross-linked 5% phenyl methysiloxane film, 25 m x 0.20 mm i.d., 0.33 µm thickness **Carrier gas:** helium **Flow rate:** 0.65 mL/min **Oven program:** 1 min at 50ºC, 10ºC/min to 100ºC, 5ºC/min to 150ºC, 25 min at 150ºC. **Injection mode:** splitless **Injector temperature:** 220ºC **Detector:** FID **Detector temperature:** 280ºC LOD= 0.30µg/L- SPME; 0.05mg/L- Headspace	Silva, F.C. et al**, Journal of Chromatographic Science**, 200, 38, 315-318
Bisphenol A Monomer used in the manufacture of epoxy resins for the lacquer lining of metal food cans; monomer of polycarbonates ; monomer of plastic used in the base paste of dental sealants. (Sun, Y et al, **Journal of Chromatography B,** 2000, 749,49-56)	**Preparation of authentic DIB-BPA sample** 1- 0.31g of Bisphenol A were added to the DIB-Cl (fluorescent reagent 4-(4,5-diphenyl-1H-imidazol-2-yl)benzoyl chloride) suspension (1g/250ml in acetonitrile); 2- 1.2ml of TEA (triethylamine) were added and the resultant clear yellow	**HPLC-PO-CL (peroxyoxalate chemiluminescence)** **Equipment:** two LC-10Advp pumps, JASCO 825-CL chemiluminescence detector **Column:** Daisopak-SP-120-5-ODS-BP, 250mm x 4.6 mm i.d., 5 µm **Mobile phase:** acetonitrile/imidazole/HNO_3 buffer (pH 7.0) (83:17, v/v) **Flow rate:** 1.0 mL/min Intra-assay Recovery = 93.86%; LSD = 7.8% with 1.14 µg/L	Sun, Y et al, **Journal of Chromatography B,** 2000, 749,49-56

Table 2. Abstract of the revised literature on analytical methods of the model migrants

Specific migration limit: 3 mg/kg (Gandara, J. S. Et al, **Journal of Chromatographic Science**, 1993, 31, 450-454)	solution was stirred at room temperature for 1 h to give white-yellowish precipitates; 3- After filtration , the precipitates were washed thoroughly with cold acetonitrile and dried in vacuum. **Sample pretreatment and derivatization reaction** 1- 100 mL of boiling water were transferred to a baby bottle and tightly capped and kept in an oven at 95% for 30 min; 2- After cool, 200 µl were transferred to a mini amber-glass vial and evaporated to dryness in a centrifugal evaporator; 3- Residue was reconstituted in 100 µL of acetonitrile; 4- An equal volume of 10mM DIB-Cl in acetonitrile and 5µl of 3M TEA in acetonitrile were added and mixed in a vortex for 20 min. **Removing the excess DIB-Cl by SPE** 1- ODS cartridges were activated with 5 mL of acetonitrile and conditioned with 5 mL of deionised water; 2- After applying the reaction mixture, the cartridge was washed with 5 mL of acetonitrile/water (70:30, v/v) and dried under vacuum; 3- DIB-Bisphenol A was eluted with 300 µL of acetonitrile; 4- 5 µL of eluate were injected.	Recovery = 92.11%; LSD = 3% with 2.28 µg/L Inter-assay LSD = 12.3% with 1.14 µg/L LSD = 7.5% with 2.28 µg/L	

Table 2. Abstract of the revised literature on analytical methods of the model migrants

		HPLC-F **Equipment:** Spectra-Physics SP8700XR **Column**: Pecosphere CRT C_{18}RC, 150 mm x 5.0 mm i.d. **Mobile phase:** 10 min isocratic elution with acetonitrile/water (35:65, v/v); 5 min linear gradient to 50% acetonitrile; 5 min linear gradient to 60% acetonitrile; 5min isocratic elution at 60% acetonitrile; 5 min linear gradient to 100% acetonitrile; 10 min isocratic elution at 100%; **Flow rate:** 1.5 mL/min **Injected volume:** 50 µL $\lambda_{excitation}$= 275 nm; $\lambda_{emission}$= 300 nm LOD = 28.8 ppb	Gandara, J. S. Et al, **Journal of Chromatographic Science**, 1993, 31, 450-454
	Residual monomer content 1- 1g of polymer (cut into small pieces) was dissolved with 10 mL dichloromethane (30 min); 2- The polymer was precipitated with propan-2-ol (10 mL) by shaking 3- After addition of 10 mL of hexane, 5 mL of the supernatant was taken to dryness; 4- Residue was dissolved with HPLC mobile phase , filtered and analysed by HPLC. **Migration conditions:** 10 d at 40ºC **Analysis of infant feed** 1- 5 drops of ammonia and 0.5 mL of ethanol were added to 1 mL of infant feed ; 2- After mixed, 2mL of hexane were added and the mixture shaken; 3- The lower layer was transferred to an SPE cartridge that had been pre-conditioned with water (2mL);	**HPLC-F** (Residual monomer content) **Equipment:** Gilson **Column**: cyano phase column, 250 mm x 4.6 mm, 5µm **Mobile phase:** hexane/propan-2-ol/dichloromethane (72:8:20 v/v/v) **Flow rate:** 1.0 mL/min **Injected volume:** 200 µL $\lambda_{excitation}$= 285 nm; $\lambda_{emission}$= 300 nm **HPLC-F** (Analysis of infant feed) **Equipment:** Gilson **Column**: C_8 bonded reverse phase column, 250 mm x 4.6 mm, 5µm **Mobile phase:** methanol/water (7:3 v/v) **Flow rate:** 1.0 mL/min **Injected volume:** 200 µL $\lambda_{excitation}$= 285 nm; $\lambda_{emission}$= 300 nm LOD = 0.03 mg/kg Recovery = 78 ± 4% with 0.3 mg/kg	Mountfort, K. A. et al, **Food Additives and Contaminants**, 1997, 14 (6-7), 737-740.

Table 2. Abstract of the revised literature on analytical methods of the model migrants

	4- Column was eluted with water (2 mL) and then by methanol (4 mL). 5- Methanol elute was evaporated to dryness at 60ºC under nitrogen; 6- Residue was dissolved in methanol/water (7:3, v/v), filtered and analysed by HPLC. **Sample Preparation for the solid portion in cans of vegetables and fruit** 1- 5 g of sample were homogenized for 2 min with 50 mL of acetonitrile and 15 g of sodium sulfate; 2- The homogenate was filtered and the residue washed with acetonitrile (30 mL); 3- The filtrate was shaken vigorously (5 min) with 50 mL of hexane saturated with acetonitrile; 4- The acetonitrile layer was transferred to a flash; 5- Hexane layer was shaken with 50 mL of acetonitrile and the acetonitrile layer combined with the other; 6- After adding 10 mL of 2-propanol to the acetonitrile layer, the extractant was evaporated to dryness under reduced pressure at 40ºC; 7- The residue was dissolved with 10 mL of acetone-heptane (2.5:97.5) and applied to a Sep-Pak Florisil cartridge; 8- After washing with 10 mL of acetone/heptane (5:95), Bisphenol A was eluted with 10 mL of acetone/heptane (20:80); 9- The eluate was evaporated to dryness under reduced pressure at 40ºC; 10- Residue was dissolved in 1 mL of mobile phase and analysed by HPLC.	**HPLC-UV** **Equipment:** HPLC Gulliver system **Column:** Wakosil II 3C18 RS, 3µm particle size, 150 mm x 4.6 mm i.d. **Column temperature:** 40ºC **Flow rate:** 0.8 mL/min **Mobile phase:** acetonitrile/water (40:60, v/v) **Volume injected:** 20µL **λ** = from 228 nm RSD = 3.9 %; Recovery (canned corn) = 85.7% with 0.5µg RSD = 2.9 %; Recovery (canned corn) = 89.3% with 1.0µg RSD = 3.9 %; Recovery (canned pineapple) = 84.5% with 0.5µg RSD = 3.5 %; Recovery (canned pineapple) = 86.5% with 1.0µg	Yoshida, T. et al, **Food Additives and Contaminants**, 2001, 18 (1), 69-75

Table 2. Abstract of the revised literature on analytical methods of the model migrants

	Sample preparation for the aqueous portion in cans of vegetables and fruit 1- 10 mL of the aqueous portion of the canned food was added to 10 mL of distilled water; 2- Aqueous portion was applied to an OASIS HLB extraction cartridge; 3- After washing with 10 mL of distilled water, Bisphenol A was eluted with 10 mL of methanol; 4- The eluate was evaporated to dryness under reduced pressure at 40ºC; 5- Residue was applied to a Sep-Pak Florisil cartridge and analysed by HPLC.		
	Analysis of Bisphenol a from coffee 1- 300 ml of water were added to 4g of coffee (decaffeinated and non-decaffeinated) in a 500 mL beaker; 2- The beaker was heated at 90ºC for 20 min; 3- After cooled to room temperature, the sample (150 mL) was poured into can and heated at 121 ºC for 30 min; 4- ISOLUTE multimode cartridge was conditionated with 5 mL methanol and 10 mL water; 5- 10 mL of sample were applied to the cartridge; 6- The cartridge was washed with 20 mL water and dried by air suction for 10 min; 7- Bisphenol A was eluted by 10 mL acetonitrile/water (40:60, v/v); 8- Solution was analysed by HPLC.	**HPLC-F** **Column**: SymmetryShield RP_{18}, 150 mm x 4.6 mm, 3.5µm **Column temperature:** 40ºC **Mobile phase:** acetonitrile/water (40:60 v/v) **Flow rate:** 1.0 mL/min **Injected volume:** 50 µL $\lambda_{excitation}$= 275 nm; $\lambda_{emission}$= 300 nm Recovery (non-decaffeinated) =85.3%±5.7 % with 50 ng/mL Recovery (non-decaffeinated) =88.7%±3.9 % with 100 ng/mL LOQ(in water and caffeine) = 2 ng/mL LOQ(coffee) = 10 ng/mL The more the caffeine content increased up to 1.0 mg/mL, the more the Bisphenol A migration grew.	Kang, J. et al, **Food Additives and Contaminants**, 2002, 19 89), 886-890

Table 2. Abstract of the revised literature on analytical methods of the model migrants

	Residual level of Bisphenol A in polycarbonate: 1- Half cut discs of polycarbonate were dissolved in chloroform; 2- After extracting the chloroform solution with 0.01M sodium hydroxide, the extract was analysed by HPLC. <u>Food simulants:</u> - water - 10% ethanol - 3% acetic acid - Miglyol 812 - Fractionated coconut oil <u>Conditions:</u> - 100ºC for 6 h (for water, 10% ethanol; Miglyol 812) - 49ºC for 6, 101, 240h (for water, 3% acetic acid, 10% ethanol) - 49ºC for 6, 96, 240 h (for Miglyol 812) **Migration** Simulants were analysed directly by HPLC except Miglyol 812: 1- An aliquot of extract was mixed with chloroform; 2- The chloroform solution was extracted with 0.01M TBAH (tetrabutylammonium hydroxide); 3- TBAH was filtered and analysed by HPLC.	**HPLC-UV** **Equipment:** Perkin Elmer **Column**: Zorbax C18, 250 mm x 4.6 mm **Mobile phase:** A- 0.1% acetic acid in Milli-Q water B- Acetonitrile/0.1% acetic acid in Milli-Q water See gradient in the paper **Flow rate:** 2.0 mL/min **Injected volume:** 400 µL **λ**= 280 nm Migration tests for **water** and **10% ethanol** extracts at **100ºC** were analysed with the above conditions, except the injection volume was 900µL. Migration tests for **water**, **10% ethanol** and **3% acetic acid** at 49ºC were analysed under the follow conditions: **HPLC-F** **Equipment:** Perkin Elmer **Column**: Phenomenex 5ODS, 250 mm x 4.6 mm **Column temperature:** 60ºc or 65ºC **Mobile phase:** methanol/Milli-Q water (6:4) **Flow rate:** 1.0 mL/min **Injected volume:** 150 µL $\lambda_{excitation}$= 235 nm; $\lambda_{emission}$= 317 nm Migration tests for **Miglyol 812** at 49ºCand 100ºC were analysed under the follow conditions: **HPLC-F** **Equipment:** Perkin Elmer **Column**: Phenomenex 5ODS, 250 mm x 4.6 mm **Column temperature:** 40ºC **Mobile phase:** acetonitrile/Milli-Q water (4:6) **Flow rate:** 1.0 mL/min **Injected volume:** 150 µL $\lambda_{excitation}$= 235 nm; $\lambda_{emission}$= no filter LOQ = 50 ng/in^2	Howe, S. R. et al, **Food Additives and Contaminants**, 1998, 15 (3), 370-375.

Table 2. Abstract of the revised literature on analytical methods of the model migrants

	Simulants: - Distilled water Heat processing: - 121ºC/90 min (tuna) - 100ºC/9 min (jalapeño peppers) - no heating process Conditions: - 25ºC for 0, 40, 70 days **Migration**: 1- Cans were opened and water evaporated by rotary evaporator at >35ºC; 2- Dry residue was redissolved in 5 ml of acetonitrile, filtered and analysed by HPLC.	**HPLC-F** (quantification) **Equipment:** Varian, Star 9012 **Pre-column:** C_{18} **Column**: Micropak C_{18} MCH-5-N-CAP, 150 mm x 5.0 mm **Column temperature:** 40ºC **Mobile phase:** 10 min acetonitrile/water (35:65), 5 min linear gradient to 50:50, 5 min at 50:50, 10 min gradient elution to 60:40, 5 min 60:40, 5 min gradient elution to 100:0, 5 min at 100:0. **Flow rate:** 1.0 mL/min **Injected volume:** 10 µL $\lambda_{excitation}$= 224 nm; $\lambda_{emission}$= 310 nm t_r = 16.8 min LOQ = 0.20 µg/L Recovery = 100.1 % ± 4.7% with 300µg/L **GC-MS** (identification) **Equipment:** Varian 3400CX **Ionisation mode:** scan mode, 70 eV, mass range: 35-500u **Column:** DB-5 fused silica capillary column, 30 m x 0.25 mm i.d., 0.25 µm thickness **Injector temperature:** 150ºC **Transference line temperature:** 200ºC **Oven program:** 30ºC/min from 90ºC to 300ºC, 10 min at 300ºC. t_r = 8.5 min m/z = 213, 228, 119, 91	Munguia-Lopez, E. et al , **J. Agric. Food Chem,** 2001, 49, 3666-3671
1-octene	**SPME extraction:** Fiber: 75 µm carboxen-poly(dimethylsiloxane) coating Conditionation: 45 min in the port injector at 280ºC Adsorption: the needle was placed in the DED (direct-extraction device) and the DED was inserted into the core of ham by pressing. Fiber was exposed to the sample during 45 min at the temperature	**GC-MS** **Equipment:** HP5890GC series II **Detector:** electronic impact at 70 eV; multiplier voltage 1756 V; rate 1scan/ s **Column:** HP-5 bonded-phase fused-silica capillary column, 50m x 0.32 mm i.d., 1.05 µm thickness **Column head pressure:** 41.3 kPa **Flow rate:** 1.45 mL/min at 40ºC **Injector temperature:** 280ºC **Injection mode:** splitless	Andres, A.I. et al, **Journal of Chromatography A,** 2002, 963, 83-88

Table 2. Abstract of the revised literature on analytical methods of the model migrants

	of the chamber. Desorption**:** into the injection port at 280ºC during the whole chromatographic run.	**Transference line temperature:** 280ºC **Oven program:** 10min at 40ºC, 5ºC/min to 200ºC, 5 min at 200ºC. **Detector mode:** full scan, mass range: 30-500u	
	Collection of headspace volatiles from heated beef **Note: -** isolation of volatiles chemicals from a fatty sample is one of the most difficult of analytic procedures - the method **steam distillation-solvent extraction** (advanced by Likens and Nickerson-1964) was applied to analyse volatiles from fat by several researchers **but volatiles still must undergo temperatures of 100ºC or higher**, which may cause further alteration of chemicals after separation of fat. **Headspace extraction:** **1-** Pure beef fat (140g) was placed in a 500 mL , two-neck, round-bottom flask; **2-** The flask was connected to an apparatus with a gas-washing bottle and a liquid-liquid continuous extractor in tandem; **3-** Beef fat was heated at 300ºC. Volatiles were purged into a 250 mL of deionised water by a stream of purified nitrogen (7.2 mL/min); **4-** Volatiles dissolved by water (10ºC) were simultaneously and continuously extracted with dichloromethane (70 mL) for 6 h; **5-** Dichloromethane extract was dried over anhydrous sodium sulfate for 12 h and removed by a rotary flash evaporator; **6-** Concentrated sample (0.3 mL) was analysed by GC.	**GC-FID** **Equipment:** HP5880A GC **Column:** DBWAX fused silica capillary column, 60m x 0.25 mm i.d. **Carrier gas:** helium **Flow rate:** 30 cm/s **Injector temperature:** 250ºC **Detector:** FID **Detector temperature:** 250ºC **Injection mode:** split, 1:30 **Oven program:** 10min at 40ºC, 2ºC/min to 200ºC **GC-MS** **Equipment:** HP5792A GC **Column:** DBWAX fused silica capillary column, 60m x 0.25 mm i.d. **Carrier gas:** helium **Flow rate:** 30 cm/s **Detector:** electronic impact at 70 eV **Injector temperature:** 250ºC **Detector temperature:** 250ºC **Oven program:** 10min at 40ºC, 2ºC/min to 200ºC	Umano. K. et al**, J. Agric. Food Chem.,** 1987, 35, 14-18.

Table 2. Abstract of the revised literature on analytical methods of the model migrants

	Headspace extraction: **1-** 10 g menhaden fish oil were mixed with cysteine (0.1, 0.25, 0.5 or 1.0 g) or cysteine (0.1, 0.25, 0.5 or 1.0 g) and trimethylamine oxide (TMAO) in a 50 mL two neck, round-bottom flask. **2-** The flask was connected to an apparatus described by Umano and Shibamoto. **3-** Samples were heated at 300ºC. Volatiles were purged into a 250 mL of deionised water by a stream of purified air (7.2 mL/min) for 6h; **4-** Volatiles dissolved by water were simultaneously and continuously extracted with dichloromethane (50 mL) for 6 h; **5-** Extract was dried over anhydrous sodium sulfate and condensed using a rotary flash evaporator and further condensed under a purified nitrogen stream; **6-** Concentrated sample (0.3 mL) was analysed by GC.	**GC-MS** **Equipment:** HP5890 GC **Column:** DBWAX fused silica capillary column, 60m x 0.25 mm i.d., 1 µm thickness **Carrier gas:** helium **Flow rate:** 30 cm/s **Detector:** electronic impact at 70 eV **Detector mode:** full scan **Injector temperature:** 250ºC **Detector temperature:** 250ºC **Oven program:** from 50 to 200ºC at 3ºC/min, 40 min at 200ºC	Horiuchi, Masahiro et al**, J. Agric. Food Chem.,** 1998, 46, 5232-5237.
Limonene	**SPME extraction:** Adsorption:, fiber was exposed to the sample headspace during 15 min at 40ºC	**GC-MS** **Equipment:** HP6890 GC **Column:** Phenomenex Zebron ZB-5 MS capillary column, 30m x 0.25 mm i.d., 0.25 µm thickness **Carrier gas:** helium **Detector mode:** full scan **Flow rate:** 0.8 mL/min **Injector temperature:** 250ºC **Injection mode:** splitless **Transference line temperature:** 230ºC **Oven program:** 2min at 36ºC, 8ºC/min to 250ºC, 10 min at 250ºC.	Bentivenga, G. et al, **La Rivista Italiana Delle Sostanze Grasse,** 2001, 78, 157-162

Table 2. Abstract of the revised literature on analytical methods of the model migrants

	Thermal desorption extraction 1- 2 L of water were purged at 40ºC for 1 h with purified nitrogen at 100mL/min; 2- A cold trap (-10ºC) was used for retention of water vapour and volatile compounds were trapped on Tenax TA.	**GC-FID** **Equipment:** Carlo Erba GC 6000VEGA series **Column:** DB1, 30 m x 0.32 mm i.d., 1.0 µm thickness A thermal desorption/cold trap device was used for transferring the volatile compounds from Tenax to the column **Carrier gas:** helium **Injection mode**. split 1:2:2, FID, sniffing port 1 and sniffing port 2 **Oven program:** 4 min at 60ºC, 2ºC/min to 140ºC, 10ºC/min to 250ºC, 5min at 250ºC. **Detector:** FID **Detector temperature:** 275ºC	Linssen, J. P. H. et al, **Food Chemistry,** 1993, 46, 367-371
	Direct thermal desorption (DTD): - has better reproducibility than simultaneous distillation-extraction - can only be applied to solid and semisolid matrices. 1- Grated samples were mixed with sodium sulphate (1:6, w/w); 2- 0.3g were introduced in a Teflon lined stainless steel tubes. **Dynamic headspace (DHS)** 1- 15 g of cheese were placed in a glass vessel at 45ºC and dynamically purged with 45 mL/min nitrogen for 120 min; 2- Volatiles were trapped on a 100 mg Tenax cartridge. **Simultaneous distillation-extraction (SDE)** 1- 8g of sample dissolved with pentane were distillated;	**Thermal desorption (TD)** **Equipment:** Perkin Elmer ATD 400 **Primary desorption:** cartridge was heated to the desired temperature 8 DTD-65ºC; DHS- 220ºC; SDE- 220ºC) with 45 mL/min He Volatiles were trapped on a Tenax GC cold trap (-30ºC) **Secondary desorption:** Tenax GC was heated at 30ºC/s up to 300ºC Volatiles were transfer to the GC column through a heated (225ºC) fused-silica transfer line. **GC-MS** **Equipment:** Fisons 8000GC **Column:** homemade FFAP/OV-1 (57:43, w/w), 25m x 0.25 mm i.d., 0.25 µm thickness **Carrier gas:** helium **Injector pressure:** 54 kPa **Detector mode:** electron-ionisation, 70eV **Oven program:** 10min at 55ºC, 3ºC/min to 180ºC, 10 min at 180ºC. **Detection mode:** full scan t_r = 7.95 min	Valero, E., **Journal of Chromatographic Science**, 2001, 39, 222-228

Table 2. Abstract of the revised literature on analytical methods of the model migrants

	2- 1 µL of the distillate was introduced into a desorption cartridge (packed with silanized glass wool by a microsyringe). **Supercritical Fluid infusion:** 1- 1 g of LDPE was placed in a 3 mL extraction cell with 10 µL of the volatiles. 2- Cell was filled with SFE-grade CO_2, pressurized, and sealed. <u>Infusion conditions:</u> Equipment: Autoprep 44 supercritical fluid extraction system Duration: 2-90 min Temperature: 40-100ºC Pressure: 100-400 atm Rate of depressurisation: 15-45 atm/min Quantity of volatile/g polymer: 1-100µL Quantity of limonene/g polymer: 0-20µL 3- The infused polymer was washed 3 times with hexane to remove surface-coated volatiles; 4- Polymer was stored at room temperature until extraction and analysis. **Volatile extraction** 1- 200 mg of infused polymer was added to 500 µL methanol (with 100µg/mL of ethyl hexanoate internal standard); 2- Samples were sealed and extract overnight at room temperature.	**GC-MS** **Equipment:** Fisons GC 8000-MD800 **Column:** DB-5, 30m x 0.25 mm i.d., 1.0 µm thickness **Carrier gas:** helium, **Carrier gas pressure:** 25 kPa **Injector mode:** splitless **Injected volume:** 1µL **Injector temperature:** 240ºC **Oven program:** 2min at 40ºC, 8ºC/min to 160ºC. **Detection mode:** full scan	Avison, S. J. et al, **J. Agric. Food Chem.,** 2001, 49, 270-275

Table 2. Abstract of the revised literature on analytical methods of the model migrants

	SPME extraction: 2g cheese were place in a 10 ml flash. Fiber: - 100 µm dimethylsiloxane (DVB) - 85 µm polyacrylate (PA) - 65 µm PDMS/divinylbenzene (PDMS/DVB) - 75 µm Carboxen/DVB Adsorption: Fiber was exposed to the sample headspace during 10 min at 20ºC. Desorption: into the injection port at 260ºC PDMS; PDMS/DVB and PA and 280ºC for Carboxen/PDMS for 5 min.	**GC-MS** **Column:** SPB-5, 60m x 0.32 mm i.d., 1.0 µm thickness **Carrier gas:** helium N55 **Carrier gas pressure:** 0.6 bar **Injector mode:** splitless **Injector temperature:** 280ºC **Detector mode:** electron impact, 70eV **Oven program:** 5min at 40ºC, 3ºC/min to 200ºC, 5 min at 200ºC **Detection mode:** full scan, m/z 15-230 t_r = 36.61 min	Peres, C. et al, **Anal. Chem.,** 2001, 73 (5), 1030-1036.
	Dynamic extraction: 5g of cheese were placed in a 20 mL vial sealed with a Teflon septum cap. **Headspace stabilization**: 20min at 30ºC. **Extraction /trapping** : septum was pierced with a double syringe: one injected helium (120mL/min for 2 min at 30ºC) and the other sent the headspace through an absorbent trap. **Desorption**: trap was heated ohmically to 220ºc for 3 min under a stream of helium (0.8 bar). Substances were transferred to the source of mass spectrometer via a deactivated silica transfer line heated to 180ºC in a chromatograph oven.	**GC-MS** **Column:** SPB-5, 60m x 0.32 mm i.d., 1.0 µm thickness **Carrier gas:** helium N55 **Carrier gas pressure:** 0.6 bar **Injector mode:** split, 9:1 **Injector temperature:** 280ºC **Detector mode:** electron impact, 70eV; full scan m/z 15-230 **Oven program:** 5min at 40ºC, 3ºC/min to 200ºC, 5 min at 200ºC t_r = 36.78 min	Peres, C. Et al, **Anal. Chem,** 2002, 74 86), 1386-1392

Table 2. Abstract of the revised literature on analytical methods of the model migrants

	Liquid extraction -<u>Fresh kiwi fruit puree:</u> 1- Cyclohexanone (10ppm) was added to 120g of fresh puree; 2- Volatiles were extracted with 80 ml methylene chloride by stirring for 1 h with a magnetic stirrer at 2ºC; 3- Mixture was centrifuged for 10 min (3000g at 5ºC); 4- Organic layer was dried with sodium sulfate and concentrated to 1 ml using a distillation- rectification system, follow by concentration to 0.1 mL using a flow of nitrogen. -<u>Commercial kiwi essence:</u> 1- 3 mL of essence was added to 10 ppm cyclohexanone; 2- Mixture was extracted twice using 2 ml of methylene chloride for each extraction and mixed between two glass syringes connected by a stainless steel lock adaptor and combined; 3- Final emulsion was centrifuged for 10 min (3000g at 5ºC); 4- Organic layer was dried and concentrated to 0.1 mL using a flow of nitrogen.	**GC-MS** **Equipment:** Agilent 6890/5973N **Column:** cross linked phenyl-methyl siloxane column, 30m x 0.25 mm i.d., 0.25 µm thickness **Flow rate:** 1 mL/min **Injector mode:** split, 10:1 **Injector temperature:** 250ºC **Ionisation source:** 280ºC **Solvent delay:** 2 min **Injected volume:** 2µL **Detector mode:** 5.27 scans/s; full scan m/z 35-300 **Oven program:** 6min at 40ºC, 2.5ºC/min to 150ºC, 90ºC/min to 250ºC	Jordan, M. J. Et al**, J. Agric. Food Chem., 2002,** 50, 5386-5390
DIPN Diisopropylnaphthlene (alkylated naphthalenes are solvents widely employed in the paper industry in the manufacture of carbonless copy paper and thermal paper; also used for the production of dielectric fluid and thermal oil substituting polychlorinated	**Extraction from paper and board prior of GC-MS analysis:** 1-2 dm^2 of sample (paperboard) were extracted using dichloromethane and ethanol by Soxhlet extraction.	**GC-MS-** compounds identification **Column:** HP5-MS, 30 m x 0.25 mm i.d., 0.25 µm **Detector mode:** full scan; m/z 50-500 **Carrier gas pressure**: 12.7 psi **Flow rate:** 1.2 mL/min **Injection volume:** 1 µL **Injector temperature:** 280ºC **Injection mode:** splitless **Headspace injection:** incubation for 1h at 120ºC; pressurization time 0.3 min, injection time 0.1 min.	Summerfield, W. and Cooper, I., **Food Additives and Contaminants**, 2001, 18(1), 77-88.

Table 2. Abstract of the revised literature on analytical methods of the model migrants

biphenyls-Boccacci M. et al, Food Additives and Contaminants, 1999, 16 (5), 207-213)	**Quantified components:** - DIPN - DBP (di-n-butyl phthalate) - DIBP (diisobutyl phthalate) **Extraction from paper and board prior of HPLC analysis:** 1 g of paper was immersed in 20mL of absolute ethanol in a 20 mL vial and agitated in an ultrasonic bath (1h). **Extraction from food and simulant prior to HPLC analysis:** **1-** 0.3dm^2 of paperboard was exposed to: - 10g of icing sugar / flour / cake - 5g of pizza base - 14g of pastry - 25g of rice - 2g of Tenax (simulant) **2-** Test were conducted at: - 20ºC /6 months - 10d/40ºC - 0.5h /150ºC - 0.5h/100ºC - 24h/40ºC **3- A)** Food containing a significant fat level (pastry and cake): were extracted with acetonitrile, filtered and injected; **B)** Tenax and dry food: were extracted in an ultrasonic bath with absolute ethanol.	**Detector temperature:** 280ºC **Oven temperature:** 2 min at 40ºC, ramp to 300ºC at 10ºC/min. **HPLC-FL-** DIPN quantification **Column:** Spherisorb ODS-2, 5µm particle size, 250 mm x 4.6mm i.d. **Mobile phase:** 70% acetonitrile, 30% water (v/v) **Volume injected:** 10 or 20 µL $\lambda_{excitation}$= 232 nm $\lambda_{emission}$= 338 nm DIPN isomers elute from 25-31 min RSD= 8%	

Table 2. Abstract of the revised literature on analytical methods of the model migrants

	Extraction from food-stuffs 1 dm^2 of artificially contaminated paperboard was put into contact with 50g of foodstuff samples: - husked rice - wheat semolina - pasta - egg pasta - maize flour and tested at intervals of 0, 3, 9, 15, 24, 40 and 60 d. DIPN was extracted according Sturaro et al (1994).	**GC-FID** **Column:** OV1, 50 m x 0.25 mm, 0.5 µm thickness **Injection mode**: splitless- 60s of closing time **Carrier gas:** hydrogen **Detector:** FID **Oven program:** 1 min at 40ºC, ∞ºC/min to 140ºC, 2 min at 140ºC, 2ºC/min to 200ºC. **Flow rate:** 1mL/min **GC-MS-** compounds confirmation **Column:** SPB 608 Supelco, 30 m x 0.25 mm i.d., 0.25 µm **Injection mode**: splitless- 60s of closing time **Detector mode:** electron ionisation, 70eV, 400µA of trap current **Flow rate:** 1.0 mL/min **Carrier gas:** helium **Oven temperature:** 2 min at 50ºC, ramp to 140ºC at 50ºC/min, 2 min at 140ºC, 2ºC/min to 240ºC t_r = 23.58 min m/z = 212.156; m/z = 197.133	Boccacci, M. et al, **Food Additives and Contaminants**, 1999, 16 (5), 207-213.
	Migrants: - o-xylene - acetophenone - benzoic acid - dodecane - naphthalene - vanillin - diphenyloxide - 2,3,4-trichloroanisole (2,3,4-TCA) - **benzophenone** - **DIPN** - Dibutyl phthalate (DBP) **Migrants determination in paper samples:** **1-** Contaminated paper strips (1cm^2-	**GC-FID** **Equipment:** Fisons 9000 **Column:** DB-1, 30 m x 0.32 mm, 0.25 µm thickness **Injection mode**: split, 15mL/min **Injector temperature:** 240ºC **Injected volume:** 1µL **Carrier gas:** helium **Detector:** FID **Detector temperature:** 290ºC **Oven program:** 3 min at 60ºC,10 ºC/min to 270ºC, 3 min at 270ºC. **Flow rate:** 1.45 mL/min **GC-MS-** compounds confirmation **Equipment:** Hewlett-Packard HP 6890 series **Column:** HP-5MS, 30 m x 0.25 mm i.d., 0.25 µm **Injector temperature**: 240ºC	Triantafyllou, V.I. et al, **Analytica Chimica Acta**, 2002, 467, 253-260

Table 2. Abstract of the revised literature on analytical methods of the model migrants

	with a migrant concentration of 250mg/L) were placed in 5mL vials; **2-** 4ml of ethanol were added and 0.5 mL of internal standard (BHT-112mg/L); **3-** Extraction was performed with gentle agitation of the vials for 1h at room temperature; **4-** Ethanol extracts were analysed by GC-FID and GC-MS. **Migrants determination in Tenax**: Paper strips were put into contact with Tenax (simulant for fatty food) for different contact periods (<4h) at 70ºC and 100ºC .	**Injection mode**: split, 15 mL/min **Injected volume**: 1µL **Detector mode:** electron ionisation, 70eV **Interface temperature:** 280ºC **Ion source temperature:** 230ºC **Flow rate:** 0.7 mL/min **Carrier gas:** helium **Oven temperature:** 3 min at 60ºC, ramp to 270ºC at 10ºC/min, 3 min at 270ºC. t_r = 23.58 min m/z = 212.156; m/z = 197.133	
Laurolactam Nylon 12 monomer	<u>Polymer:</u> - Nylon 12 <u>Simulants:</u> - isooctane - 10% aq ethanol - 50% aq ethanol - 95% aq ethanol - olive oil <u>Conditions:</u> - 10 d at 40ºC <u>Internal standard:</u> Capryllactam (210µg/mL) **1-** 1g oil was dissolved in eluent and filled up to a total weight of 10g and injected in the SEC; **2-** Fraction eluted between 23-34 min was collected (~75 mL) and evaporated in a rotary evaporator at 60ºC to about 1mL;	**GC-FID** **Equipment:** Hewlett Packard 5890 **Column:** DB624, 30 m x 0.32 mm, 1.8 µm thickness **Injection mode**: split 1:20 **Injector temperature:** 220ºC **Carrier gas:** hydrogen **Column head pressure:** 65 kPa **Detector:** FID **Detector temperature:** 240 ºC **Oven program:** 2 min at 180ºC, 10ºC/min to 240ºC, 5 min at 240ºC **Injected volume**: 1 µL $t_{r\ laurolactam}$= 9.5 min $t_{r\ capryllactam}$= 6.6 min LOD = 0.04µg/mL LOQ= 0.15µg/mL **Preparative HPLC** **1-** 50g or 50 dm^2 of polyamide 12 were extracted with 500mL of 95% ethanol/water mixture for 3 d at 60ºC **2-** Extract was evaporated to dryness and residue purified **Equipment:** Shimazu fraction collector (FRC-10A)	Stoffers, N. H., Brandl, F., et al **Food Additives and Contaminants,** 2003**,** 20, (4), 410-416

Table 2. Abstract of the revised literature on analytical methods of the model migrants

	3- Then was quantitatively transferred into a 10mL flask and dried using a stream of nitrogen; **4-** Residue was redissolved in 10mL methanol and injected in the HPLC/MS. Isooctane samples and standards (0.5mL) were evaporated using a stream of nitrogen and redissolved in 0.5mL of methanol prior to injection.	**Injected volume:** 50µL Fractions belonging to the peaks were collected **HPLC-UV** **Equipment:** Shimazu Class 10A **Column:** Hypersil ODS5 5µm particle size, 125 mm x 4 mm i.d. **Column temperature:** 40ºC **Flow rate:** 1mL/min **Mobile phase:** gradient from 70% water/ 30%methanol to 10% water/90% methanol in 8 min, where was held for 10 min. **Volume injected:** 50µL λ = 207 nm $t_{r\ laurolactam}$= 9.4 min; LOD = 1.5 µg/mL; LOQ = 5.7 µg/ mL $t_{r\ dimer}$= 10.8 min; LOD = 1.2 µg/mL; LOQ = 4.4 µg/mL $t_{r\ trimer}$= 12.2 min; LOD = 0.5 µg/mL; LOQ = 2.0µg/mL $t_{r\ tetramer}$= 12.9 min **SEC** **Equipment:** Gynkotek N480 **Column:** Pharmacia XK26/40, 26mm i.d., bed height 370mm, filled with Bio-beads SX-3 **Mobile phase:** ethyl acetate/cyclohexane (56:44) **Flow rate:** 3mL/min **Injected volume**: 4mL **HPLC-MS** **Equipment:** Thermo Finnigan LCQ Deca **Ionisation:** APCI (atmospheric pressure chemical ionisation) **Vaporizer temperature:** 450ºC **Corona current:** 5.0 mA **Heated capillary:** 225ºC **Scan range:** 100-1200 **Column:** Hypersil ODS5 5µm particle size, 125 mm x 4 mm i.d. **Flow rate =** 0.8 mL/min **Mobile phase:** 70% water/ 30%methanol for 3 min, gradient to 10% water/90% methanol in 6 min, where was held for 6 min returning to 70% water/ 30%methanol for 10 min. **Volume injected:** 15µL $m/z_{\ laurolactam}$ = 198.2 $m/z_{\ dimer}$ = 395.4	

Table 2. Abstract of the revised literature on analytical methods of the model migrants

		m/z_{trimer} = 592.6 $t_{r\ laurolactam}$= 8.1 min; LOD = 0.05 µg/mL; LOQ = 0.2 µg/ mL $t_{r\ dimer}$= 8.9 min; LOD = 0.04 µg/mL; LOQ = 0.17 µg/ mL $t_{r\ trimer}$= 9.9 min; LOD = 0.03 µg/mL; LOQ = 0.14 µg/ mL $t_{r\ tetramer}$= 10.8 min In olive oil: LOD = 0.57µg/g LOQ = 2.1µg/g	
Triacetin 1,2,3-propanetriol, triacetate	**Extraction**: 1- 150 mL of aq 0.5 N sulphuric acid and a few glass beads were added to a distillation flask; 2- 25 mL of water were added to a flask at the condenser outlet end of the steam distillation unit. The end of the outlet tube was immersed in the water; 3- Steam distillation unit collected 200 mL of distillate. 4- The distillate was added to a separatory funnel and 50 ml of methylene chloride were added. 5- The separatory funnel was shaken and then the methylene chloride was drained off. 6- The extraction was repeated 3 times and extracts combined; 7- The sample was evaporated in a rotary evaporator to 5 mL; 8- The residual was transfer for a centrifuge tube with 2 x 2 mL rinses of methylene chloride; 9- The sample was evaporated under a constant stream of nitrogen to 1 mL; transferred for an autosampler vial and injected.	**GC-FID** **Column:** HP-101 fused silica **Injection mode**: split 1:20 **Injector temperature:** 220ºC **Carrier gas:** helium **Flow rate:** 2.3 mL/min **Detector:** FID **Detector temperature:** 250 ºC **Oven program:** 2 min at 70ºC, 5ºC/min to 220ºC, 3 min at 220ºC **Injected volume**: 1 µL	Health Canada. Tobacco control programme. 1999 (http://www.hc-sc.gc.ca/hecs-sesc/tabac/pdf/T-311e4.PDF)

Table 2. Abstract of the revised literature on analytical methods of the model migrants

ATBC (Acetyl tributyl citrate) Plasticizer (improve properties such as flexibility, elasticity and processibility) Generally used in Saran (trade name for vinylidene chloride films copolymerised with up to 20% vinyl chloride).	**Migration**: 1- Circular pieces of PVDC/PVC (with 5% of ATBC) were brought into two-side contact with 105 mL of olive oil; 2- Samples were irradiated at 0 to 2ºC with high-energy electron beam radiation (10MeV) at 20 and 50 kGy; 3- Samples were stored at 18 to 20ºC and oil analysed between 1 and 288h.	**GC**	Goulas, A. E., et al, **Journal of Food Protection**, 1998, 61 (6), 720-724
	Extraction and clean-up: 1- Internal standard (deuterated-ATBC-1mg/mL in acetone-hexane 1:1) was added to homogenized food (30-50g); 2- 150mL of acetone-hexane (1:1, v/v) were added and mixture for 5 min in a homogeniser; 3- Supernatant was decanted from the residue and extraction repeated (with 150mL of solvent); 4- After combining the extracts , solution was dried over sodium sulphate and evaporated to dryness on a rotary evaporator; 5- A portion of the extracted lipid was dissolved in dichloromethane-cyclohexane (1:1, v/v) to a final solution of 0.3g lipid/mL ; 6- After centrifugation, solution was cleaned-up by size-exclusion chromatography (SEC). <u>Food items used:</u> cheese, fruit, vegetables, soups, cakes, puddings and meat dishes	**SEC** **Column:** Pharmacia, 1m x 25mm i.d., filled with bed 80cm Bio-beads SX-3 **Mobile phase:** dichloromethane/cyclohexane (1:1,v/v) **Flow rate:** 3mL/min **Injected volume**: 1.5mL **Collected fraction:** 186-204 mL Collected fraction was evaporated to dryness and transferred to a small vial using acetone. Samples were taken to dryness under nitrogen and stored at –20ºC prior to GC/MS analysis. **GC-MS** **Equipment:** Carlo Erba 4160 GC **Column:** BP5, 12 m x 0.33 mm i.d. **Injection mode**: on-column **Detector mode:** electron ionisation, 70eV with 250µA trap current **Ion source temperature:** 250ºC **Flow rate:** 4 mL/min **Carrier gas:** helium **Oven temperature**: ramp from 70ºC to 210ºC at 39ºC/min, 7 min at 210ºC. m/z = 185 (fragment ion for ATBC)	Castle, L. et al, **Journal of Chromatography,** 1988, 437, 281-286.

Table 2. Abstract of the revised literature on analytical methods of the model migrants

	Determination in fat: 1- Internal standard (1-60 ppm di-2-ethylhexyladipate solution in HB 307-mixture of triglycerides) and an antifoam solution were added to 10 mL of fat; 2- The vessel was connected to a Tenax absorption column and subsequently heated at 210ºC in an oil-bath. While heating the vessel nitrogen was passed through the fat at 170mL/min for 30min; 3- Tenax (15 mg) was extracted with 1-3 mL hexane; 4- Hexane extract was analysed by GC-MS. **Determination in fatty food** 1- Fat content of the food was determinate; 2- A sample food (containing at least 10 mL of fat) was mixed with twice its weight of dry sodium sulphate; 3- Mixture was extracted with diethyl ether in a Soxhlet apparatus for 6-7h. 4- Ether was removed by evaporation.	**GC-MS** **Equipment:** Finnigan 4000 GS-MS **Column:** DB5, 30 m x 0.32 mm i.d. **Injection mode**: split **Injected volume:** 1µL **Carrier gas:** helium **Oven temperature**: 3 min at 60ºC, ramp to 300ºC at 40ºC/min, 15 min at 300ºC. m/z = 129 and 185 (fragment ion for ATBC)	Van Lierop, J. B. H. et al, **Journal of Chromatography**, 1988, 447, 230-233.
	<u>Migrants:</u> - 6 adipated (DMA, DEA, DBA, DIBA, DEHA, DIDA) - 3 phthalates (DEHP, DINP, DIDP) - 1 Citrates (ATBC) Plastisols were preparing with the plasticizer (with 50 parts per hundred of ATBC).	Microwave sample preparation system **Equipment:** MSP-1000 **Extraction temperature:** 120ºC **Extraction time:** 10 min SFE system **Equipment:** SFX 220 **Extraction temperature:** 95ºC **Extraction pressure:** 7000 psi **Extraction time:** 2 min of static time and then 30 min of dynamic	Cano, J.M. et al, **Journal of Chromatography A,** 2002, 963, 401-409.

Table 2. Abstract of the revised literature on analytical methods of the model migrants

	Microwave-assisted extraction (MAE) 0.5 g of sample (cut into pieces) were put in a vessel containing 25mL of methanol and extracted by a microwave sample preparation system. **Supercritical Fluid extraction (SFE):** 0.2 g of sample were placed inside an extraction cartridge.	**GC-FID** **Equipment:** Shimadzu GC-9A **Column:** SPB-5, 15 m x 0.53 mm, 0.50 µm thickness **Injector temperature:** 300ºC **Injected volume:** 1µL **Detector:** FID **Oven program:** 1 min at 110ºC, 10 ºC/min to 260ºC, 20 min at 260ºC.	
BHT Antioxidant According the Codex Alimentatius antioxidants limits are: 75 ppm for BHT and 125 ppm for BHA 150 ppm for gallates 200 ppm for any combination of these antioxidants (Dieffenbacher, D. **Deutsche Lebensmittel-Rundschau**, 1998, 94 (11), 381-385) ADI (daily intake) by the Joint Expert Committee on Food Additives (JECFA) of the World Health Organization (WHO): 0-0.3 mg/kg body weigh (Tombesi, N. et al, **Journal of Chromatography A**, 2002, 963, 179-183)	**Fatty food simulants** -sunflower oil -ethanol 95% **Migration**: 1- 1g of plastic were totally immersed in 300 ml of food simulant and stored under nitrogen for 7 days at 2 different temperatures: 4ºC and 20ºC; 2- Ethanol samples and film pieces were analysed after 1, 2, 3, 4 and 7 weeks of storage. Sunflower oil was analysed after 4 and 7 weeks of storage; 3- a) Ethanol samples were evaporated to dryness using a Rotavapor at 8 rpm and 30ºC and dissolved in heptane to a volume of 3 mL; b) 1g of film pieces was extracted with 2 x 20 mL heptane for 2 x 24 h. Extracts were evaporated to dryness in a Rotavapor at 6 rpm and 25ºC and dissolved with to 2ml with heptane; c) Sunflower oil was extracted according IUPAC 2.432 method	**GC-MS** **Equipment:** Hewlett-Packard GC 6890 **Column**: 50% phenyl-50 methyl polysiloxane, 30 m x 0.32 mm i.d. **Injection mode**: split, 1:20 **Injector temperature:** 290ºC **Detector mode:** ion mode **Ion source temperature:** 280ºC **Flow rate:** 1 mL/min **Carrier gas:** helium **Oven temperature**: 5 min at 100ºC, ramp to 290ºC at 50ºC/min, 15 min at 290ºC. m/z = 205 (fragment ion for BHT) LOD = 3µg/mL Recovery after ethanol evaporation = 45% ± 9%	Wessling, C., et al, **Food Additives and Contaminants**, 1998, 15 (6), 709-715.

Table 2. Abstract of the revised literature on analytical methods of the model migrants

	IUPAC method: Extraction: 1- Melt the fat sample at 10ºC above its melting point; 2- Weight 2g of sample and add 10mL of methanol. Shake vigorously (5 min) and centrifuge for 5 min at 2000 rpm; 3- Inject the upper methanol layer. For fats with melting points higher than 35ºC gently heat the portion in methanol to 40ºC with shaking.	**IUPAC method** **HPLC-UV** **Column:** Silica RP-18, 5µm particle size, 250 mm x 4 mm i.d. **Flow rate:** 6mL/min for 5 min until polar lipids are eluted **Mobile phase:** gradient from 30%acetonitrile + 1% acetic acid/ 70%water + 1% acetic acid to 100% acetonitrile + 1% acetic acid and hold for 4 min. **Volume injected:** 20µL λ = 280 nm **Alternative method:** Just change the mobile phase **Mobile phase**: gradient from 30%(methanol/ potassium dihydrogen phosphate buffer 0.02M 1:1) / 70%methanol to 100% methanol and hold for 4 min.	Dieffenbacher, D. **Deutsche Lebensmittel-Rundschau**, 1998, 94(11), 381-385
	Acetonitrile extraction from lipids: 1- 5mL of acetonitrile was added to 2g of lipids (with a known amount of BHT added); 2- Vials were tightly capped with a brief purge with N_2 stream and vigorously shaken for 2 min; 3- The extracted was filtered and injected. **Acetonitrile extraction of smoke-flavoured sausage:** 1- 5g of minced sample (with a known amount of BHT added) were homogenized in 5 mL acetonitrile and filtered through No 5C filter paper; 2- Extraction and filtration were repeated twice; 3- Filtrates were combined and made up to 25 ml;	**HPLC-F** **Equipment:** Simadzu LC 10 **Column**: Lichrosorb RP-18, 250 mm x 4.0 mm i.d., 5 µm **Mobile phase:** A- water/acetonitrile/acetic acid (66.5:25.5:5) B- acetonitrile/acetic acid (95:5) See elution program in the paper **Flow rate:** 1.0 mL/min **Injected volume:** 10 µL $\lambda_{excitation}$= 280 nm; $\lambda_{emission}$= 310 nm $\lambda_{max\ excitation\ BHT}$= 290 nm; $\lambda_{max\ emission\ BHT}$= 320 nm LOD = 810 ng Recovery (fish oil)= 99.2% with 5 µg/g Recovery (sausage) = 85.9% with 5µg/g Recovery (sausage) = 89.9% with 200µg/g Recovery (with Bligh and Dyer procedure) = 99.5% **GC-MS** (peak assignment) **Equipment:** Shimadzu QP 1000 **Column:** Quadrex 65 HT, 15 m x 0.25mm **Source:** electron impact ionisation	Yankah, V. et al, **Lipids**, 1998, 33(11), 1139-1145

Table 2. Abstract of the revised literature on analytical methods of the model migrants

	4- A solution aliquot was filtered and then injected. **Preparation of GC-MS samples:** HPLC eluants were evaporated by flushing to dryness with a N_2 stream. After dissolved in n-hexane they were subjected to GC analysis. **SPME extraction:** Fiber: 100 µm polydimethylsiloxane film Adsorption: 15mL solution was exposed to the sample during 30 min at 20-25ºC (with a magnetic stirring velocity of 2200 rpm). Desorption: into the injection port at 250ºC for 4 min.	**GC-MS** **Equipment:** HP 6890 GCS equipped with an HP 5972 MS **Ionisation mode:** scan mode, 70 eV, mass **Column:** HP-5 capillary column, 25 m x 0.25 mm i.d., 0.25 µm thickness **Carrier gas:** helium **Flow rate:** 1.0 mL/min **Oven program:** 5 min at 40ºC, 20ºC/min to 250ºC, 5 min at 250ºC. **Detector mode:** full scan, range: 35-500u LOD= 4.2 µg/L LOQ = 13.9 µg/L Recovery = 84%; LSD = 7% with 25.5 µg/L Recovery = 99%; LSD = 16% with 38.3 µg/L Recovery = 119%; LSD = 14% with 51.1 µg/L	Tombesi, N. et al, **Journal of Chromatography A**, 2002, 963, 179-183
Triclosan (TIP) **2,4,4'-trichloro-2'-hydroxydiphenyl ether** - bis-phenol and a nonionic germicide with low toxicity and a broad spectrum of antimicrobial activity; - have been incorporated into a variety of personal hygiene products: hand soaps, deodorants, shower gels, mouthwashes and toothpastes;	**Experiment 1:** Lean beef surfaces were inoculated with: - *B. thermophacta* - *Salmonella typhimurium* - *E. coli O157:H7* - *B. Subtilis* and covered with TIP, vacuum package and stored for 24 h at 4ºC. **Experiment 2:** Prerigor beef surfaces were inoculated with:	**Experiment 1:** Only *B. thermophacta* were slightly reduced. Experiment 2: There was a slight reduction in the population of the organisms after initial application with TIP. Experiment 3: There were no significant effect (P<0.05). TIP did not reduced population of *E. coli* O157:H7. -Additional experiments suggest that the presence of fatty acids or adipose may diminish the antimicrobial activity of TIP on meat surfaces.	Cutter, C. N., **Journal of Food Protection,** 1999, 62(5), 474-479.

Table 2. Abstract of the revised literature on analytical methods of the model migrants

- Incorporated in plastics (during the extrusion process) inhibits: *Brochothrix thermosphacta* ATCC 11509; *Salmonella Typhimurium* ATCC 14028; *Staphylococcus aureus* ATCC 12598, *Bacillus subtilis* ATC 6051, *Shigella flexneri* ATCC 12022, *Escherichia coli* ATCC 25922 and several strains of *E. coli* O157:H7 -triclosan is added during the extrusion of plastic and fibers to manufacture products like: cutting boards, garbage bags, carpet, surgical gauze, toothbrushes, toys and bathroom fixtures.	- *E. coli O157H7* - *Salmonella typhimurium* - *B. Thermosphacta* and incubated at 4ºc for 24h, wrapped in TIP or control plastic, vacuum packaged and stored at 4ºC for up to 14 days. **Experiment 3:** TIP wrapped, vacuum packaged beef samples were temperature abused at 12ºC and stored for 14 days.	-This study demonstrates that while antimicrobial activity is detected against bacterial cultures in antimicrobial plate assays, **plastic containing 1.5 ppm of triclosan does not effectively reduce bacterial populations on refrigerated, vacuum-packaged meat surfaces.**	

Table 3. Results of preliminary experiments on some interesting properties to develop analytical procedures

No.	MODEL MIGRANT	Spectroscopic Properties				GC-FID analysis	Remaining fraction in acetonitrile phase after 1 extraction with equal volume of olive oil
		Fluorescence	Ultraviolet	MS-APCI$^+$	MS-APCI$^-$		
1	Irganox 1076		λmax (ε): 204 (33400), 218 (10600), 273 (4000)	475, 419, 167			
2	DPBD	λex: 330 λem: 375	λmax (ε).:206 (47300), 232 (45000), 315 (92200), 328 (105900), 344(69600)			Yes	0.32
3	Chimassorb 81		λmax (ε):206 (23700), 243 (13000), 288 (16600), 324 (11100)	327, 439			0.18
4	Uvitex OB	λex: 374 λem: 432	λmax (ε):207 (41600), 263 (12100), 355 (42100), 373 (47300)	431		Yes	0.24
5	Caprolactam		λmax (ε):204 (5900), 224 (2200)	114		Yes	
6	Benzophenone		λmax (ε).:208 (27100), 251 (19900)	183, 105		Yes	0.58
7	Diphenyl Phthalate		λmax (ε):206 (45800), 227 (17100)	225, 149, 319, 177	144, 121, 148, 169		0.87
8	DEHA			129, 147, 241, 259, 113, 371		Yes	
9	Styrene	λex: 250 λem: 305	λmax (ε):208 (14000), 247 (15000)			Yes	0.53
10	Bisphenol A	λex: 280 λem: 307	λmax (ε): 207 (35000), 227 (33900), 278 (9000)		227		

Table 3. Results of preliminary experiments on some interesting properties to develop analytical procedures

No.	MODEL MIGRANT	Spectroscopic Properties				GC-FID analysis	Remaining fraction in acetonitrile phase after 1 extraction with equal volume of olive oil
		Fluorescence	Ultraviolet	MS-APCI$^+$	MS-APCI$^-$		
11	**1-Octene**		λmax (ε): 205 (9500), 222 (4200), 274 (1100)			Yes	
12	**Limonene**		λmax (ε): 204 (9700)			Yes	
13	**DIPN**						
14	**Laurolactam**		λmax (ε): 203 (7700), 222 (2900)	198		Yes	
15	**Triacetin**			159, 99, 275, 117		Yes	
16	**ATBC**			259, 185, 213, 157, 273, 329, 361, 425, 403, 217	341, 211, 111, 139, 267		
17	**BHT**		λmax (ε): 204 (25200), 217 (10000), 276 (3300)		219	Yes	0.33
18	**Triclosan**		λmax (ε): 206 (43900), 229 (16500), 281 (7000)		289, 253		0.47

Table 4. Guidelines suggested to prepare analytical procedures to determine migrants in foods

Model migrant n°	Compounds	Type of food	Sample Preparation	Analysis procedure
1	Irganox 1076	Non fatty	Extraction with weakly or non polar solvents and concentration step. Solvent change if HPLC is used (***) Extraction/dilution with polar solvents (*)	GC-FID or MS (**) Column type DB5, with medium to high phase ratio (β), on column or splitless injection mode HPLC-UV or APCI (+) (**) Column type C8 is better than a C18. Mobile phase: acetonitrile, Detection at. λ = 205 or 218 nm by UV and 475 (m/z) by MS
		Fatty	Extraction with weakly non polar solvents followed by SEC-GPC clean up and concentration step. Solvent change if HPLC is used (***) Extraction with polar solvents not miscible with fat (*)	
2	DPDB	Non fatty	Extraction with polar to non polar solvents (**)	HPLC-UV-or FL (***) Column type C18. Mobile phase: acetonitrile, Detection at. λ = 328 nm by UV and . λex = 330 nm, λem = 375 nm by fluorescence
		Fatty	Extraction with polar solvents not miscible with fat (**)	
3	Chimassorb 81	Non fatty	Extraction with weakly or non polar solvents and concentration step. Solvent change if HPLC is used (***) Extraction/dilution with polar solvents (*)	HPLC-UV or APCI (+) (***) Column type C18. Mobile phase: acetonitrile/water, Detection at. λ = 288 nm by UV and 327 (m/z) by MS GC-FID or MS (*) Column type polar, with medium to high phase ratio (β), on column or splitless injection mode
		Fatty	Extraction with weakly non polar solvents followed by SEC-GPC clean up and concentration step. Solvent change if HPLC is used (***) Extraction with polar solvents not miscible with fat (*)	

Table 4. Guidelines suggested to prepare analytical procedures to determine migrants in foods

Model migrant n°	Compounds	Type of food	Sample Preparation	Analysis procedure
4	**Uvitex OB**	Non fatty	Extraction with weakly or non polar solvents and concentration step. Solvent change if HPLC is used (***) Extraction/dilution with polar solvents (*)	HPLC-UV or Fl or APCI (+) (***) Column type C18. Mobile phase: acetonitrile, Detection at. λ = 373 nm by UV, λex = 374 nm, λem = 432 nm by fluorescence and 431 (m/z) by MS
		Fatty	Extraction with weakly non polar solvents followed by SEC-GPC clean up and concentration step. Solvent change if HPLC is used (***) Extraction with polar solvents not miscible with fat (*)	
5	**Caprolactam**	Non fatty	Extraction/dilution with polar solvents (***)	GC-FID or MS (***) Column type polar, with medium to high phase ratio (β), split or splitless injection mode HPLC-UV or APCI (+) (**) Column type C18. Mobile phase: acetonitrile/water, Detection at. λ = 224 nm by UV and 114 (m/z) by MS
		Fatty	Extraction with polar solvents not miscible with fat (***)	
6	**Benzophenone**	Non fatty	Extraction with polar to non polar solvents (**)	GC-FID or MS (***) Column type apolar, with medium to high phase ratio (β), on column or splitless injection mode HPLC-UV or APCI (+) (***) Column type C18. Mobile phase: acetonitrile/water, Detection at. λ = 251 nm by UV and 183 (m/z) by MS
		Fatty	Extraction with polar solvents not miscible with fat (**)	

Table 4. Guidelines suggested to prepare analytical procedures to determine migrants in foods

Model migrant n°	Compounds	Type of food	Sample Preparation	Analysis procedure
7	**Diphenyl phthalate**	Non fatty	Extraction with polar to non polar solvents (**)	GC-FID or MS To check HPLC-UV or APCI (+) or (-) (***) Column type C18. Mobile phase: acetonitrile, Detection at. λ = 227 nm by UV and 225 (m/z) by APCI(+) or 144 (m/z) APCI(-)
		Fatty	Extraction with polar solvents not miscible with fat (**)	
8	**DEHA**	Non fatty	Extraction with weakly or non polar solvents and concentration step. Solvent change if HPLC is used (***) Extraction/dilution with polar solvents (*)	GC-FID or MS (***) Column type apolar, with medium to high phase ratio (β), on column or splitless injection mode HPLC-APCI (+) (***) Column type C18. Mobile phase: acetonitrile/water, Detection at 129 (m/z) by MS
		Fatty	Extraction with weakly non polar solvents followed by SEC-GPC clean up and concentration step. Solvent change if HPLC is used (***) Extraction with polar solvents not miscible with fat (*)	
9	**Styrene**	Non fatty	Separation by Volatilization (***) Extraction or dilution with polar solvents (*)	GC-FID or MS (***) Column type apolar, with medium to low phase ratio (β), static or dynamic headspace HPLC-UV or Fl (**) Column type C18. Mobile phase: acetonitrile, Detection at. λ = 247 nm by UV, λex = 250 nm, λem = 305 nm by fluorescence
		Fatty	Separation by Volatilization (***) Extraction with polar solvents not miscible with fat (**)	

Table 4. Guidelines suggested to prepare analytical procedures to determine migrants in foods

Model migrant n°	Compounds	Type of food	Sample Preparation	Analysis procedure
10	**Bisphenol A**	Non fatty	Extraction or dilution with polar solvents (***)	HPLC-UV or Fl or APCI (-) (***) Column type C18. Mobile phase: acetonitrile/water, Detection at. λ = 227 nm by UV, λex = 280 nm or 225 nm, λem = 307 nm by fluorescence and 227 (m/z) by MS
		Fatty	Extraction with polar solvents not miscible with fat (***)	
11	**1-octene**	Non fatty	Separation by volatilization (***)	GC-FID or MS (***) Column type apolar, with medium to low phase ratio (β), static or dynamic headspace
		Fatty	Separation by volatilization (***)	
12	**Limonene**	Non fatty	Separation by olatilization (***) Extraction or dilution with polar solvents (acetonitrile, methanol or isopropanol) (**)	GC-FID or MS (***) Column type polar, with medium to low phase ratio (β), static or dynamic headspace
		Fatty	Separation by olatilisation (***) Extraction with polar solvents not miscible with fat (acetonitrile, methanol or isopropanol) (**)	
13	**DIPN**	Non fatty	Extraction or dilution with polar solvents (**)	GC-FID or MS (***) Column type apolar, with medium to high phase ratio (β), on column or splitless injection mode HPLC-UV or Fl or APCI (+) or (-) (***) Column type C18. Mobile phase: acetonitrile/water, Detection. To check
		Fatty	Extraction with polar solvents not miscible with fat (**)	

Table 4. Guidelines suggested to prepare analytical procedures to determine migrants in foods

Model migrant nº	Compounds	Type of food	Sample Preparation	Analysis procedure
14	**Laurolactam**	Non fatty	Extraction with weakly or non polar solvents and concentration step. Solvent change if HPLC is used (***) Extraction/dilution with polar solvents (*)	GC-FID or MS (***) Column type apolar, with medium to low phase ratio (β), split injection mode HPLC-APCI (+) (**) Column type C18. Mobile phase: acetonitrile/water, Detection at. 198 (m/z)
		Fatty	Extraction with weakly non polar solvents followed by SEC-GPC clean up and concentration step. Solvent change if HPLC is used (***) Extraction with polar solvents not miscible with fat (*)	
15	**Triacetin**	Non fatty	Extraction with polar to non polar solvents (**)	GC-FID or MS Column type apolar, with medium phase ratio (β), split injection mode HPLC-APCI (+) Column type C18. Mobile phase: acetonitrile/water, Detection at. 159 (m/z)
		Fatty	Extraction with polar solvents not miscible with fat (***)	
16	**ATBC**	Non fatty	Extraction with weakly or non polar solvents and concentration step. Solvent change if HPLC is used (***) Extraction/dilution with polar solvents (*)	GC-FID or MS (**) Column type apolar, with medium to low phase ratio (β), split injection mode HPLC-APCI (+) or (-) (**) Column type C18. Mobile phase: acetonitrile/water, Detection at. 259 (m/z) by APCI(+) or 341 (m/z) APCI(-)
		Fatty	Extraction with weaklyor non polar solvents followed by SEC-GPC clean up and concentration step. Solvent change if HPLC is used (***) Extraction with polar solvents not miscible with fat (*)	

Table 4. Guidelines suggested to prepare analytical procedures to determine migrants in foods

Model migrant nº	Compounds	Type of food	Sample Preparation	Analysis procedure
17	**BHT**	Non fatty	Extraction or dilution with polar or non polar solvents (**)	GC-FID or MS Column type apolar, with medium phase ratio (β), split or splitless injection mode HPLC-UV or APCI (-) (***) Column type C18. Mobile phase: acetonitrile/water, Detection at. λ = 217 nm by UV and 219 (m/z) APCI(-)
		Fatty	Extraction with polar solvents not miscible with fat (**)	
18	**Triclosan**	Non fatty	Extraction or dilution with polar or non polar solvents (**)	HPLC-UV or APCI (-) (**) Column type C18. Mobile phase: acetonitrile/water, Detection at. λ = 229 nm by UV and 289 (m/z) APCI(-)
		Fatty	Extraction with polar solvents not miscible with fat (**)	

(***) Appropriate option

(**) Possible option

(*) Difficult option

European Commission

EUR 22552 EN – DG Joint Research Centre, Institute for Health and Consumer Protection
Compilation of Analytical Methods for Model Migrants in Foodstuffs: Review of Analytical Methodologies
Authors: P. Paseiro - C. Simoneau - R. Franz

Luxembourg: Office for Official Publications of the European Communities
2006 – 85 pp. – 21 x 29.7 cm
EUR - Scientific and Technical Research series; ISSN 1018-5593

ABSTRACT

D2-2

Compilation of analytical methods for model migrants in foodstuffs:

Collection of method descriptions

This publication corresponds to project deliverable D2 of the EU-Project QLK1-CT2002-2390, Foodmigrosure.

The report consists of a compilation of analytical methods developed for the quantitative determination of all model migrants in selected foodstuffs. Depending on each combination analyte/food matrix, more purification steps were tailored to satisfy the method required criteria: detection limits, calibration curves linearity, repeatability, recovery and stability checks of the model migrants in the foodstuffs.

The analytical work started with a 'triangular' approach to select those three foods on each corner of the triangle representing the extremes of different food components, fat, protein and carbohydrate which could influence the sampling and work-up procedure prior to the analytical determination. 3 different foods were selected that could be purchased (with a similar specification) in different countries and were thought to represent major food categories, both with respect to consumption and towards the physical-chemical properties influencing the migration process from Food Contact Materials (FCM). In this report the collection of analytical methods for all 18 model migrants and at least for the three 'triangle corner foods' were compiled in a harmonised written format. These methods were successfully applied to all of the 25 foods selected for the project, some of them after minor modifications to meet the specific requirements of a particular foodstuff. This method compilation represents a unique collection of valuable and useful analytical methods which are applicable to any kind of foodstuffs and beyond this to other chemical compounds when considering physical-chemical analogies. The use of these methods is recommended either directly or as templates for the analysis of potential migrants from FCM in foods.

Mission of the JRC

The mission of the Joint Research Centre is to provide customer-driven scientific and technical support for the conception, development, implementation and monitoring of EU policies. As a service of the European Commission, the JRC functions as a reference centre of science and technology for the Union. Close to the policy-making process, it serves the common interest of the Member States, while being independent of special interests, whether private or national.