Risk assessment derived from migrants identified in several adhesives commonly used in food contact materials

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Keyworks

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Abstract

Adhesives are used to manufacture multilayers materials, where their components pass through the layers and migrate to the food. Nine different adhesives (acrylic, vinyl and hotmelt) and their migration in twenty one laminates for future use as market samples have been evaluated and risk assessment has been carried out. A total of 75 volatiles and non volatile compounds were identified by gas chromatography-mass spectrometry and ultra-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry. Most of the compounds migrated below their specific migration limit (SML), lowest observed adverse effect level (LOAEL), no observed adverse effect level (NOAEL) and values recommended by Cramer. Six compounds classified as high toxicity class III according to Cramer classification, migrated over their SML and exposure values recommended by Cramer, when they were applied in the full area of the packaging.

Nevertheless, these adhesives fulfill the threshold in the real application as they are applied in a small area of the packaging.

1. Introduction

Adhesives are commonly used in the packaging industry. In most of the applications, they are used to manufacture multilayer materials, where the adhesive is applied on the full area of two or more different substrates forming the laminates [substrate-adhesive-substrate]. They can be also applied on a partial area forming boxes or pouches. The substrates used can be different materials as polypropylene, polyethylene, cardboard, etc. according to the final use of the packaging (Ashley et al., 1995).

The adhesives are complex formula of substances such as a polymer, antioxidants, tackifiers, solvents, plasticizers, fillers, adhesion promoters, etc. which provide specialized functions for the adhesives (Petrie, 2000 Chapter 9, p. 319-341). Besides, they can also contain impurities from raw materials or by-products as result of a side reaction between different ingredients. These substances are called NIAS (non intentioned added substances) (Felix et al., 2012; Isella et al., 2013) which are unknown by adhesive producers.

One of the main parameters that must be considered is the potential migration of these compounds present in the adhesives to the food in contact with the multilayer materials. Although in the most common applications, the adhesive is not in direct contact with the packed food, it has been demonstrated that volatile and non-volatile compounds can migrate from the adhesive through the different layers, except aluminium, to the food

(Athenstadt et al., 2012; Aznar et al., 2011; Canellas et al., 2010a; Nerin et al., 2012; Sendon et al., 2012; Vera et al., 2011; Vera et al., 2013; Vera et al., 2014)

All components of food contact materials must comply with the Framework Regulation (EC) N 1935/2004 that requires that materials and articles, must not transfer their constituents to food in quantities which could endanger human health. However there is no specific legislation in the EU for adhesives. Manufacturers currently follow the provisions of the European "Plastics Directive" and the Spanish recent legislation governing food contact materials other than plastics . Both regulations provide positive lists of authorized substances with their specific migration limits (SML).

Previously to migration assay, it is very important to carry out a screening to identify the most of compounds present in the adhesives (Canellas et al., 2010b; Canellas et al., 2012; Nerin et al., 2009; Vera et al., 2012), and consequently, to determine their possible risks as potential migrants to the food when the laminates are used as food packaging materials. The screening of unknown non-volatile compounds involves an important analytical challenge, as it requires the most powerful techniques to provide more structural information to elucidate the molecular structure of the non volatile compounds (Zweigenbaum, 2011). The time-of-flight mass (TOF) analyzer combined with quadrupole provides the sensitivity and selectivity required for screening these types of samples. It provides the possibility of acquiring full scan mass spectra with high sensitivity and high resolution mass spectrometry of any ionizable components in the sample (Hernandez et al., 2008; Lacorte and Fernandez-Albaz, 2006).

The first aim of this work was to carry out a screening analysis of nine different adhesives in order to obtain a list of the possible migrant compounds that can be found in laminates containing these adhesives. The techniques selected to determine the volatile and semivolatile compounds were the solid phase microextraction in headspace mode coupled

to gas chromatography and mass spectrometry (HS-SPME-GC-MS) and liquid extraction coupled to gas chromatography and mass spectrometry (LE-GC-MS). The technique used to identify the non volatile compounds was ultra-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (UPLC-MS/QTOF).

Subsequently, migration experiments were carried out on twenty one laminates manufactured with the adhesives above mentioned, in order to evaluate the mass transfer of the compounds detected and the risks of exposure for future potential consumers

2. Materials and methods.

2.1. Standards

The following compounds were used as standards to confirm the identification and for calibration plots in quantitative analysis: N-butyl ether (142-91-6), 2-propenoic acid 2-methylpropyl ester (2210-28-8), Propanoic acid butyl ester (590-01-2), Butanoic acid butyl ester (109-21-7), 1-hexanol-2-ethyl (104-76-7), Acetic acid 2-ethylhexyl ester (103-09-3), 1-butoxy-2-ethylhexane, 2-propenoic acid 6-methylheptyl ester (29590-42-9), 2,4,7,9-tetramethyl,5-decyn-4,7-diol (126-86-3), Cyclododecane (294-62-2), 2,4,7,9-tetramethyl,5-decyn-4,7-diol ethoxylate (9014-85-1), Phenol, 2-(1-phenylethyl) (4237-44-9), Isopropyl myristate (110-27-0), Bis (2-ethylhexyl) maleate myristate (142-16-5), Decanal (112-31-2), Butyl acrylate (141-32-2), Mono-(2-ethylhexyl) phthalate (4376-20-9), Diacetin (25395-31-7), Triacetin (102-76-1), Butylated Hydroxy Toluene (128-37-0), Bis (2-ethylhexyl) maleate (142-16-5), 1,2,3-Trimethylbenzene (526-73-8), Decane (124-18-5), Undecane (1120-21-4), Dodecane (112-40-3), Tridecane (629-50-5), Tetradecane (629-59-4), 3,5-di-tert-butyl-4-hydroxybenzaldehyde (1620-98-0) II, Pentadecane (629-62-9), Acenaphthalene (83-32-9), Hexadecane (544-76-3), Diethyl phthalate (84-66-2),

Heptadecano (629-78-7), 3,5-di-tert-butylbenzoquinone (719-22-2), Octadecane (593-45-3), Nonadecane (629-92-5), Chrysene octahydro (2090-14-4) III, Fluorerene decahydro (5744-03-6) III, Eicosane (112-95-8), Heneicosane (629-94-7), Docosane (629-97-0), Tricosane (638-67-5), Tetracosane (646-31-1), Methyl styrene (98-83-9), Styrene (100-42-5), 3(2H)-isothiazolone, 2-methyl- (2682-20-4), 5-Chloro-2-methyl-1,2-thiazol-3(2H)-one (137662-59-0), Polyethylene glycol (25322-68-3), 5-Chloro-2-methyl-1,2-thiazol-3(2H)-one (137662-59-0) 1,2-benzothiazol-3(2H)-one (2634-33-5), Polypropylene glycol (25323-30-2), 5-chloro-2-methylisothiazol (26172-55-4), Triethylamine (121-44-8), Dimethylol propionic acid (4767-03-7), Sodium 3-[(2-aminoethyl)amino] propanoate (84434-12-8) and Adipic acid (124-04-9). All were of analytical quality.

Water and methanol of HPLC grade were supplied by Scharlau Chemie S.A (Sentmenat, Spain). Tenax TA 80/100 mesh and PDMS fiber of 100 μm of thickness were supplied by Supelco (Bellefonde, USA).

2.2. Adhesive samples and laminates.

Twenty one laminates forming the structure [substrate 1–adhesive–substrate 2] have been studied in this work. They were provided by a Spanish company for future use as food packaging. They were not printed but produced in the same run as regular packages. The substrates and the adhesives used for their manufacturing were also separately provided. Nine different adhesives had been used in the manufacture of the laminates: 4 acrylics (AC), 3 vinyl (V), and 2 hotmelts (HM). The substrates used were couche paper, mate or gloss polypropylene (PP), cellulose acetate, polyethylene terephthalate (PET), polylactic acid (PLA), offset paper and cardboard.

Table 1 shows the different laminates studied, their substrates (gramage or thickness) and adhesives used in the manufacture of these samples analyzed, and therefore the amount of adhesive applied per m^2 (gramage) of the laminate.

2.3. GC-MS

A CTC Analytics system from Agilent Technologies (Madrid, Spain) was used as autosampler. The GC system was Agilent 6890 Series connected to 5973 series mass selective detector. Chromatographic separations were carried out on a DB-5 (30 m x 0.25 mm x 0.25 μm) from Agilent Technologies (Madrid, Spain). The oven temperature program was as follows: initial temperature at 40°C (2 min), a temperature rate of 15ºC/min from 40 to 300ºC, and 2 minutes at the final temperature. Helium was used as gas carrier at a flow of 1 mL/min.

HS-SPME-GC-MS analyses were carried out with a polydimethylsiloxane (PDMS) fiber of 100 μm of thickness. Injection was performed in splitless mode and extraction conditions were as follows: 80ºC extraction temperature, 15 min extraction time and 1 min desorption time at 250 ºC. Acquisition was performed in SCAN mode (50-350 m/z). Liquid injection (LE-GC-MS) was carried out in splitless mode, 1 μL of sample was injected. Acquisition was performed in SCAN mode for identification purposes and in SIM mode for quantitative analysis.

2.4. UPLC separation.

Chromatography was carried out in an Acquity ™ system using an Acquity UPLC BEH C18 column of 17 μm particle size (2.1 mm x 100 mm), both from Waters (Milford, MA, USA). The solvents used as mobile phase were water and methanol both with 0.1 %

formic acid. The column flow was 0.3 mL/min and the column temperature was 35ºC. The gradient used here was 5-95% methanol 0.1% formic acid (0-24min) and the volume of sample injected was 5 µL.

2.5. Mass spectrometry detector/ QTOF

The detector was an API source (atmospheric pressure ionization) with an electrospray interface (ESI) coupled to a Xevo G2 mass spectrometer consisting of a hexapole, a quadrupole, a collision cell and a time of flight analyzer (QTOF) supplied by Waters (Milford, MA, USA).

The electrospray probe was used in positive (ESI+) and negative (ESI-) modes as well as in sensitivity analyzer mode. The mass range considered was from 50 to 1000 Da. Corona voltage was 2.5 kV for (ESI+) and 0.5 kV for (ESI-). The sampling cone voltage was optimized between 20 and 50V. Finally, 30 V was selected for the migration screening because more peaks were detected. Other MS parameters were as follows: the source temperature was 150º C, the desolvation gas temperature 450ºC and the desolvation gas flow 650Lh^{-1}. MS^{E} mode was selected for the acquisition, which alternates between two functions: function 1 acquiring low-energy exact mass precursor ion spectra and function 2 acquiring elevated-energy exact mass fragment ions with collision ramp energy from 5 to 40 V was used.

MassLynx v.4.1 software (Waters, Milford MA, USA) was used to analyze the samples.

2.6. Identification of the compounds presents in the adhesives samples

The first aim of this work was to identify the potential migrant compounds (volatiles and non volatile compounds) from the adhesives used in the manufacture of the laminates.

For this purpose, firstly, 1 gram of the cured adhesives was analyzed by HS-SPME-CG-MS to identify the most volatile compounds. After that, adhesives were dissolved with methanol 1:100 and analyzed by LE-GC-MS and UPLC-MS/QTOF to determine semi volatile and non-volatile compounds respectively. Two replicates of each sample were analyzed.

For the identification of volatile compounds NIST 08 mass spectral search program version 2.0 was used. This database compared the mass spectra of each peak with the spectra of the compounds contained in the library. Then the standards of the compounds were analyzed to confirm the identification.

For the identification of non volatile compounds, two software tools as Elemental composition and Mass Fragment were used to help in the structural elucidation of unknown compounds. The first one provides the most probable molecular formula for the accurate masses of the compounds (M+), protonated compounds (M+H+) and unprotonated (M−H−) and the second one provides the exact mass fragments of unknown compounds submitted to elevated-energy. Besides, Chemspider (www.chemspider.com) and Scifinder (scifinder.cas.org) databases were also used to find the molecular structure with the exact mass and the mass fragments obtained in each case.

Therefore, spectra coming from each peak were studied and molecular formulas were proposed. Then, it was necessary to use the databases mentioned above and to know the typical composition of an adhesive in order to elucidate the possible compounds that could be present in the sample. Once the candidates were proposed, the fragmentation spectra were used to work with the accurate masses of the fragments in order to find out if they could be generated from the candidates obtained in the databases and then confirm

their identification. Finally, the standards of the compounds were analyzed to confirm the identification.

2.7. Migration tests

Once the migrants were identified, the migration test was carried out. Tenax® was selected as food simulant for most of the laminates which contained paper or cardboard in their structures, and therefore, the use of liquid food simulants were not possible. Besides, these kinds of laminates will be used for dry food packaging as breadcrumbs, flour, jelly powders and mash potato and Tenax ® is recommended simulant for the migration test .

Previous to migration test, Tenax® was purified by Soxhlet extraction with acetone during 6 hours and dried.

The migration test with Tenax ® was performed following the procedure optimized in previous works (Aznar et al., 2011; Canellas et al., 2010a; Vera et al., 2011). The cutouts of each laminate and substrate, 5 x 5 cm in size, were placed in Petri dishes and covered with 1 gram of Tenax ® forming a uniform layer (4 g Tenax per dm^2 laminate in accordance with UNE-EN 14338 (2004)). Tenax® was applied on the side of the laminate that will be in contact with food (left substrates shown in Table 1). This system was kept in the oven at 40 ºC for 10 days. After that, it was extracted two consecutive times with 3.4 mL of methanol. The total solution was concentrated under a stream of N_2 to 200 µL. Finally, the extracts were analyzed by GC-MS and UPLC-MS/QTOF. Three replicates of each laminate and substrate were studied.

For building the calibration curves, solutions of the compounds at different concentration levels were prepared in methanol and analyzed by GC-MS and UPLC-MS/QTOF. Three replicates of each concentration level were analyzed to determine the reproducibility.

3. Results and Discussion

The information on adhesives is usually very restricted, as it belongs to the know-how of the producer companies. For this reason, it is very difficult to know *a priori* what kind of migrants can be expected. Thus, the first step of the study was to identify the potential migrants in the cured adhesives. Migration tests were later carried out from the multilayer structures, using Tenax as simulant. This way, the correlation between the migrants and their origin could be achieved. The following paragraphs show the results obtained.

3.1. Screening of compounds from adhesives.

The identification of these compounds was carried out by comparing their retention times and mass spectra with those of the pure standards. A total of 62 different volatile and semivolatile compounds were detected in the adhesives analyzed by GC-MS. They are shown in Table 2 and Table 3. Besides, 12 non-volatile compounds were detected in the adhesives analyzed by UPLC-MS/Q-TOF. They are shown in the table 4.

Volatile compounds

Acrylic adhesives

Twenty three compounds were detected in acrylic adhesives (Table 2). Two of these compounds could not be identified and only their main chemical structures are provided. Three compounds were classified as class III of toxicity according to Cramer rules: 2,4,7,9-tetramethyl,5-decyn-4,7-diol, 2,4,7,9-tetramethyl,5-decyn-4,7-diol ethoxylate and phenol, 2-(1-phenylethyl). The first and the second compounds are used as industrial defoaming agent and non-ionic surfactant respectively. The surfactant presents a range of ethoxylation in the 2,4,7,9-tetramethyl,5-decyn-4,7-diol molecule depending on the water solubility, foaming and wetting characteristics needed in the adhesive.

The compounds found in all acrylic adhesives were 1-hexanol-2-ethyl and acetic acid 2-ethylhexyl ester, which are probably impurities of the methyl methacrylate used to manufacture this type of adhesive (Canellas et al., 2010a).

Vinyl adhesives

Only two volatile compounds were detected in vinyl adhesives, diacetin and triacetin, which are commonly used as plasticizers or humectants in the adhesives (Aznar et al., 2011).

Hotmelt adhesives

Thirty nine compounds were detected in these two hotmelt adhesives. Four compounds were classified as class II of toxicity according to Cramer rules (BHT, 3,5-di-tert-butylbenzoquinone, cis-Jasmone and Cis-Calamenene) and nine compounds as class III (acenaphthalene, octahydro-1(2H)-naphthalenone, chrysene octahydro, fluorerene decahydro, 3,5-ditertbutyl-1,7-dihydroxi-8-methylnaphthalene, 4-(3-hydroxy-2,2,6-trimethyl-cyclohex-1-enyl) pent-3-en-3-one, 1H indene, 2,3-dihydro-1,1,3-trimethyl-3 phenyl and 2-methyl-7phenyl indole). They are polycyclic aromatic compounds which

are probably coming from the wax, oil or hydrocarbon resin used in the manufacture of this kind of adhesives. Also several alkanes were found (from decane to hexacosane) coming from the paraffin.

Non volatile compounds

Twelve non volatile compounds were detected in the adhesives; they are shown in the Table 4.

The identification of these was a tedious and complex task, which would require some software and database tools (above mentioned). Thanks to the high resolution MS and the software tools, all compounds were identified.

The spectra of each chromatographic peak were studied in order to elucidate the compounds. Firstly, taking the accurate mass of the molecular ion in each spectrum, different possibilities for molecular formula were established. Once the molecular formula of each accurate mass were known, it was necessary to use the databases of chemical compounds mentioned above and to know the typical composition of an adhesive, in order to elucidate the compounds that could be likely present in the sample and to obtain a list of candidates for the identification. Then, using the high energy function, the spectra of fragmentation were obtained. The accurate masses of the fragments were considered to find out if they could be generated from the candidates obtained in the databases and then to confirm their identification. The software MassFragment from Waters (Milford, MA, USA) was used in order to match each fragment obtained by UPLC-Q-TOF with a fragment of the candidate proposed by the databases.

Figure 1 shows the high energy spectra of the compound 1,2-benzothiazol-3(2H)-one obtained by UPLC-Q-TOF. In the figure, fragments of the molecules and accurate mass of each fragment are shown.

Four compounds classified as class III of toxicity according to Cramer were identified here. 3(2H)-isothiazolone, 2-methyl, 1,2-benzothiazol-3(2H)-one (both found in all adhesives) and 5-chloro-2-methyl-1,2-thiazol-3(2H)-one are used as biocides. The compound 2,4,7,9-tetramethyl,5-decyn-4,7-diol was also detected by GG-MS (Table 2).

3.2. Migration test.

Tenax ® was selected as food simulant because the multilayer materials contained paper or cardboard in their structures.

Migration results are shown in Tables 2, 3, and 4. The values of migration and their limit of detections are expressed as µg compound per kg of stimulant (using 6 dm^2 of laminate in contact with 1 kg of food simulant established by the EU Regulation)

In order to evaluate the possible human risk, the compounds were checked in the positive lists of both plastic legislations: European legislation 10/2011 and Spanish legislation Real Decreto 847/2011 . The migration values were compared with their SMLs.

For the rest of the identified compounds which were not in any of the positive lists, the estimated daily intake (EDI) established by the FDA (Food and Drug Administration of United States) was calculated using the following equation (FDA, 1995. Recommendations for Chemistry Data for Indirect Food Additive Petitions):

$$EDI\left(\frac{mg}{personxday}\right) = migration\ \left(\frac{mg}{Kg}\right) x1Kg(food\ intake\ per\ person\ and\ day)xCF$$

Where CF is the fraction of the daily diet expected to be in contact with a specific packaging material (for adhesives this is 0.14). The EDI values were compared with their LOAEL (Lowest observed adverse effect level) and NOAEL (No observed adverse effect level) values found in bibliography, and also with the maximum values recommended for human exposure threshold (HET) (in milligram per person per day) established by Cramer for each toxicity class. The values of daily intake for Classes I, II, and III are 1.8, 0.54 and 0.09 mg per person per day, respectively (Threshold of toxicological concern (TTC), 2005)

Volatile compounds

EDI (mg/person/day) values were calculated from the values of migration expressed as μg compound per Kg of simulant as was mentioned above. The potential risk for the human health of each group of adhesives was evaluated taking into account migration expressed as μg compound per Kg of simulant and EDI values. These values were compared with the SMLs found in the legislation, LOAEL (Lowest-observed-adverse-effect level) or NOAEL (No-observed-adverse-effect level). In case these experimental values were not available, the values found were compared to the theoretical values of Human Exposure Threshold (mg/person/day) established in the TTC according to Cramer toxicity classes.

The migration values of the laminates manufactured with acrylic adhesives are shown in Table 2. As observed, all compounds detected previously in AC02 and AC03 (from lam06 to lam15) were not found in the migration test (migration values below their LODs).

However, several compounds were found in the laminates which contained AC01 and AC04. The common migrant compounds in both adhesives were 1-hexanol-2-ethyl, acetic acid 2-ethylhexyl ester and N-butyric acid 2-ethylhexyl ester. In all cases, their migration values were higher in the lam 22 (corresponding to AC04) than in the laminates

manufactured with AC01 (from 01 to 05). This could be explained by the fact that these last laminates contained couche paper in their structures. This is a type of paper that has been coated to achieve certain qualities, such as surface gloss, smoothness or ink absorbency. Kaolinite and calcium carbonate, are the most often treatments used for coating papers in commercial printing (Canellas et al., 2010a). This could be the reason why this coating reduced the diffusion coefficients and consequently prevent or delay the migration process.

Only four compounds out of all migrant compounds appeared in the positive lists , 1-butyl acrylate (without SML), hexanol-2-ethyl (SML 30mg/Kg), 2-ethylhexyl acrylate (SML 0.05 mg/Kg) and Bis-(2-ethylhexyl) adipate (SML 18 mg/Kg). Among the rest of compounds, only three had LOAEL or NOAEL and corresponded to 2,4,7,9-tetramethyl,5-decyn-4,7-diol and 2,4,7,9-tetramethyl,5-decyn-4,7-diol ethoxylate (LOAEL of 12.000 mg/person/day for both compounds) and mono-(2-ethylhexyl) phthalate (LOAEL of 11 mg/person/day and NOAEL of 5mg/person/day). EDI values for the rest of the compounds were below the maximum values recommend by Cramer according with their toxicities

The migration of laminates 17 to 19 (laminates with vinyl adhesives) were quantified by UPLC-MS/QTOF (Table 4), because the compounds previously identified (diacetine and triacetine) had lower LODs by this technique than by GC-MS. These compounds can be used without SML restriction according to the European legislation 10/2011 .

For the laminates manufactured with hotmelt adhesives (Table 3), most of the compounds previously identified migrated to Tenax ®. Three compounds were found in the positive lists : styrene (without SML), diethyl phthalate (SML 10 µg/Kg) and methyl styrene (SML 50 µg/kg), and their values of migration were higher than their SMLs. For the rest

of compounds, alkanes had a NOAEL of 6000 mg/person/day, 1,2,3-Trimethylbenzene a NOAEL of 143 mg/person/day and LOAEL of 429 mg/person/day and acenaphthalene a NOAEL and LOAEL of 175 and 350 mg/person/day respectively. Their migration values were below these LOAEL or NOAEL. However, the migration values converted to EDI of 3,5-ditertbutyl-1,7-dihydroxi-8-methylnaphthalene, 4-(3-hydroxy-2,2,6-trimethyl-cyclohex-1-enyl) pent-3-en-3-one and 1H indene, 2,3-dihydro-1,1,3-trimethyl-3 phenyl and 2-methyl-7phenyl indole were higher than the value recommended by Cramer for the compounds of class III of toxicity (0.09 mg/person/day). It should be noted that the study was done for laminates where the surface is completely covered with adhesive. However, in the case of hotmelt adhesives, the real application is on a partial area of the packaging to provide the geometry shape for boxes or pouches and therefore, if they only cover a small part of the area (usually less than 5%) their migration will be lower than the Human Exposure Threshold established by Cramer. Therefore, the knowledge of the surface covered by the adhesive with respect to the total volume of the food packaged (Aznar et al., 2011) is required in order to recalculate these values of migration.

Non volatile compounds

The migration for non volatile compounds is shown in Table 4. , Only two compounds out of twenty one laminates studied migrated; diacetine and triacetine, for the lam17 manufacture with V01. These compounds can be used without SML restriction according to the European legislation 10/2011 as explained above. The number of non volatile compounds (2 out of 13) that migrated was lower than the number of volatile compounds (54 out of 62), since non volatile compounds have higher molecular weight and consequently, lower diffusion coefficients than volatile compounds.

Figure 2 shows the EDI (mg/person/day) of the compounds that migrated from adhesives to the food simulants over their LODs, their Cramer toxicity class and Human Exposure Threeshold (HET), provided by the Toxtree. This figure allowed us to compare the migration of different adhesives under study and to give a global overview of the study. Fifty six out of the 74 compounds identified migrated above their LOD. Most of the compounds were classified in the Cramer class I, the less toxic class.

Fifteen out of the twenty three compounds identified in acrylic adhesives migrated over their LOD, and two of them were classified as Cramer class III, the most toxic class according to this classification. Nevertheless, all these compounds migrated below their SML and LOAEL or NOAEL as explained above.

In the case of vinylics and hotmelt adhesives, all the compounds identified migrated to the food simulant. Ten compounds in hotmelt adhesives were classified as Cramer class III and the values of EDI of six of them were over the HET established by Cramer when the study was done with laminates where the surface was completely covered by adhesive. Moreover, most of the migration values of the compounds classified as Cramer classes I and II were higher in hotmelt adhesives than in acrylic or vinylic adhesives.

Conclusion

The migration from nine different types of adhesives used to manufacture twenty one laminates for food contact material and the risk assessment have been studied. The packaging samples have been analyzed by GC-MS and UPLC-MS/QTOF. These methodologies have demonstrated to be useful and sensitive techniques, not only to identify the most of volatile and non volatile compounds present in the adhesives, but also, to evaluate the concentration of migrants in order to estimate the risk assessment. A wide range of compounds were detected, some of them of class III of toxicity. Most of

the compounds did not appear in the positive list of compounds for European legislation neither in the Spanish legislation concerning adhesives, but their migration were below the LOAEL, NOAEL or the values recommended by Cramer. Only, two laminates manufactured with hotmelt adhesives did not comply with the legislations when the adhesive was applied on the whole packaging surface, because the migration values of diethyl phthalate and methyl styrene were higher than their SML. Also, the migration of several compounds of class III of toxicity was higher than 0.09 mg/person/day recommended by Cramer. Therefore, it is necessary to recalculate the values of migration using the real proportion of the surface covered by the adhesive with respect to the volume of food contained in the packaging. In most of the applications, these types of adhesives were used to give the geometric shape to pouches and boxes, where the adhesive is applied on a small area (less than 5%). Subsequently, the adhesives here studied comply with Regulation (EC) No 1935/2004, since the migration of compounds coming from the adhesive does not endanger the human health.

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Table1: Sample code, substrates, adhesives used for the laminates manufactured and grams of adhesive per $m^2$of laminate.

Sample code	Substrates	Adhesive type	Adhesive code	Gramaje of adhesive (g/m^2)
Lam01	Couche Paper ($60g/m^2$) / matte PP (15 µm)	Acrylic	AC01	11
Lam02	Couche Paper ($60g/m^2$) / gloss PP (12 µm)	Acrylic	AC01	11
Lam03	Couche Paper ($60g/m^2$) / cellulose acetate (15 µm)	Acrylic	AC01	11
Lam04	Couche Paper ($60g/m^2$) / PET (12 µm)	Acrylic	AC01	11
Lam05	Couche Paper ($60g/m^2$) / PLA (20 µm)	Acrylic	AC01	11
Lam06	Couche Paper ($60g/m^2$) / matte PP (15 µm)	Acrylic	AC02	11
Lam07	Couche Paper ($60g/m^2$) / gloss PP (12 µm)	Acrylic	AC02	11
Lam08	Couche Paper ($60g/m^2$) / cellulose acetate (15 µm)	Acrylic	AC02	11
Lam09	Couche Paper ($60g/m^2$) / PET (12 µm)	Acrylic	AC02	11
Lam10	Couche Paper ($60g/m^2$) / PLA (20 µm)	Acrylic	AC02	11
Lam11	Couche Paper ($60g/m^2$) / matte PP (15 µm)	Acrylic	AC03	11
Lam12	Couche Paper ($60g/m^2$) / gloss PP (12 µm)	Acrylic	AC03	11
Lam13	Couche Paper ($60g/m^2$) / celullosa acetate (15 µm)	Acrylic	AC03	11
Lam14	Couche Paper ($60g/m^2$) / PET (12 µm)	Acrylic	AC03	11
Lam15	Couche Paper ($60g/m^2$) / PLA (20 µm)	Acrylic	AC03	11
Lam16	Offset paper ($80g/m^2$) / PET (12 µm)	Acrylic	AC04	300 µm
Lam17	Offset paper ($80g/m^2$) /PET (36 µm)	Vinylic	V01	300 µm
Lam18	Offset paper ($80g/m^2$) /PET (36 µm)	Vinylic	V02	300 µm
Lam19	Offset paper ($80g/m^2$) /PET (36 µm)	Vinylic	V03	300 µm
Lam20	Offset paper ($80g/m^2$) /offset paper ($80g/m^2$)	Hotmelt	HM01	180
Lam21	Offset paper ($80g/m^2$) /offset paper ($80g/m^2$)	Hotmelt	HM02	180

Table 2: Volatile compounds identified by GC-MS on acrylic adhesives and their toxicity class according with Cramer Class (TC). Their LODs expressed as µg compound per Kg and their values of migration expressed also as µg compound per Kg of simulant for the different laminates manufacture with these acrylic adhesives by GC-MS.

N	Compound (TC)	LOD	AC01					AC02					AC03					AC04
			Lam 01	Lam 02	Lam 03	Lam 04	Lam 05	Lam 06	Lam 07	Lam 08	Lam 09	Lam 10	Lam 11	Lam 12	Lam 13	Lam 14	Lam 15	Lam 16
1	N-butyl ether (I)	0.35						<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	442±74
2	2-propenoic acid 2-methylpropyl ester (I)	1.80	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	
3	Propanoic acid butyl ester (I)	1.01						<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	345±48
4	Butanoic acid butyl ester (I)	13.8						<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	201±52
5	1-hexanol-2-ethyl (I)	7.42	142±11	180±14	143±12	51.0±4.1	8.2±1.1	<LOD	<LOD	<LOD	<LOD	7.2±1.0	<LOD	<LOD	<LOD	<LOD	<LOD	1030±190
6	Acetic acid 2-ethylhexyl ester (I)	0.16	112±8.7	115±9.2	103±8.3	101±8.1	2.4±4.1	<LOD	<LOD	<LOD	<LOD	3.4±1.1	<LOD	<LOD	<LOD	<LOD	<LOD	632±130
7	1-butoxy-2-ethylhexane (I)	3.50	<LOD	<LOD	<LOD	<LOD	<LOD											
8	2-propenoic acid 6-methylheptyl ester (I)	1.80	51.4±4.2	103±4.2	94±7.5	105±8.4	2.8±3.1											
9	Unknown 1 (structure of ester)(I) [1]		121±8.5	110±8.8	103±8.2	105±8.3	2.9±5.0											
10	N-butyric acid 2-ethylhexyl ester (I)[2]	4.2	32.3±2.6	74.5±5.9	51.2±4.1	60.6±4.8	6.0±2.1											74.6±18
11	2,4,7,9-tetramethyl,5-decyn-4,7-diol (III)	0.52	852±59	284±23	266±21	141±11	22.3±1.6											
12	Unknown 2 (structure of ester) [1]		<LOD	<LOD	<LOD	<LOD	<LOD											
13	Cyclododecane (I)	1.30	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	
14	2,4,7,9-tetramethyl,5-decyn-4,7-diol ethoxylate (III)	0.53	102±7.1	104±8.3	100±7.5	110±8.4	3.8±1.1											
15	Phenol, 2-(1-phenylethyl) (III)	2.10						<LOD	<LOD	<LOD	<LOD	<LOD						
16	Isopropyl myristate (I)	8.40						<LOD	<LOD	<LOD	<LOD	<LOD						
17	Bis (2-ethylhexyl) maleate myristate (I)	8.40	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD					
18	Decanal (I)	5.42											<LOD	<LOD	<LOD	<LOD	<LOD	
19	Butyl acrylate (I)	2.07																110±13.8
20	2-ethylhexyl acrylate (I)[2]																	47.3±2.9
21	Pentyl propanoate (I) [3]																	145±34
22	Bis-(2-ethylhexyl) adipate (I) [2]																	19.2±5.9
23	Mono-(2-ethylhexyl) phthalate **(I)**	0.38																314± 64

[1] Quantified with Acetic acid 2-ethylhexyl ester, [2] quantified with 2-ethyl-hexyl acetate and [3] quantified with propanoic acid butyl ester.

Table 3: Volatile compounds identified by GC-MS on hotmelt adhesives and their toxicity Cramer class (TC). Their LODs expressed as µg compound per Kg and their values of migration expressed as µg compound per Kg of simulant for the different laminates manufacture with these adhesives by GC-MS.

N	Compound (TC)	LOD	Lam20	Lam21
24	N-butyl ether (I)	0.35	34.5±0.38	117±3.98
25	1,2,3-Trimethylbenzene(I)	0.49	6.63±1.64	34.3±5.65
26	Decane (I)	0.62	3.02±0.21	
27	Undecane (I)	0.51	1.78±0.55	1.74±0.57
28	Dodecane (I)	0.07	3.90±0.36	19.8±6.06
29	Tridecane (I)	0.06	4.35±1.37	8.96±2.53
30	Tetradecane	0.05	19.6±5.68	253±76.2
31	3,5-di-tert-butyl-4-hydroxybenzaldehyde (II)	0.17	34.6±8.48	43.2±12.0
32	Pentadecane (I)	0.07	5.34±1.31	8.54±2.63
33	Acenaphthalene (III)	4.02	92.0±16.3	49.9±16.7
34	Hexadecane (I)	0.08	83.0±15.5	1130±173
35	Diethyl phthalate (I)	0.48	10.4±1.87	34.7±11.8
36	Heptadecano (I)	0.08	6.22±1.66	10.6±3.56
37	Octahydro-1(2H)-naphthalenone [1] (III)		75.9±26.2	110±18.3
38	3,5-di-tert-butylbenzoquinone (II)	2.38	136±7.32	159±44.3
39	cis-Jasmone [1] (II)		24.2±5.44	243±43.7
40	Octadecane (I)	0.11	275±88.6	2190±401
41	1,1,6-Trimethyl-1,2,3,4-tetrahydronaphthalene [2] (I)	6.20	607±98.0	762±161
42	Cis-Calamenene (II) [2]		450±95.2	645±102
43	Camphor (III) [1]		68.0±14.1	85.8±20.5
44	Nonadecane (I)	0.11	10.0±1.52	10.7±3.07
45	Chrysene octahydro (III)	1.82	11.5±2.41	14.2±2.02
46	Fluorerene decahydro (III)	28.1	28.0±6.70	23.7±5.65
47	Fenchane [3](I)		53.2±10.1	71.8±15.3
48	Eicosane (I)	0.11	502±103	1010±703
49	Heneicosane (I)	0.10	62.1±10.8	45.1±12.2
50	Docosane (I)	0.09	1023±180	5610±876
51	Tricosane (I)		74.0±11.9	55.1±14.3
52	Tetracosane (I)	0.09	560±58.1	2240±421
53	Pentacosane [4](I)		69.6±3.65	43.6±8.52
54	3,5-ditertbutyl-1,7-dihydroxi-8-methylnaphthalene [5] (III)		301±62.3	
55	Hexacosane [4](I)		139±16.7	45.3±8.51
56	Methyl styrene (I)			161±28.2
57	Styrene (I)			89.3±14.3
58	4-(3-hydroxy-2,2,6-trimethyl-cyclohex-1-enyl) pent-3-en-3-one (III) [1]			1230±134
59	1H indene, 2,3-dihydro-1,1,3-trimethyl-3 phenyl (III) [6]			1540±361
60	Unknown 3 (structure of indene) [6]			631±98.7
61	2-methyl-7phenyl indole (unconformed) (III) [6]			611±98.2
62	Unknown 4 (structure of indene) [6]			242±40.7

[1] Quantified with 1,4 naphthalenedione as standard [2] Quantified with naphthalene as standard [3] Quantified with decane as standard [4] Quantified with tetracosane as standard [5] Quantified with 3,5-ditertbutyl-4-hydroxibenzaldehyde as standard [6] Quantified with Acenaphthalene as standard

Table 4: Nonvolatile compounds identified on the different adhesives by UPLC-MS/QTOF. Their toxicity class according with Cramer Class (TC). Their migration values for the different laminates expressed as µg of compound per Kg of simulant.

N	Compounds (TC)	Detected mass	Adhesives	LOD	Migration
63	3(2H)-isothiazolone, 2-methyl (III)	116.0161	AC02, AC03, V01, V02, V03	4.0	
64	Polyethylene glycol (I) (from n=2 to n=18)	173.0791 (n=2), (n+1)= +44.02	AC01, AC02, AC04	6.2	
65	5-Chloro-2-methyl-1,2-thiazol-3(2H)-one (III)	149.9780	AC02, V01, V02, V03	7.0	
66	1,2-benzothiazol-3(2H)-one (III)	152,0168	AC02, AC03, V01, V02, V03	5.1	
67	Polypropylene glycol (from n=5 to n=10) (I) Polypropylene glycol (from n=2 to n=16)	313.1976 (n=5) (n+1)= +58.04	AC01	4.2	
68	2,4,7,9-tetramethyl,5-decyn-4,7-diol (III)	249.1832	AC01	*	*
69	Bis(2-ethylhexyl) maleate myscirate (I)	363.2513	AC01	*	*
70	Bis-(2-ethylhexyl) phthalate (I)	413.2628	AC04	*	*
71	Diacetin (I)	177.0762	V01, V02, V03	9.1	Lam17→650±51 Lam18→ <LOD Lam19→ <LOD
72	Triacetin (I)	219.0868	V01, V02, V03	9.3	Lam17→930±65 Lam18→ <LOD Lam19→ <LOD
73	Oxydi-2,1-ethanediyl bis(2-acetoxypropanoate) (unconfirmed) (I)	335.1341	V01, V02, V03		
74	2-(2-(2-methoxyethoxy)ethoxy)ethyl methacrylate (unconfirmed) (I)	233.1388	V01, V02, V03		

* Quantified by CG-MS

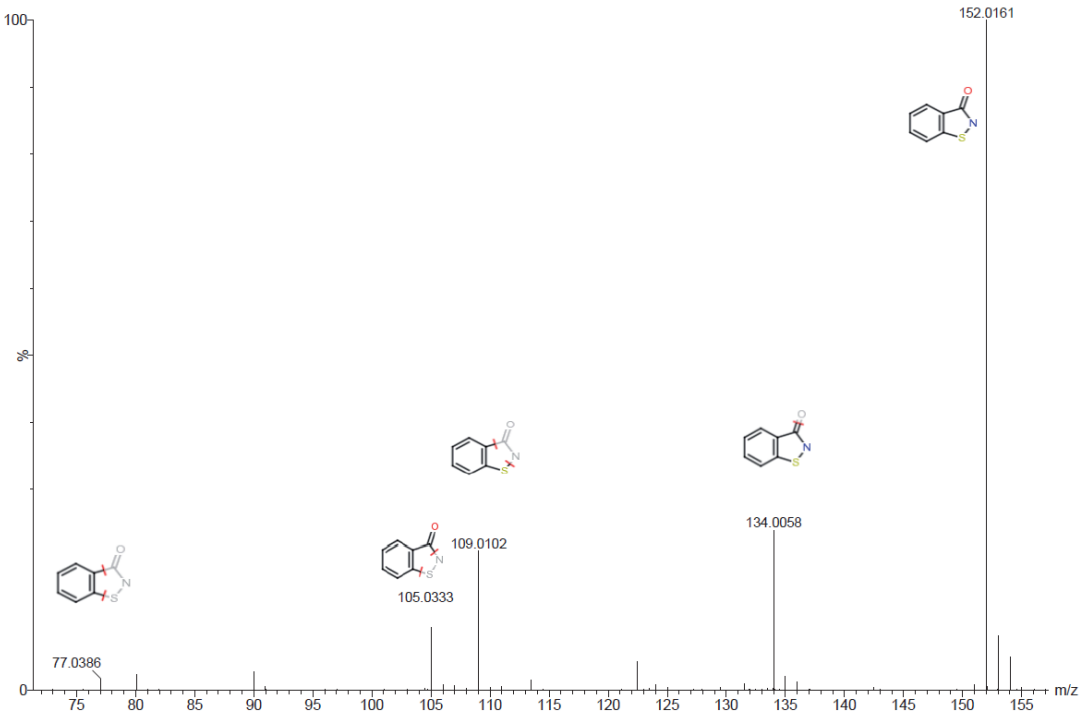

Figure 1. High energy spectrum of the compound 1,2-benzothiazol-3(2H)-one obtained by UPLC-MS/Q-TOF.

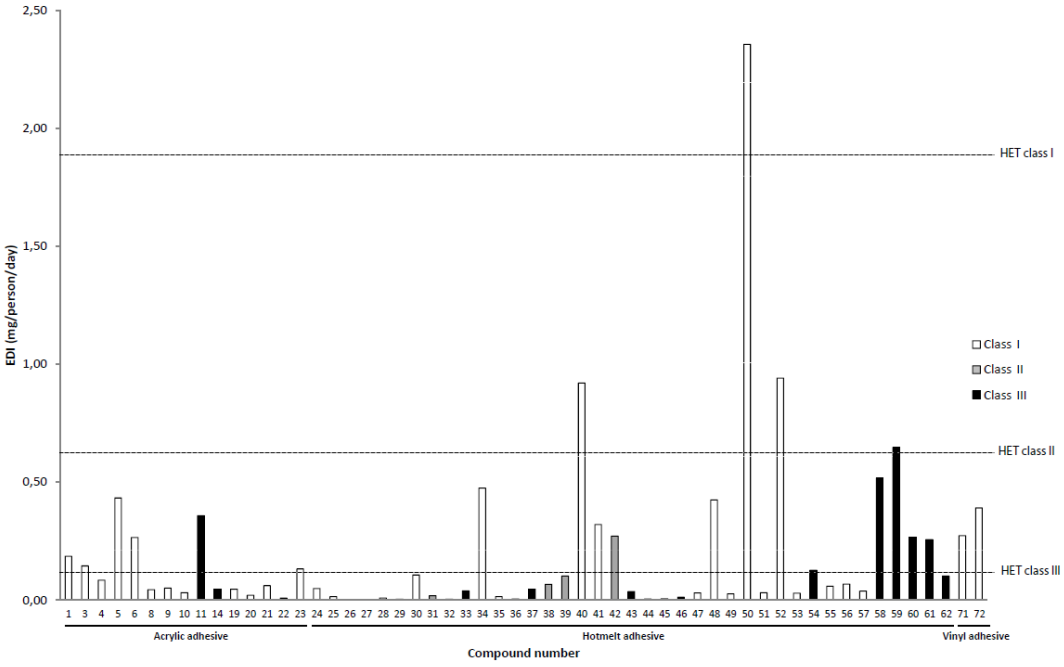

Figure 2. EDI (mg/person/day) of the adhesives compounds that migrated to the food simulants over their LODs, Toxtree toxicity class and Human Exposure Threeshold (HET) according the Toxtree toxicity.