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# Effect of several variables in the polymer toys additive migration to saliva

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ABSTRACT

Capacity to migrate of a representative group of polymeric additives, dyes, antioxidants, hindered amine light stabilizers (HALS) or antistatics, from plastic toys to saliva was analyzed to protect children in their habits of sucking and biting. Most of target additives appear no-regulated in toys normative but adverse effects on human health of some of them have been demonstrated and their presence in others commercial articles normative has been included.

In order to offer an effective and easy tool to perform these controls, migration tests by dynamic and static contact, followed by a preconcentration step by liquid–liquid extraction (LLE) and ultra performance liquid chromatographic analysis with ultraviolet-visible and evaporative light scattering detections (UPLC-UV/Vis-ELSD) have been optimized to evaluate the migrated amounts of the additives in saliva simulant. The detection limits of the migration methodologies were ranged from $8.68 \times 10^{-2}$ to $1.30 \times 10^{-3}$ mg migrated (L simulant)$^{-1}$.

Influence of several variables on this mass transport, as time, temperature and friction, was also analyzed to achieve the most aggressive methodology to protect consumers.

Migration of several studied additives, whose presence has been demonstrated in several purchased commercial toys, has been observed.



## 1. Introduction

Production and consumption of toys have been strongly growing up during last decades over the world together with the need of people to adopt improved living conditions. However, technological developments in the toys market have raised new issues with respect to the safety of toys and have increased consumer concerns. During the last years, the children exposure to toxic additives contained in polymeric toys has received special attention. Presence of toxic additives into these articles constitutes a category of pollutants that cannot be neglected since children are considered more sensitive to environmental influences than adults. In fact, a lot of harmful toys articles have already had to be recalled from the market.

In order to avoid the use of unsuitable toys materials and consequently to protect the public from possible health hazards, European parliament and the council of European Union have recently adopted the Directive 2009/48/CE [1] about safety of toys. Concretely, the harmonized standards, appearing as a tool to know the conformity with the safety requirements of the Directive, about organic additives of toys are: EN 71–9 [2] about safety of toys containing requirements for certain organic compounds in toys, EN 71-10 [3] specifying sample preparation and extraction and EN 71-11 [4] specifying methods of analysis.

However, there are a wide amount of additives in the market used in polymeric manufacture [5–8]. In fact, in the normative EN 71-9 [2] it is established that not all the potentially toxic compound could be studied, leaving it for future editions.

Two azo dyes, one anthraquinone dye, three phenolic antioxidants, two hindered amines light stabilizers and one monoester antistatic (most of them no-regulated in toys normative and regulated in food and packaging food) were chosen for this study. Legislation of other commercial articles where these additives appear regulated and the toxicity data which has been found for them in the literature are showed in Table 1 [9–17].

The toxicity each of these compounds was documented (Table 1). It is remarkable that synthetic azo dyes may be reduced to aromatic amines by intestinal bacterial species of the gastrointestinal tract. These amines can be metabolically activated to DNA-binding intermediates that are mutagenic and carcinogenic [11]. On the other hand, allergic contact dermatitis was also found for anthraquinone dyes as Solvent Blue 35 [13]. Moreover,

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**Table 1**
Studied additives in the three samples with its CAS number, chemist classification, structure and toxicity.

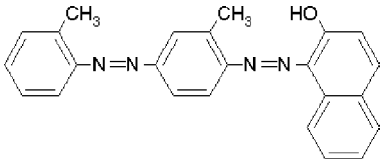
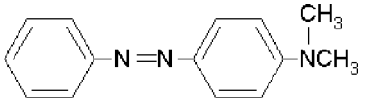
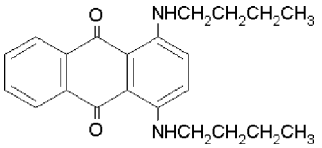
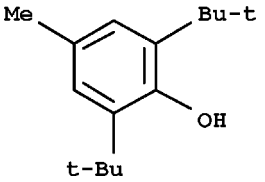
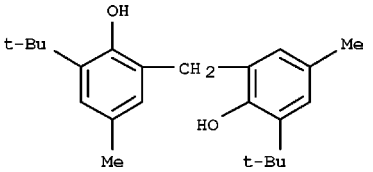
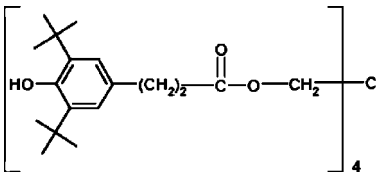
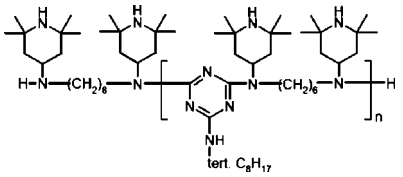
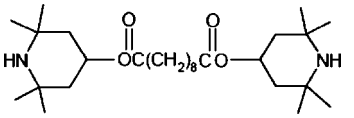
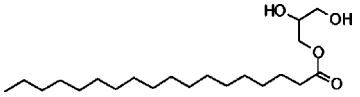
| Name, used shorthand, purity, N° CAS and molecular mass. | | Scientific name | Chemist classification | Structure | Toxicity and legislation limits data |
|---|---|---|---|---|---|
| Dyes | Sudan IV (80%) [85–83–6] 380.44 g mol$^{-1}$ | 2-Naphthalenol, 1-[[2-methyl-4-[(2-methylphenyl)azo]phenyl]azo]-(9CI) | Azo dye. Solvent type. |  | Category 3 carcinogen to humans (IARC) [9]. According to Cramer rules [10] (software Toxtree): classified in high toxicity group (class III). Reduced to form potentially toxic amines aromatic by intestinal bacterial species [11]. Banned as additive in food [12]. |
| | Dimethyl Yellow [60–11–7] 225.29 g mol$^{-1}$ | Benzenamine, N,N-dimethyl-4-(phenylazo)- (9CI) | Azo dye. Solvent type. |  | Category 2B carcinogen to humans (IARC) [9]. According to Cramer rules [10] (software Toxtree): classified in high toxicity group (class III). Action limit=10 mg kg$^{-1}$ in toys [5]. |
| | Solvent Blue 35 (98%) [17354–14–2] 350.45 g mol$^{-1}$ | 9,10-Anthracenedione, 1,4-bis(butylamino)-(9CI) | Azo dye. Solvent type. Anthraquinone derivate. |  | According to Cramer rules [10] (software Toxtree): classified in high toxicity group (class III). Allergic contact dermatitis was reported [13]. |
| Antioxidants | BHT, 99% [128–37–0] 220.35 g mol$^{-1}$ | 2,6 ditertbutyl-*p*-cresol | Phenol (primary antioxidant) |  | $LD_{50}$ = 2-10 mg kg$^{-1}$ in rats [14]. According to Cramer rules [10] (software Toxtree): classified in intermediate toxicity group (class II). Displayed estrogenic activities [15]. Specific Limit Migration (SML)=3.0 mg kg$^{-1}$ in packaging food [16]. |

Table 1 (*Continued*)

| Name, used shorthand, purity, N° CAS and molecular mass. | | Scientific name | Chemist classification | Structure | Toxicity and legislation limits data |
|---|---|---|---|---|---|
| | Antioxidant 2246 (AO 2246) [119–47–1] 340.50 g mol$^{-1}$ | 2,2-methylenebis(6-tert-butyl-4-methylphenol) | Phenol (primary antioxidant) |  | $LD_{50}$ = 5,0 g kg$^{-1}$ in rats and 11 g kg$^{-1}$ in mice [14]. According to Cramer rules [10] (software Toxtree): classified in high toxicity group (class III). SML (T) = 1.5 mg kg$^{-1}$ in packaging food [16]. |
| | Irganox 1010 (I 1010) [6683–19–8] 1177.63 g mol$^{-1}$ | Pentaerythritol tetrakis(3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate | 2,2,6,6-tetramethylpiperidine derivate |  | Decrease of weight in mice [14]. According to Cramer rules [10] (software Toxtree): classified in high toxicity group (class III). SML=60 mg kg$^{-1}$ in packaging food [16]. |
| Hindered amine light stabilizers (HALS) | Chimassorb 944 [71878–19–8] Oligomer | (Poly[[6-[1,1,3,3-tetramethylbutyl)amino]-1,3,5-triazine-2,4-diyl][(2,2,6,6-tetramethyl-4-piperidinyl)imino]-1,6-hexanediyl[(2,2,6,6-tetramethyl-4-piperidinyl)imino]]) | |  | According to Cramer rules [10] (software Toxtree): classified in high toxicity group (class III). SML=3.0 mg kg$^{-1}$ in packaging food [16]. |
| | Tinuvin 770 [52829–07–9] 480.72 g mol$^{-1}$ | (Bis (2,2,6,6,-tetramethyl-4-piperidyl) sebaceate) | 2,2,6,6-tetramethylpiperidine derivate |  | According to Cramer rules [10] (software Toxtree): classified in high toxicity group (class III). Toxics effects on myocardium [17]. |
| Antistatic | Atmer 129 [31566–31–1] 358.31 g mol$^{-1}$ | Octadecanoic acid monoester with 1,2,3-propanetriol | Ester lipid acid. Glycerol monostearate |  | According to Cramer rules [10] (software Toxtree): classified in low toxicity group (class I). |

estrogenic activities of phenolic antioxidants as the studied ones were reported [15].

Regarding the potential risk of coming into contact with toxic additives in the children's habit of biting and sucking polymeric toys, it is necessary to know the identity of the material, their additives and the qualitative and quantitative migration of components of the plastic toy into saliva.

Regarding the additive migration, the most studied field during the last decades has been packaging food articles [18–20]. Several studies about migration kinetics [21], influence of several variables in the migration [22], mathematical models to predict the experimental migration [23,24], etc., have been developed. Moreover, migration of several monomers and additives from food packaging to food is widely regulated [16].

In the toy field, most of studies about the migration of toxic additives from polymeric toys into saliva were reported for the well-known phthalates plasticizers in polyvinyl chloride (PVC) [25–29], being also widely studied in others articles [30,31]. In order to establish safety-in-use under the most severe conditions encountered in practice, horizontal, magnetic, ultrasonic and rotatory (Head over Heels) dynamic agitation tests as simulation of biting and sucking the plastic were developed [27,28]. The obtained results with some of them were comparable with in vivo release [28]. Nowadays, these compounds together with others as azo dyes are banned in toys by the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) [32], which covers restriction of chemicals, in concentrations higher than 0.1% by mass of plasticized material. On the other hand, in the normative EN 71-10 [3], migration tests with saliva are only proposed for monomers and solvents with the Head over Heels agitation as simulation test.

In order to know the extent to which the target compounds can be extracted from polymeric material under conditions relevant to real-life activities, migration methods are necessary. Migration is a complex process depending in part on the diffusivity of the migrating substance. Diffusivity or the diffusion coefficient is defined as the tendency of a substance to diffuse through the polymer bulk phase. Migration can, therefore, be considered a mass transport process under defined conditions of test (i.e., time, temperature, and the nature and volume of the contact phase).

Then, the aim of this study was to develop a methodology to know the levels of concentration and migration capacity of a representative group of additives from polymeric toys into saliva. Moreover, effect of different variables, as time, temperature and friction in the migration of several toxic additives used in these applications was studied.

## 2. Experimental

### 2.1. Chemicals and solvents

All studied additives of each sample are shown in Table 1. All dyes and the antioxidant BHT were supplied by Sigma Aldrich (Steinheim, Germany) and the antioxidant 2246 and Irganox 1010, the two amine light stabilizers (HALS) and the antistatic were supplied by Ciba (Basel, Switzerland).

Individual stock standard solutions of each compound (1000 mg $L^{-1}$) were prepared in tetrahydrofuran (THF). Work standard solutions were prepared from individual stock standard solution by dilution with acetonitrile (ACN).

The following chemicals were used as solvent, eluent or additive: *n*-hexane, methanol, and THF HPLC-gradient grade for instrumental analysis were supplied by Merck (Darmstadt, Germany); ACN was from J.T. Baker (Deventer, Holland) HPLC-gradient grade for instrumental analysis; potassium phosphate dibasic anhydrous (99%) and potassium hydroxide (86%) were from Fluka (Steinheim, Switzerland); water was purified using a Milli-Q Ultrapure water purification system from Millipore (Bedford, MA, USA); hydrochloric acid 37% for analysis, and sodium hydroxide (98%) for analysis were obtained from Panreac Química (Barcelona, Spain).

The saliva simulant were prepared with the following composition [29]: 745.5 mg potassium chloride, 525.5 mg potassium carbonate anhydrous, 753.1 mg potassium phosphate dibasic anhydrous, 327.3 mg sodium chloride, 147.0 mg calcium chloride dehydrate and 166.7 mg magnesium chloride hexahydrate, all these salts were supplied with pour analysis grade by Fluka (Buchs, Switzerland) dissolved in 1000 mL ultrapure water adjusted to pH 6.8 with 3 M hydrochloric acid solution.

### 2.2. Samples

#### 2.2.1. Laboratory samples

Isplen polypropylene (PP) 070 G2 M from Repsol YPF (Madrid, Spain) was selected in this work as polymer used in toys articles. PP film was extruded at laboratory by using a Brabender DSE 20 double screw extruder (Duisburg, Germany) with five heating zones and the following zone temperature settings: 210/200/200/200/200/200 °C. PP granules with the additives and the concentrations shown in Table 2 were re-extruded several times to obtain a homogeneous distribution of the compounds ($n = 5$), obtaining so the three samples for this study. Irgafos 168 was added as secondary antioxidant for the stabilization of polymer in the extrusion [33] for the three samples. Samples with film shape of 0.30 mm thickness were got. The showed concentrations in the Table 2 were chosen because to be the most common used in the manufacture of plastics [34].

#### 2.2.2. Commercial samples

Thirteen commercial toys with the same colour than the chosen dyes for this study were purchased in local shops. Illegal labelling in the packets of these toys was observed. The polymer matrix of these commercial samples was identified by Fourier transform infrared spectrometry (FTIR).

### 2.3. Determination of the initial additive concentration in the polymer

Microwave-assisted extraction (MAE) was performed using a Milestone microwave laboratory system ETHOS 1 (Sorisde, Italy) equipped with a 12-vessel position carousel. Duplicate 1 g samples cut into small pieces of approximately 0.5 cm × 1 cm were put into the polytetrafluoroethylene (PTFE) microwave extraction vessels and mixed with 20 mL of dichloromethane.

The samples were extracted for 15 min at 60 °C and 2 min were needed to reach the selected extraction temperature. After the extraction, vessels were allowed to cool to room temperature before opening and the final extracts were filled with methanol to the mark of a 20 mL graduated flask. A portion of 10 mL of extract was passed by 0.2 μm filters and analyzed by ultra performance liquid chromatographic analysis with ultraviolet-visible and evaporative light scattering (UPLC-UV/Vis-ELSD) system. The other 10 mL were put into a pear-shaped recovery flask and the volume was reduced until the last drop at 200 mbar and 30 °C by rotary evaporator. The drop was diluted with 1 mL of acetonitrile, passed by 0.2 μm filters and analyzed by UPLC-UV/Vis system. This last step was carried out to be able to determinate the concentration of Tinuvin 770, since this compound has the maximum of absorption at 200 nm and the dichloromethane at 230 nm.

**Table 2**
Laboratory samples used for this study with their additives and added concentrations in extrusion process.

| | Dye (concentration) | Antioxidant (concentration) | HALS (concentration) | Antiestatic (concentration) |
|---|---|---|---|---|
| Sample 1 | Sudan IV (0.25%) | I 1010 (0.25%) | Chimassorb 944 (0.5%) | – |
| Sample 2 | Dimethyl Yellow (0.005%) | BHT (0.25%) | – | Atmer 129 (0.5%) |
| Sample 3 | Solvent Blue 35 (0.25%) | AO 2246 (0.25%) | Tinuvin 770 (0.5%) | – |

The lowest chosen concentration was for Dimethyl Yellow, since it appears in normative UNE-EN 71-9 with an action limit of 10 mg $kg^{-1}$ in toys [2].

### 2.4. UPLC-UV/Vis-ELSD

UPLC analyses were performed using an Acquity system from Waters (Milford, MA, USA) with a gradient pump and automatic injector. Analytes were separated using a stainless steel column 50 mm × 2.1 mm packed with Acquity UPLC BEH $C_{18}$, 1.7 μm particle size (Waters) kept at 30 °C. Needle-over-fill injection mode was selected. The detection systems were an Acquity UPLC photodiode array (Waters) model and Acquity UPLC Evaporative Light Scattering Detector (Waters). The signal acquired from the detector was recorded by a personal computer operated under the Empower Pro software (Waters). All solvents were passed by 0.2 μm filters. The linear solvent gradients of two chromatographic methods used in this study are shown in Table 3. The flow rate was 0.5 mL $min^{-1}$ and the injection volume was 3 μL.

### 2.5. Specific migration tests in saliva simulant

#### 2.5.1. Specific dynamic migration test

Ten circular portions of each test sample (approximately 2 g) with 2.6 cm of diameter and 10.6 $cm^2$ of total surface each one (selected to correspond to the surface area of child's open mouth [29]) were placed in a 100 mL glass flask with 50 mL saliva simulant solution, the used one by Earls et al. [29], and 4 g of glass balls (to simulate the action of biting). This flask was rotated for different times in a Head over Heels rotator at 60 rpm. Then, the saliva simulant solution was replenished and a second 50 mL fresh portion of saliva simulant was added repeating again the process. This replenishment is more representative of the realistic use of toy. Finally, the two portions of saliva were combined, following the preconcentration step (Section 2.5.3) and the analysis by UPLC-UV/Vis-ELSD system.

These migration experiments were performed twice for each sample and a single blank test was also included.

**Table 3**
Chromatographic methods to determinate the studied additives.

Developed method by UPLC-UV/VIS system for quantification of HALS [7]

| Time (min) | Solvent A (%) | Solvent B (%) |
|---|---|---|
| 0 | 20 | 80 |
| 1 | 0 | 100 |
| 4.5 | 0 | 100 |
| 5 | 20 | 80 |

Solvent A: aqueous 10 mM $K_2HPO_4$ solution adjusted to pH 11.5 with 0.2% aqueous 2 M KOH
Solvent B: ACN
10 min conditioning between injections
λ integration: 220 nm: Tinuvin 770; 230 nm: Chimassorb 944

Developed method by UPLC-UV/VIS-ELSD system for quantification of dyes, antioxidants [5] and antistatic [8]

| Time (min) | 10 min conditioning between injections. | Methanol (%) |
|---|---|---|
| 0 | 40 | 60 |
| 0.8 | 30 | 70 |
| 0.9 | 10 | 90 |
| 4.0 | 0 | 100 |
| 4.5 | 0 | 100 |
| 5.0 | 40 | 60 |
| 6.0 | 40 | 60 |

ELSD: Atmer 129
λ integration: 220 nm: BHT and I 1010; 230 nm: Sudan IV; 260 nm: Solvent Blue 35; 420 nm: Dimethyl Yellow

#### 2.5.2. Specific static migration test

The static migration test was developed by totally immersing of three 3.5 cm × 3 cm pieces (approximately 1.2 g) of each sample in 50 mL of saliva simulant for ten days at ambient temperature and 40 °C. Then, the saliva simulant solution was replenished and a second 50 mL portion of saliva was added repeating again the process. The two portions of saliva were combined, following the preconcentration step (Section 2.5.3) and the analysis by UPLC-UV/Vis-ELSD system.

These migration experiments were performed twice for each sample and a single blank test was also included.

#### 2.5.3. Preconcentration step

Due to the low migration levels of these compounds from polymer to simulants, a preconcentration step by liquid–liquid extraction (LLE) following the migration tests and prior to analysis by UPLC-UV/Vis-ELSD system was required. The portions of saliva simulant after migration test were adjusted at different pHs depending on the determination of each additive with solutions 2 M of KOH (aq) and HCl (aq.): I 1010, BHT, Atmer 129, Sudan IV and Dimethyl Yellow at pH 2.8, Chimassorb 944 and Tinuvin 770 at pH 10.5 and Solvent blue 35 and AO 2246 at simulant saliva pH (6.8). Then, the simulant was extracted by LLE with three successive 5 mL portions of *n*-hexane in a separatory funnel (250 mL) for 10 min at 230 rpm with an agitator Rotabit Selecta, being resting for 2 min. Organic extracts were combined in a pear-shaped recovery flask and the volume was reduced until the last drop (≈10 μL) at 200 mbar and 30 °C by rotary evaporator. The drop was diluted with 1 mL of acetonitrile, passed by 0.2 μm filters and analyzed by UPLC-UV/Vis-ELSD system.

## 3. Results and discussion

### 3.1. Determination of the initial additive concentration in the polymer

The concentration of additives in the polymers as reported in the previous section was determined by MAE extraction using dichloromethane as solvent, followed by UPLC-UV/Vis-ELSD method. The process was repeated to ensure complete extraction of the polymer sample. Complete extraction was assumed if the amount of found additive in the second extract was less than 10% of the first. Total level of additive was obtained by summing the amounts found in all extracts. The detection limits of the extraction methodology were ranged from 2.6 to 75.8 mg (kg sample)$^{-1}$.

**Table 4**
Values of additive concentration means, standard deviations and variation coefficients of the extraction of laboratory samples by MAE-UPLC-UV and ELSD methods to evaluate the repeatability ($n = 10$, in the same day).

| Additive | Mean (mg of additive by kg sample) | Standard deviation | RSD (%) |
|---|---|---|---|
| Dimethyl Yellow | 38.32 | 3.29 | 8.59 |
| BHT | 1432.09 | 117.66 | 8.22 |
| Atmer 129 | 4240.89 | 613.13 | 14.46 |
| Sudan IV | 1522.53 | 76.10 | 5.00 |
| I 1010 | 684.41 | 70.75 | 10.34 |
| Chimassorb 944 | 980.41 | 133.92 | 13.66 |
| Solvent Blue 35 | 1636.25 | 88.73 | 5.42 |
| Tinuvin 770 | 2410.26 | 169.09 | 7.02 |

All of extraction results of the laboratory samples, being most of them in broad agreement with the levels of additives blended into the polymers in the extrusion moulding, are showed in Table 4. The differences between the additived and the extracted amount of additives may be due to the extrusion process or the incidence of the passing of time, light, changes in ambient temperature, etc., since known amounts of additives were spiked in the extraction solvent, finding recoveries around 100% after the extraction. The extraction method precision in mg of additive by kg of sample was evaluated by repeatability ($n = 10$, in the same day), obtaining relative standard deviation levels less than 15% (Table 4).

The only no extracted additive was the antioxidant 2246. Losing additive in the sample during the time was corroborated by several assays.

### 3.2. Influence of the contact time on the migration of additives

Specific dynamic migration tests at room temperature in saliva simulant during 1 and 2 h and 1, 2, 5 and 10 days were tested for all laboratory samples, with a LLE followed by UPLC-UV/Vis-ELSD method. Percentages of migrated amounts regarding extracted amounts by MAE are presented in the Fig. 1.

Migration values by this type of tests were not observed for the two HALS (Chimassorb 944 and Tinuvin 770). However, it could be observed at the different times for the others additives (Fig. 1). Migration values in the range of 0.02–0.9% (0.006–0.75 mg migrated (L simulant)$^{-1}$) were quantified. These values must be taken into account due to growing concern about the children exposure to toxic additives. Moreover, although migration levels of the target compounds do not appear regulated in the normative about safety of toys, migration levels less than 0.75 mg (L simulant)$^{-1}$ for certain monomers and solvents do [2].

Regarding the time influence in the migration, a prolonged time of contact may result in an increase in the migrated amount of Dimethyl Yellow, BHT, Sudan IV and I 1010 and in a relative constancy for Solvent Blue 35 were observed. The lack of increase in migrated percentage with the time for this last compound may be due to lack of good reproducibility between migration results. However, no migration at higher times than 2 h was observed for the antistatic Atmer 129. Therefore, stability in the time for this additive Atmer 129 was studied. Solution of simulant saliva only spiked with known amounts of this additive was left at ambient temperature during 1 and 2 h and 1, 2, 5 and 10 days and the solutions were subjected to ELL-UPLC-UV/Vis-ELSD. Again, chromatographic peak of antistatic Atmer 129 was not observed at higher times than 2 h. Then, a change in the chemical of monoester in saliva simulant was thought to happen with time. Its study was left for following works.

The best ability to migrate was observed for Dimethyl Yellow (the only target additive regulated in toys by concentration levels (Table 1) [2]) and for the antistatic Atmer 129. The ability to migrate of this last one seems to be logical taking into account its behaviour in polymeric matrix. The antistatic Atmer 129 is incorporated to polymer in extrusion step and then it works migrating itself to surface and interacting with atmospheric moisture reducing surface resistivity and hence dissipating high electric charge densities to give a long-lasting antistatic effect to the plastic [34]. In spite of its ability to migrate, high toxicity data for this compound was not found in the literature (Table 1).

### 3.3. Influence of friction and temperature on the migration of additives

Static migration test at room temperature and at 40 °C and dynamic migration test at room temperature in saliva simulant during ten days (time of the highest migration levels for nearly all of additives) were tested for all samples. Migration was observed in at least one of the test for all additives, except for Atmer 129, due to the indicated one above.

In this way, an analysis of influence of two variables as temperature (being about 40 °C the temperature in saliva of mouth) and agitation (being this factor in the children's habit to suck and bit the plastic of toys) on the migration were carried out for all additives and obtained results are shown in the Fig. 2.

Migration of Sudan IV, Solvent Blue 35 and I 1010 was only observed in dynamic migration testing at ambient temperature. Regarding the migration of these additives, the influence of agitation and friction was significant whereas an increase of temperature in the testing did not lead to increase the migration for these additives.

Migration of HALS (Chimassorb 944 and Tinuvin 770) was practically only observed in static migration testing at 40 °C. In contrast with the influence of agitation and friction for dyes and antioxidants, the temperature is the most significant factor for the HALS.

Migration of Dimethyl Yellow was observed in the three experimental testing and migration of BHT was observed in all of them except for static testing at room temperature. In the two cases, the effect of temperature in the migration is more influent than the effect of agitation and friction. Migration percentage of BHT was lower than the obtained one with I 1010, having this last antioxidant higher molecular mass. This fact may be attributed to better mobility of the I 1010 molecule as it was established by Katan [35].

In the Fig. 2 is shown the values of migration percentage (w/w) for all additives in the experimental testing regard to calculated concentration values by MAE. Dimethyl Yellow is the dye with the highest migration percentage value in the experimental testing where the maximum migration was achieved, being the only additive of studied ones that it appears in the toys normative as it was described above [2]. On the other hand, lower migration levels were found for the others dyes, Sudan IV and Solvent Blue 35 that are banned in others normative about food and toxic effect data were found (Table 1). Tinuvin 770 shows the highest value of migration percentage of all compounds and toxic effects in myocardium were reported, being regulated in normative about food packaging (Table 1). The studied antioxidants and the Chimassorb 944 are regulated with specific migration limits in the Directive 2002/72/CE [16] but not higher migration values than these limits were found in any assay (Table 1).

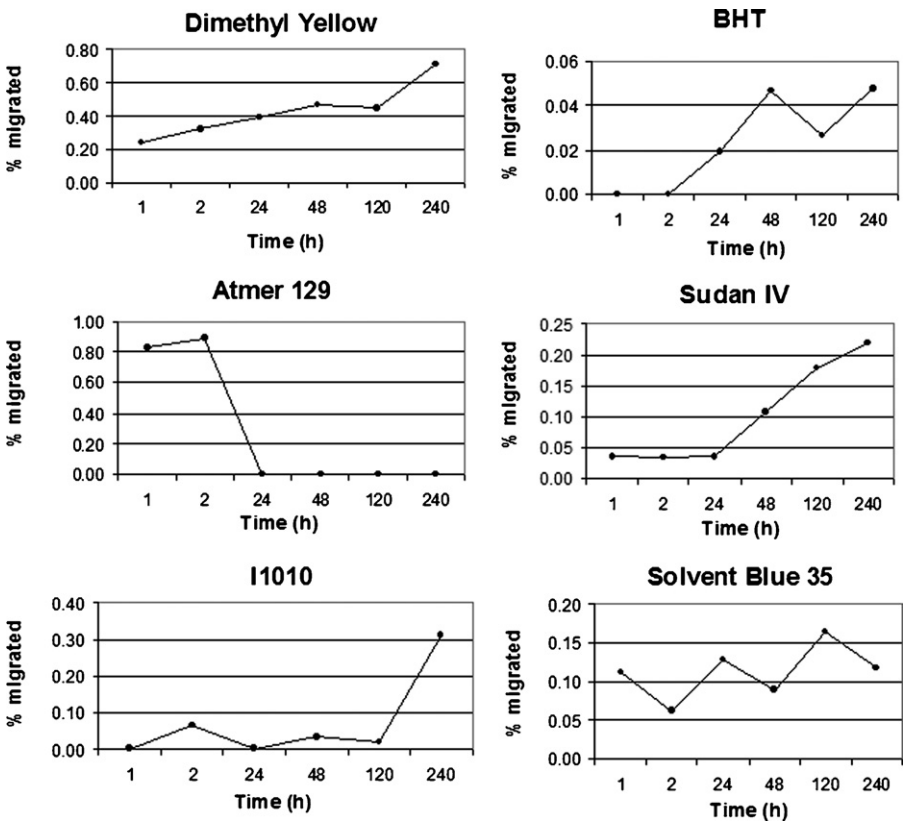



**Fig. 1.** Experimental detected migrated percentage (w/w) for studied additives at 1, 2 h and 1, 2, 5 and 10 days by dynamic testing at ambient temperature.

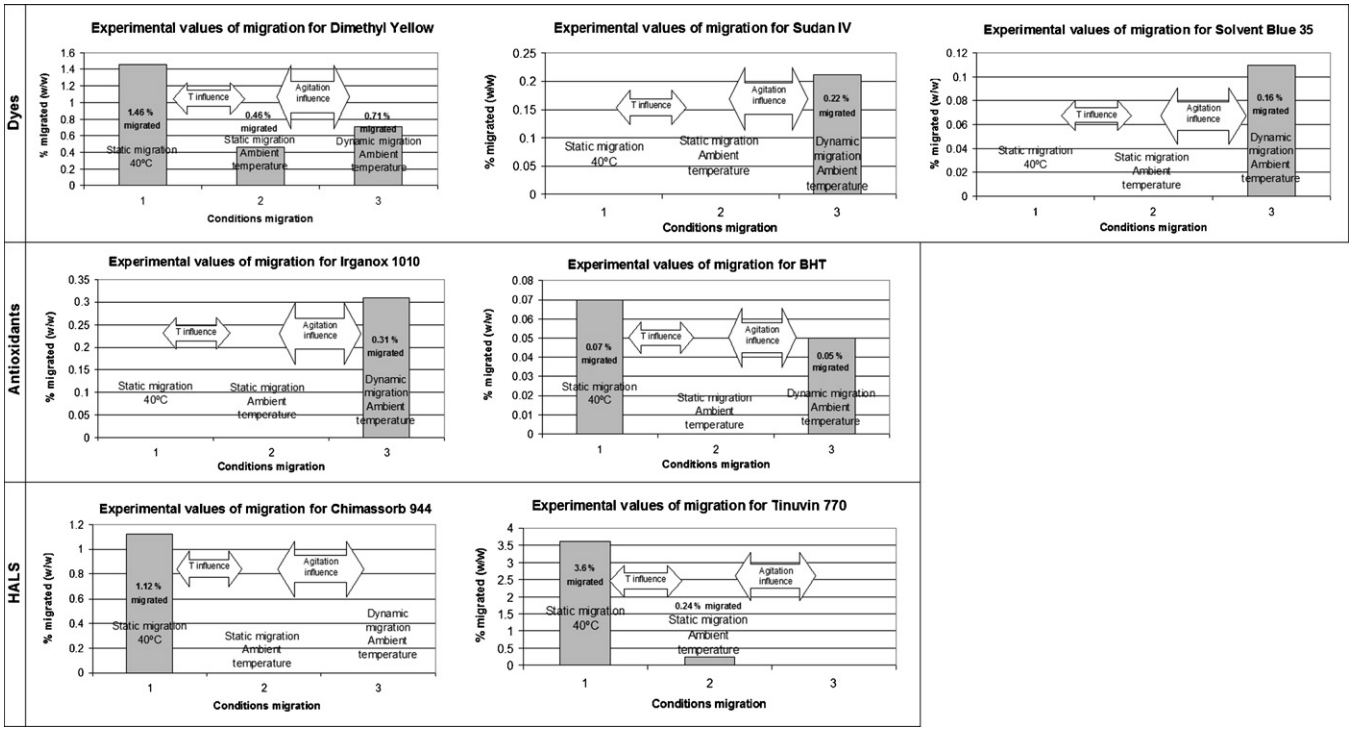



**Fig. 2.** Obtained experimental values of migrated percentage (w/w) by three migration testing for the detected additives: static testing at two temperatures and dynamic testing at ambient temperature.

**Table 5**
Obtained results for commercial toys samples of concentration by MAE-UPLC-UV method and dynamic migration testing-ELL-UPLC-UV method.

| Sample | Additive | mg content (kg of sample)$^{-1}$ | mg migrated (L simulant)$^{-1}$ | % Migrated |
|---|---|---|---|---|
| Blue boat | BHT | 1128.09 | 0.03 | 0.11 |
| | Solvent Blue 35 | 103.61 | 0.01 | 0.22 |
| Blue cat | Solvent Blue 35 | 544.30 | – | – |
| Red washers | I 1010 | 46.80 | – | – |
| Blue bear | Solvent Blue 35 | 898.71 | – | – |
| Yellow bear | I 1010 | 20.08 | 0.01 | 5.62 |
| Red duck | I 1010 | 347.62 | 0.01 | 0.17 |

The additives with higher tendency to migration were: Dimethyl Yellow (1.46%), Chimassorb 944 (1.12%) and Tinuvin 770 (3.6%), all of them in static testing at 40 °C.

Dynamic migration test at room temperature was the assay where was obtained migration for higher number of compounds (five compounds). However, migration levels by static migration test at 40 °C were obtained for four compounds and at room temperature for two compounds.

As it was found by others authors studying phthalates [26–29], dynamic migration test was the most aggressive methodology. These authors found higher values of migration by this method than the found ones by ultrasonic extraction and in vivo release.

### 3.4. Specific migration test in commercial samples

Dynamic migration tests during ten days at room temperature followed by a LLE step and injection by UPLC-UV/Vis-ELSD were developed on all commercial samples that showed a detectable content of target additives after the extraction assays. The detection limits of the migration methodology were ranged from $8.68 \times 10^{-2}$ to $1.30 \times 10^{-3}$ mg migrated (L simulant)$^{-1}$.

Twenty five commercial toys samples were tested and amount of studied additives was found in six of them, detecting migration in three of samples. Obtained results of concentration (mg kg$^{-1}$), mg migrated by litre of simulant and percentage of migration are shown in Table 5. The found additives in the commercial samples were Solvent Blue 35, BHT and I 1010. In some of them, values of migration of these compounds were quantified.

In order to corroborate the possibility to establish a correlation between the real content of the target additives and their migrated amount, the obtained values of laboratory and commercial samples were compared. In extruded samples in laboratory, the found percentages of migration for BHT, I 1010 and Solvent Blue 35 were 0.05%, 0.31% and 0.16%, respectively, being lower than some of the found ones in the commercial sample in spite of the fact that higher concentrations of additive were quantified in laboratory samples. With regard to this no found proportionality, the identified different polymer matrix by Fourier transform infrared spectrometry must be taken into account since polymer matrixes as acetonotrile–butadiene–estirene (ABS) were identified. So factors as migrant–polymer affinity must be also considered. An increase in crystallinity of the polymeric matrix reduces the diffusion rates because these domains tend to be in the form of small platelets are nearly impermeable to small molecules due to the dense packing of the polymer chains [36].

The migration values found in the analysis of commercial toys for the antioxidants, BHT and I 1010, are lower than their specific migration limits regulated in the Directive 2002/72/CE for food packaging (Table 1).

In spite of that, toxicity data of dye Solvent Blue 35 as allergic contact dermatitis [13], BHT as estrogenic activities [15] and I 1010 as decrease of weight in mice [14] was reported (Table 1) and concentration and migration values of these additives were detected in tested commercial toys samples.

## 4. Conclusions

Complete procedures of extraction and migration tests with liquid chromatography determination, to detect simultaneously the presence and the migration from toys to saliva of several harmful additives of different nature, were successfully optimized.

Depending on the additive, variables as temperature, friction or contact time were significant, seeing the need to take them into account when migration tests are developed to be close to real situations and in that way, to establish safety-in-use.

In order to be able to establish a correlation between the real content of the additives and their migrated amount, the solubility of the migrant in the different polymeric matrix, had to be considered.

The proposed migration tests resulted to be simple and with a wide field of application, mainly when it is necessary to measure a lot of samples, to identify the presence of several additives of different nature like the target ones and quantify them in screening activities of notified bodies [1] or control agencies. The detection limits of the migration methodologies were ranged from $8.68 \times 10^{-2}$ to $1.30 \times 10^{-3}$ mg migrated (L simulant)$^{-1}$.

The potential risk to the migration of toxic additives of different utility and use from toys to saliva in the children's habit to suck and bit the plastic was experimentally evaluated. Although found additive migration was not higher than the migration limits appearing in others article normative, each additive must be analyzed for the specific use of the article in where is contained since toxicity data as estrogenic activities or allergic contact dermatitis was found in the literature.

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