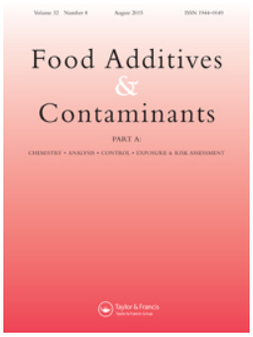

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Migration of Tinuvin P and Irganox 3114 into milk and the corresponding authorised food simulant

Zsolt Bodai[a], Csaba Kirchkeszner[a], Márton Novák[a], Zoltán Nyiri[a], József Kovács[b], Norbert Magyar[b], Béla Iván[c], Tamás Rikker[d] and Zsuzsanna Eke[a,d]*

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Migration of Tinuvin P (UV stabiliser) and Irganox 3114 (antioxidant) from high-density polyethylene (HDPE) was studied. HDPE pieces were soaked in either milk (1.5% or 3.5% fat content) or 50% (v/v) ethanol–water mixture – the food simulant for milk as specified in Regulation No. 10/2011/EC. The obtained extracts were analysed by LC-MS/MS. For statistical assessment variography was used. It proved to be a useful tool for making a distinction between the early migration range and the equilibrium, despite the variance of the data. Regulation No. 10/2011/EC specifies 10 days of contact time for milk at 5°C. Our experiments with the food simulant with 24 dm^2 kg^{-1} surface/mass ratio showed that both Tinuvin P and Irganox 3114 need less than 1 h to reach equilibrium. Furthermore, 10-day experiments with daily sampling showed that these additives are stable in milk, as well as in the food simulant. The effect of the concentration of the additives in HDPE was studied in the 0.01–5% (m/m) range. For both Tinuvin P and Irganox 3114 and all three extractants the migrated amount became independent of the concentration of the additive in the HDPE approximately at 1% (m/m). For Tinuvin P the food simulant gave a close estimate for the milk samples. However, using the food simulant for modelling the migration of Irganox 3114 into milk gave an overestimation with a factor of minimum 3.5. In the case of Tinuvin P special care must be taken, since the recommended amount in the HDPE can result in additive concentrations near or even over the specific migration limit (SML). However, Irganox 3114 cannot reach the SML either in milk or in the food simulant.

Keywords: migration; additives; antioxidant; UV stabiliser; milk; food simulant; semi-variogram; LC-MS

Introduction

Food packaging is important for several reasons: it protects foods from physical impact, preserves quality and also displays information. Even though packaging materials are obviously necessary, we have to take care of the probable migration of compounds from the packaging material into the food.

In 2011 the European Union issued a regulation on plastic materials and articles intended to come into contact with food (European Commission 10/2011/EC 2011). This is because nearly all polymer products used for a large variety of everyday and advanced applications, including food packaging and storage, contain additives, such as stabilisers, plasticisers, processing aids, colorants etc., added to improve processing and application characteristics of such materials. Tinuvin P (UV stabiliser) and Irganox 3114 (an antioxidant) are two of the hundreds of chemicals for which migration levels are limited. SMLs set the maximum permitted amount of a given chemical released from a material or article into foods or food simulants. They are expressed as mg of substance per kg of food (mg kg^{-1}). Six food simulants are listed in the regulation: Tenax for solid food and 3% (m/v) acetic acid, 10%, 20% and 50% (v/v) ethanol–water mixture and vegetable oil for liquid food. For milk, a 50% (v/v) ethanol–water mixture should be used for modelling the migration from plastics. Recommended contact time for both food simulants and the food itself is based on the storage procedure and the shelf-life of the food; in the case of milk (except ultra-high-temperature processed milk) 10 days of contact time at 5°C is approved.

In several cases contact times less than 10 days (Garde et al. 2001; Sanches Silva, Cruz, et al. 2007; Sanches Silva, Freire, et al. 2007; Galotto et al. 2011) proved to be enough to reach the equilibrium concentration. On the other hand, sometimes more than 10 days of contact time was necessary (Garde et al. 2001; Marcato et al. 2003; Sanches Silva, Cruz, et al. 2007; Sanches Silva, Freire, et al. 2007; Sanches Silva et al. 2008; Reinas et al. 2012). To the best of our knowledge, no such information for Tinuvin P and Irganox 3114 is available yet. Since shorter contact time and thus quicker migration trials may have financial benefits, determination of the correct contact time is favourable. However, measurement error may obscure

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the margin between the early migration range (with increasing concentration) and the equilibrium. Statistical methods may help to make the distinction. In earth and environmental science many studies are known to use the basic function of geostatistics, the semi-variogram to determine the sufficient temporal (Kovács et al. 2012) or spatial sampling range (Hatvani et al. 2014). Semi-variogram can be a useful mathematical tool for the determination of the appropriate contact time.

The purpose of the above cited experiments was the determination of diffusion coefficient at different temperatures, with different polymeric thickness or different fat content of the food, etc. The comparison of the food and the corresponding authorised food simulant gets less emphasis or is missing. Sanches Silva and co-workers showed that authorised food simulant for orange juice is not suitable in the case of diphenylbutadiene (DPBP), probably due to the fibre content of the orange juice (Sanches Silva et al. 2008). Reinas and co-workers published the fact that Irgafos 168 and Irganox 1076 migrate slower into rice than the authorised food simulant (Tenax) (Reinas et al. 2012). These results suggest that testing the suitability of the authorised food simulants to mimic specific food is also important. This is especially true for milk – a complex, colloidal system with diversified fat content.

Tinuvin P and Irganox 3114 have been measured mostly from standard solution or food simulant using HPLC (Nerín et al. 2003; Block et al. 2006; Reingruber et al. 2010; Li et al. 2014). A method for the determination of these additives from milk is also available (Bodai et al. 2014).

The aim of this study was to determine the appropriate contact time between high-density polyethylene (HDPE) and a 50% (v/v) ethanol–water mixture (authorised food simulant) for Tinuvin P and Irganox 3114. Migration studies were performed with in-house-prepared HDPE sheets. Semi-variogram, which is widely used in geostatistics, was applied for the determination of the appropriate contact time. We also aimed to test the suitability of the food simulant for substituting milk. Therefore 10-day migration studies were performed using 1.5% and 3.5% fat containing milk as well as with the food simulant. The effect of additive concentration in the HDPE sheets was also examined.

Materials and methods

Chemicals and materials

Tinuvin P (99.8%, CAS: 2440-22-4, log*P*: 3.19; predicted by MarvinSketch) and Irganox 3114 (99.5%, CAS: 27676-62-6, log*P*: 13.17; predicted by MarvinSketch) were donated by BASF SE (Ludwigshafen, Germany). Additive-free HDPE, Irganox 1010 and Irgafos 168 were provided by the Laboratory of Plastics and Rubber Technology at the Budapest University of Technology and Economics. Tetrahydrofuran (HPLC grade) was purchased from Merck Ltd (Budapest, Hungary). Methanol (HPLC grade) was bought from LGC Promochem Ltd (Budapest, Hungary). Ammonium formate (HPLC-MS grade) was obtained from Fluka (Sigma Aldrich Co., Budapest, Hungary). Sodium hydroxide and sodium chloride (reagent grade) were bought from Sigma Aldrich.

UHT milk (in a Tetra Pak container) was used during the experiments to avoid milk spoilage. Milk with fat contents of 1.5% and 3.5% was purchased in local food stores and stored in a refrigerator at 5°C.

Instrumentation

An Agilent 1100 HPLC equipped with a degasser (G1322A), a binary pump (G1312A), an autosampler (G1313A), a column thermostat (G1316A) and a variable wavelength detector (G1314A) was used for chromatographic separation. Detection was performed with an Applied Biosystem API 2000 triple quadrupole mass spectrometer. HPLC-grade water was produced with a Direct-Q 5 (Millipore) system. Julabo FT902 cryostat and Hermle Z206A and Z230A centrifuges were used during sample preparation. Tinuvin P and Irganox 3114 was added to HDPE in a Brabender W50 EHT internal mixer. HDPE sheets were formed in a Fontijne SRA 100 Hydraulic press.

High-density polyethylene

HDPE, Irganox 1010, Irgafos 168 (to provide stability against thermal oxidation) and either Tinuvin P or Irganox 3114 were homogenised in an internal mixer at 190°C for 10 min and 50 rpm. The homogenised, melt mixture was transferred to the compression-moulding machine at 140 kN (190°C, 5 min), then it was cooled with water. The thus obtained 1.0-mm-thick (21.5 × 15.0 cm) sheets were cut into 1 × 1 cm pieces. All sheets were prepared to contain 0.1% (m/m) Irganox 1010 and 0.1% (m/m) Irgafos 168. The Tinuvin P or Irganox 3114 contents of the sheets were set at 0.01%, 0.05%, 0.1%, 0.5%, 1%, 3% and 5% (m/m). BASF's recommendation for Tinuvin P is 0.1–0.5% (m/m) (Technical Data Sheet of Tinuvin P) and that for Irganox 3114 is 0.05–0.3% (m/m) (Technical Data Sheet of Irganox 3114). Since all HDPE sheets contained either Tinuvin P or Irganox 3114 (never their mixture), all migration experiments proved the lack of either Irganox 3114 or Tinuvin P in both the food simulant and the milk.

Migration experiments

Milk, food simulant and HDPE were stored at 5°C at least for 24 h before the migration experiments started. Migration of Tinuvin P and Irganox 3114 was studied by

soaking 1 × 1 cm pieces (1.0 mm thickness) of HDPE into 1 g of either a 50% (v/v) ethanol–water mixture (food simulant) or milk. This is a simple and easily reproducible setup which, however, provides high surface/mass of simulant or milk ratio (24 $dm^2\ kg^{-1}$). Samples were gently shaken for the HDPE to be fully covered with the liquid. An appropriate time later the HDPE pieces were removed and the obtained extract was analysed immediately.

The contact time necessary to reach equilibrium (minimum contact time) was determined with 2 × 5 parallel samples (five for Tinuvin P and five for Irganox 3114). The HDPE containing the studied additive in 0.5% (m/m) concentration was soaked in the food simulant for 2, 5, 10 min, 0.5, 1, 2, 4, 6 and 8 h at 5°C.

Migration into food simulant and milk (1.5% and 3.5% fat content) was compared with experiments based on Regulation No. 10/2011/EC: samples were stored in a refrigerator at 5°C for a maximum of 10 days. First, HDPE containing 0.5% (m/m) Tinuvin P or Irganox 3114 was studied with all three extractants for 10 days. Thirty samples for each extractant–additive pair were placed in a refrigerator. Out of the 30 samples 3 were removed and analysed every day. These experiments provided information on the migration behaviour of the additives into the different extractants, the intra-day reproducibility of the experiments and the stability of the additives in the course of the 10-day migration experiments. A semi-variogram was used to confirm the non-correlation of the data. HDPE sheets with varying additive concentrations (0.01%, 0.05%, 0.1%, 0.5%, 1%, 3%, 5% (m/m)) were also tested with all three extractants. For all extractant–additive pairs, three parallel samples were prepared, stored at 5°C and analysed on the 10th day.

Analytical method

The analytical method used for the determination of Tinuvin P and Irganox 3114 has already been described in detail (including validation) by Bodai et al. (2014). The present paper mentions the main points only. Food simulant samples were injected into the HPLC-MS either directly or after 10-fold dilution with tetrahydrofuran (in case HDPE contained at least 0.5% (m/m) Tinuvin P). Milk samples were prepared with liquid–liquid extraction and low-temperature purification: after removal of the HDPE piece, 50 µl of 0.02 mol l^{-1} sodium hydroxide solution were added and then 5 ml tetrahydrofuran were used for extraction. Samples were vortexed for 1 min then sonicated for 10 min. Phase separation was assisted with 0.25 g sodium chloride and centrifugation (2000 rpm) for 10 min. A total of 1.5 ml aliquots were taken from the upper level into 2 ml HPLC vials, which were then sealed. The sealed vials were transferred into a 15 ml centrifuge tube that had been filled with 2.15 ml of −50°C acetone. Samples were kept in a cryostat at −50°C. In these centrifuge tubes, samples were centrifuged for 2 min (5500 rpm). After centrifugation, the fat-free supernatant was immediately transferred into another HPLC vial. In the case of Tinuvin P, 10-fold dilution was applied whenever its concentration in the HDPE was at least 0.5% (m/m). Otherwise, the transferred supernatant was injected directly into the HPLC-MS.

Separation was performed on a Kinetex pentafluorophenyl (100 mm × 2.1 mm × 2.6 µm) column with a 0.25 ml min^{-1} flow rate. A total of 1 mmol l^{-1} ammonium formate (the pH was adjusted to 2.8 with formic acid) in water was used as eluent A and 0.1% (v/v) formic acid in methanol was used as eluent B. Gradient separation was started with 10% B then with a linear gradient B was increased to 95% within 3 min. This composition was held for 13 min, then the eluent composition was lowered to 10% B immediately.

An electrospray ion source was used in positive ionisation mode at 4500 V. Nitrogen was used as the curtain gas, nebuliser and auxiliary gas and also as a collision-activated dissociation gas. Positive ions were acquired using MRM mode with two–two transitions. Tinuvin P was measured in protonated form and Irganox 3114 as a NH_4^+ adduct.

The method was successfully validated for Tinuvin P and Irganox 3114 in milk (1.5% and 3.5% fat) with external calibration. For Tinuvin P the accuracy was between 94% and 97%; for Irganox 3114 they were 94% and 98%. Tinuvin P got better intra-day precision than Irganox 3114. Maximal RSD% values were 5% for Tinuvin P in contrast to the 12% for Irganox 3114.

Semi-variogram

Many functions are at hand that can be used for determining spatial and/or temporal dependence. Of these, the semi-variogram was used (Webster & Oliver 2007; Oliver 2010; Molnár et al. 2014), which can be calculated with the Matheron algorithm (Matheron 1965):

$$\gamma(\mathrm{h})=\sum_{\mathrm{i}=1}^{\mathrm{N(h)}}[\mathrm{Z}(\mathrm{x_i})-\mathrm{Z}(\mathrm{x_i}+\mathrm{h})]^2 \qquad (1)$$

where $\gamma(h)$ is the semi-variogram; $N(h)$ is the number of pairs within the lag distance h; and $Z(x)$ and $Z(x + h)$ are the values of two sampled parameters at distance h measured in time or space from each other.

As discussed by Kovács et al. (2012), the most important properties of the function are as follows:

- C_0 (nugget effect) is the value of $\gamma(h)$ where the function starts. It provides information on the error of the sampling and the measurement.

- Nugget effect type of the semi-variogram, when the semi-variogram does not have a rising part, the empirical semi-variogram's points align parallel to the abscissa. In this case no range can be estimated, i.e. the sampling frequency is insufficient.
- The sill is equal to the variance ($C + C_0$), where C is the reduced sill.
- The range is the distance where the samples still have an influence on each other (Webster & Oliver 2007), i.e. where the function stabilises and reaches the variance after a rising part.

The estimation of sampling frequency using semi-variograms is based on the fact that samples outside the temporal or spatial variogram range are uncorrelated (Chiles & Delfiner 1999). If the sample is therefore taken outside the range, inferences can only be made on quantities depending on the marginal distribution of the process and the interdependence structure remains undetectable. Thus, to describe the processes, samples should be taken inside the spatial, or – in this case – temporal range (Hatvani 2014).

Empirical semi-variograms can be approximated by numerous types of theoretical functions, e.g. Spherical (Figure 1A) and Gaussian (Figure 1B) types. Of these, the best fit should be chosen. One of the easiest ways of determining the sufficient sampling frequency in the case of a theoretical semi-variogram model is finding h graphically, where the fitted function reaches the sill value. This h value is called range. In the case of a Gaussian model, the function approaches the sill asymptotically, so the range is often estimated at 95% of the sill value. In this study the ranges were determined at a higher h (where the $\gamma(h)$ is very close to the sill value), because we wanted to add a safety factor to the estimated minimum contact time.

Validation of the semi-variogram

Diffusion coefficients (D) and partition coefficients ($K_{P/F}$) were determined to confirm the suitability of semi-variogram method. Equation (2) (Crank 1975; Garde et al. 2001; Helmroth et al. 2002; Han et al. 2003; Sanches Silva, Cruz, et al. 2007; Sanches Silva et al. 2008) was used to calculate these values based on Fick's second law. The first step was to calculate the positive roots of equation (3). The more roots are determined, the more reliable are the results. However, according to some previous studies (Sanches Silva, Cruz, et al. 2007; Sanches Silva et al. 2008), the first 12 positive roots make the results feasible. $K_{P/F}$ was calculated with equation (4) and the root of the mean-square error % (RMSE%) with equation (5) (Helmroth et al. 2002; Sanches Silva et al. 2008):

$$\frac{M_{F,t}}{M_{F,\infty}} = 1 - \sum_{n=1}^{12} \frac{2\alpha(1+\alpha)}{1+\alpha+\alpha^2 q_n^2} \exp\left(\frac{-Dq_n^2 t}{L_p^2}\right) \tag{2}$$

$$\tan(q_n) = -\alpha q_n \tag{3}$$

$$\alpha = \frac{1}{K_{P/F}} \frac{V_F}{V_P} \tag{4}$$

$$RMSE\% = \frac{1}{M_{P,0}} \sum_{i=1}^{n} \sqrt{\left(\left(M_{F,t}\right)experimental, i - \left(M_{F,t}\right)predicted, i\right)^2} 100 \tag{5}$$

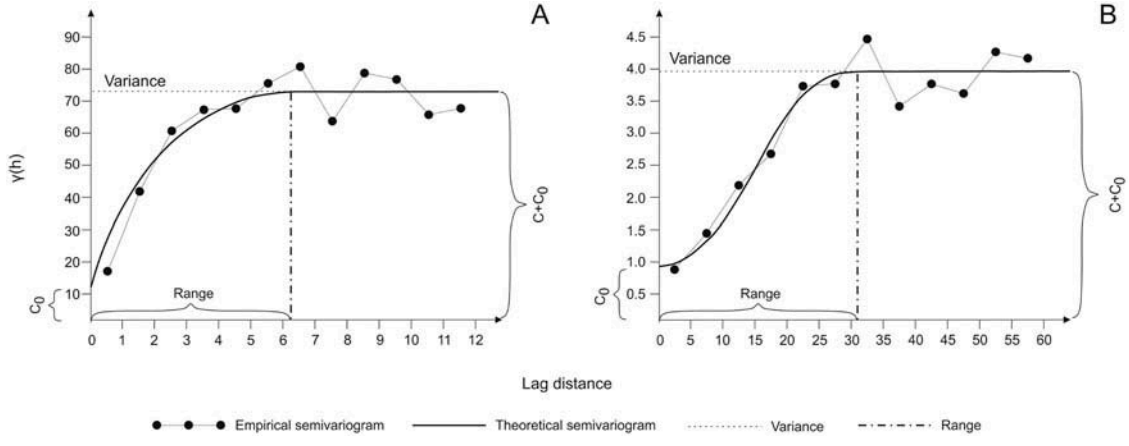

Figure 1. Examples on Spherical (A) and Gaussian (B) models of empirical semi-variograms.

where $M_{F,t}$ is the amount (μg) of additive migrated into the food or food simulant at time t; $M_{F,\infty}$ is the amount (μg) of additive migrated into the food or food simulant at equilibrium; α is the mass ratio of additive in food or food simulant and in the packaging film at equilibrium; D is the diffusion coefficient ($cm^2\ s^{-1}$); q_n is the nth positive root of equation (3); t is the migration time (s); L_p is the thickness of the HDPE (cm); $K_{P/F}$ is the partition coefficient of the additive between the polymer and the food or food simulant; V_P is the volume of the polymer (cm^3); V_F is the volume of the food or food simulant (cm^3); $M_{P,0}$ is the initial amount of the additive in the polymer (μg); and N is the number of the experimental points per migration study. $M_{F,\infty}$ was calculated with the average mass data corresponding to the last three sampling times. Calculations were performed with R (version 3.2).

D and $K_{P/F}$ were first calculated based on all the measured data. The calculation was then repeated with a decreased number of data. Data with the longest contact time were omitted one by one. Finally, the calculation was performed for only the first two contact times (2 and 5 min).

Results and discussion

Minimum contact time

Preliminary experiments showed for both Tinuvin P and Irganox 3114 their migration from HDPE into either milk or its authorised food simulant (50% (v/v) ethanol–water mixture) is very fast. Ten days are far too long to reach equilibrium. This means migration studies can be performed with much shorter contact times. The minimum contact time to be applied is the time necessary to reach equilibrium. Its value was determined by soaking HDPE pieces with 0.5% (m/m) additive concentration in the food simulant for 2, 5, 10 min, 0.5, 1, 2, 4, 6 and 8 h at 5°C. Results are shown in Figure 2. In the case of Tinuvin P, apparently 1 h is enough to reach equilibrium. For Irganox 3114, it is difficult to decide by visual examination of the diagram: possibly 0.5 h is enough.

For quantitative determination of the proper contact time semi-variograms were fitted to the data. After fitting a Gaussian model, the following ranges were estimated: 57 min for Tinuvin P (Figure 3A) and 23 min for

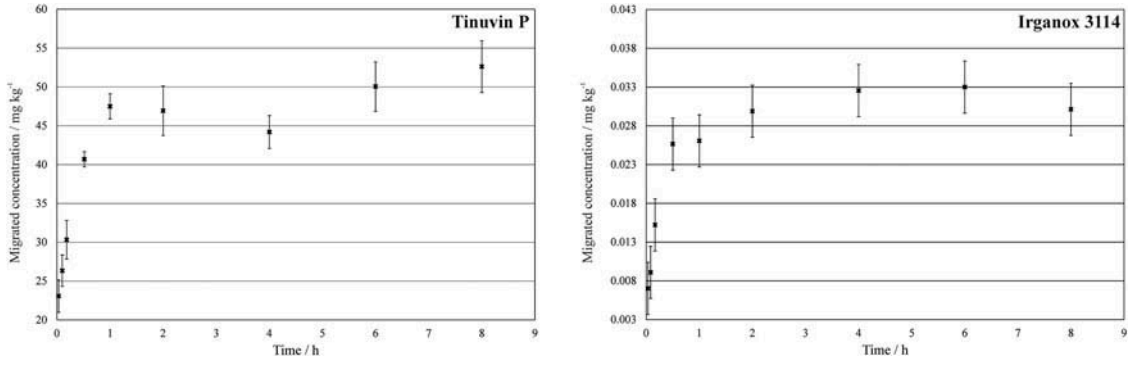

Figure 2. Migrated concentration of Tinuvin P and Irganox 3114 into a 50% (v/v) ethanol–water mixture as a function of contact time. Error bars represent the mean ± standard deviation of migrated concentrations (n = 5).

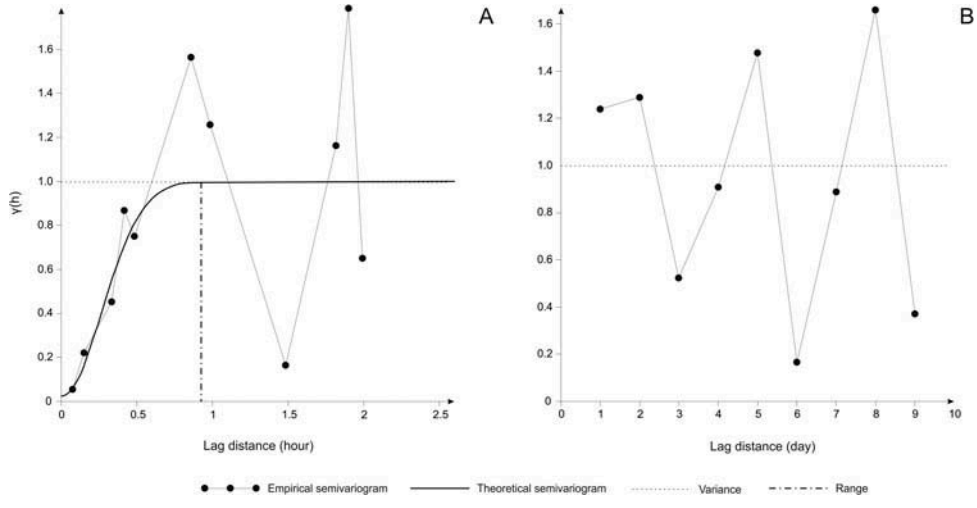

Figure 3. Empirical semi-variograms of Tinuvin P sampled for 8 h (A) and sampled daily for 10 days (B).

Irganox 3114. In geostatistics the general practice is to sample the phenomena in question inside the temporal and/or spatial range to be able to investigate its interdependence structure. In the case of multiple parameters describing the investigated phenomena, the range of the most variable parameter should be considered as the sufficient sampling frequency. Here, the idea is the opposite. Sampling should be taken after the time data are uncorrelated. This criterion can only be met if the largest temporal range (57 min) is considered as the sufficient sampling frequency. This means that to study the migration of Tinuvin P and Irganox 3114, the minimum contact time for food simulant samples should be 1 h and 10 days of contact time is unnecessary in the case where 24 $dm^2\ kg^{-1}$ surface/mass of simulant are applied.

Validation results of the variography

Suitability of the variography was confirmed by calculating diffusion and partition coefficients with a decreasing number of data. Without leaving any data, the diffusion coefficient for Tinuvin P was $6.91 \cdot 10^{-7}$ $cm^2\ s^{-1}$ and for Irganox 3114 it was $6.94 \cdot 10^{-7}$ $cm^2\ s^{-1}$. $K_{P/F}$ was 0.47 in the case of Tinuvin P and 0.19 for Irganox 3114. Computing RMSE% gave good results. Without leaving any data, RMSE% of Tinuvin P was 0.00079 and 0.00095 for Irganox 3114.

The high-diffusion coefficients for both Tinuvin P and Irganox 3114 may be explained by the limited miscibility of these polar compounds and the non-polar HDPE. This limited miscibility can lead to phase separation even during processing (compression moulding at 190°C), and thus this results in higher additive concentrations in the surface region than in the bulk material in both cases. This increased concentration on the surface is, however, a useful tool with regard to efficient UV protection.

Omitting the last data did not change diffusion or partition coefficients significantly at the beginning. Diffusion and partition coefficients with a decreasing number of data are shown in Table 1. The same results were obtained with partition coefficients (data not shown).

Table 1 clearly shows that inclusion of the data belonging to the longest contact times does not change the resulting diffusion and partition coefficients. Once equilibrium is reached, additional data points with longer contact times have little to add to the determination of these values. In contrast the shortest contact time range providing diffusion and partition coefficients similar to those with the longest contact times indicate the beginning of the equilibrium. In the case of Tinuvin P the big change is between 30 min and 1 h. Data points over 1 h have minimal influence on the calculated diffusion and partition coefficients. For Irganox 3114 the inclusion of the 30-min contact time almost doubles the calculated diffusion coefficient and decreases the partition coefficient with nearly one-third. Data points over 30 min have a notably lower influence. Therefore, in the case of Tinuvin P 1 h and for Irganox 3114 30 min proved to be enough to reach equilibrium. These results are in good accordance with the ranges obtained with variography (57 and 23 min respectively) and thus confirm the suitability of variography for the determination of minimum contact time.

Ten-day migration studies

Based on the storage procedure and the shelf-life of milk (except ultra-high-temperature processed milk) Regulation No. 10/2011/EC specifies 10 days of contact time at 5°C. Even though our experiments with the food simulant showed that 1 h is enough to reach equilibrium, we decided to follow the regulation in the course of our migration experiments. During the 10 days we analysed the extractants daily for the samples with HDPE containing 0.5% additive. Figure 4 shows a typical example: the results of Irganox 3114 migrating into 3.5% fat milk. The concentration of both additives in all extractants was nearly constant; no correlation with the contact time was detected. This is in accordance with the short time needed to reach equilibrium, and it also means that both additives were stable during the 10-day experiments.

The average migrated concentration of Tinuvin P was 18 mg kg^{-1} in 1.5% fat milk, 39 mg kg^{-1} in 3.5% fat milk

Table 1. Calculated diffusion and partition coefficients with all and with a decreased number of data for Tinuvin P and Irganox 3114.

	Tinuvin P			Irganox 3114		
Data point range	$K_{P/F}$	D ($cm^2\ s^{-1}$)	RMSE%	$K_{P/F}$	D ($cm^2\ s^{-1}$)	RMSE%
2 min–8 h	0.47	$6.91 \cdot 10^{-7}$	$7.90 \cdot 10^{-4}$	0.19	$6.94 \cdot 10^{-7}$	$9.50 \cdot 10^{-4}$
2 min–6 h	0.47	$6.93 \cdot 10^{-7}$	$7.96 \cdot 10^{-4}$	0.19	$6.95 \cdot 10^{-7}$	$8.33 \cdot 10^{-4}$
2 min–4 h	0.47	$6.92 \cdot 10^{-7}$	$7.96 \cdot 10^{-4}$	0.19	$6.93 \cdot 10^{-7}$	$8.34 \cdot 10^{-4}$
2 min–2 h	0.45	$7.31 \cdot 10^{-7}$	$7.96 \cdot 10^{-4}$	0.20	$6.84 \cdot 10^{-7}$	$8.37 \cdot 10^{-4}$
2 min–1 h	0.43	$7.60 \cdot 10^{-7}$	$7.93 \cdot 10^{-4}$	0.20	$6.85 \cdot 10^{-7}$	$8.36 \cdot 10^{-4}$
2 min–30 min	2.69	$4.30 \cdot 10^{-8}$	$3.95 \cdot 10^{-3}$	0.19	$7.28 \cdot 10^{-7}$	$8.30 \cdot 10^{-4}$
2 min–10 min	2.69	$3.80 \cdot 10^{-8}$	$4.41 \cdot 10^{-3}$	0.27	$3.89 \cdot 10^{-7}$	$7.75 \cdot 10^{-4}$
2 min–5 min	3.58	$1.40 \cdot 10^{-8}$	$2.99 \cdot 10^{-2}$	0.57	$6.22 \cdot 10^{-8}$	$3.49 \cdot 10^{-3}$

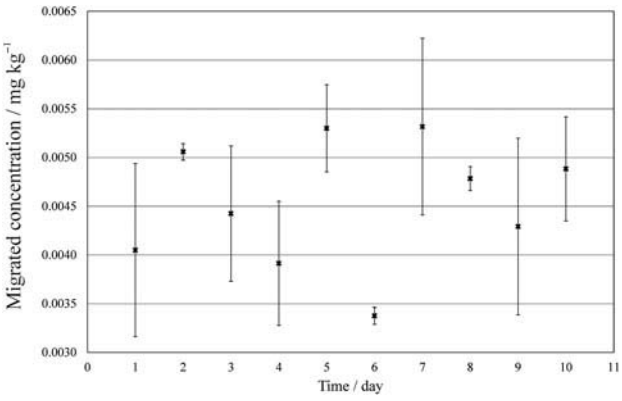

Figure 4. Migrated concentration into 3.5% fat containing milk as a function of contact time in the case of Irganox 3114. Error bars represent the mean ± standard deviation of migrated concentrations (n = 3).

and 54 mg kg^{-1} in food simulant. RSD% values were 15%, 16% and 19% respectively. In the case of Irganox 3114, 0.0047 mg kg^{-1} migrated into 1.5% fat milk, 0.0045 mg kg^{-1} into 3.5% fat milk and 0.039 mg kg^{-1} into food simulant. The RSD% values were 25%, 26% and 18% respectively. These RSD values are distinctly higher than those observed during the validation of the analytical method. This increased variance may originate from various sources: inter-day precision of the analytical measurement is better than its intra-day precision; inhomogeneity of the additives in the HDPE; and inaccuracy in the size of the HDPE pieces.

Although the results showed no apparent correlation, we decided to confirm it with a statistical method. A semivariogram was used again to examine the correlation of the migration concentration into each extractant (milks with 1.5%, 3.5% fat and the food simulant). The semivariograms obtained from the 10-day samplings were all of nugget effect type, i.e. the rising part of the functions were missing. The points were arranged around the variance (Figure 3B), thus the measured concentrations proved to be uncorrelated.

The effect of the concentration of the additives in the HDPE on the migrated amount was studied using HDPE sheets containing 0.01%, 0.05%, 0.1%, 0.5%, 1%, 3% or 5% (m/m) Tinuvin P or Irganox 3114. (BASF's recommendation for Tinuvin P is 0.1–0.5% (m/m) and that for Irganox 3114 is 0.05–0.3% (m/m) in HDPE.) The results are presented in Figure 5. The trend in the data is the same for all additive–extractant pairs: at low concentrations a fast increase was observed in the migrated amount, but approximately at 1% (m/m) the migrated concentrations became constant, i.e. independent of the original concentration of the additive in the HDPE. This saturation of the extractants is probably due to the high log$P$ values of the additives: 3.19 for Tinuvin P and 13.17 for Irganox 3114. The saturation concentrations can be easily compared by examining the average concentrations obtained for 1%, 3% and 5% (m/m) HDPE sheets: a 1.5% fat content milk dissolved 41 mg kg^{-1}, a 3.5% fat content milk 58 mg kg^{-1} and the food simulant 63 mg kg^{-1} Tinuvin P. The same values for the less polar Irganox 3114 are 0.010, 0.011 and 0.046 mg kg^{-1} respectively.

For both additives the concentrations measured in the food simulant were higher than that for the milk samples at all HDPE concentration levels. For Tinuvin P the difference in the result of the milk and the food simulant is notably small, in some cases insignificant. The food simulant gave a close estimate of the milk samples for Tinuvin P. In the case of Irganox 3114 the results for 1.5% and 3.5% fat milk are nearly identical. On the other hand, the food simulant dissolved significantly more additives than the milk. Using the food simulant for modelling the migration of Irganox 3114 into milk gives an overestimation by a factor of minimum 3.5.

The SML for Tinuvin P is 30 mg kg^{-1}; for Irganox 3114 it is 5 mg kg^{-1}. The results show that in the case of Tinuvin P the migrated concentration can reach the SML if it is used at approximately 0.5% (m/m) in HDPE. This means that special care must be taken, since BASF's recommendation is 0.1–0.5% (m/m). For Irganox 3114 even the saturated concentration is two orders of magnitudes lower than the SML.

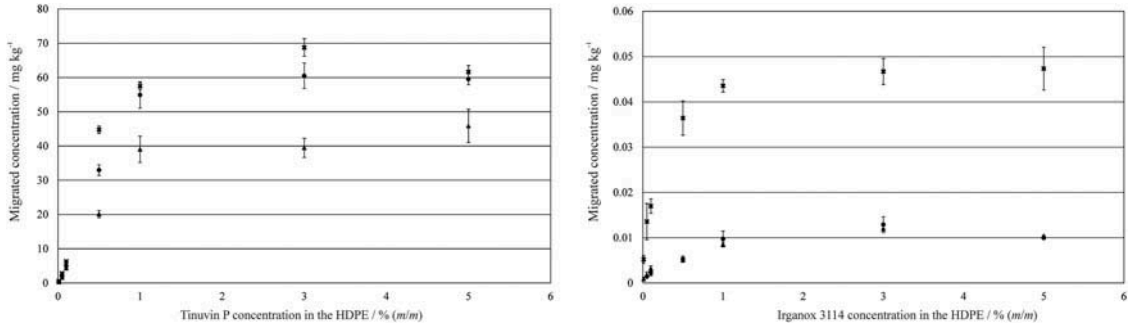

Figure 5. Migrated concentration into milk and food simulant from HDPE with different concentration of additives. A ▲ represents the mean migrated concentration into 1.5% milk, a ● into 3.5% milk and a ✕ into food simulant. Error bars represent the mean ± standard deviation of migrated concentrations (n = 3).

Conclusions

Regulation No. 10/2011/EC specifies SMLs for hundreds of chemicals used in plastic materials and articles intended to come into contact with food. To make the measurement of migration easier, it allows food simulants to be used (e.g. a 50% (v/v) ethanol–water mixture for milk). It also gives a recommendation on the contact time and temperature of the migration experiments based on the storage procedure and the shelf-life of the food. For milk (except ultra-high-temperature processed milk) the recommendation is 10 days at 5°C. Our experiments with HDPE containing either Tinuvin P or Irganox 3114 showed that the equilibrium between HDPE and a 50% (v/v) ethanol–water mixture can be reached within 57 and 23 min respectively. However, a minimum contact time has to be determined for every combination of polymer, additive and food simulant or foodstuff. Results obtained with semi-variogram were confirmed by computing diffusion and partition coefficients with a decreasing amount of data. The results achieved with this method correlate well with those calculated with the semi-variograms. Thus, the semi-variogram proved to be a useful mathematical tool for making a distinction between the early migration range (with increasing concentration) and the equilibrium, despite the relatively high variance of the data.

Ten days of migration with daily concentration measurements was studied with 1.5% and 3.5% fat content milk and food simulant using HDPE pieces containing 0.5% (m/m) Tinuvin P or Irganox 3114. The measured concentrations showed no correlation with contact time. The apparent uncorrelation, confirmed also by variography, is in accordance with the short time needed to reach equilibrium, and it also means that both additives were stable during the 10-day experiments. These results show that in the case of this two additives 10 days are far too much for testing the migration. Using shorter contact times may speed up migration studies considerably.

Migration experiments using HDPE pieces with varying additive content showed that an ethanol–water mixture (50% (v/v)) is an acceptable food simulant for milk for both Tinuvin P and Irganox 3114. The same trend was obtained for all extractant–additive pairs: at low concentrations a fast increase was observed in the migrated amount, but approximately at 1% (m/m) the measured concentrations became independent of the original concentration of the additive in the HDPE. For both additives the concentrations measured in the food simulant were higher than that for the milk samples at all HDPE concentration levels. For Tinuvin P the food simulant gave a close estimate. However, using the food simulant for modelling the migration of Irganox 3114 into milk gives an overestimation with a factor of minimum 3.5. This overestimation is reassuring regarding food safety. However, it is not detrimental for producers, since the concentrations measured for this additive were far below the SML even in the food simulant.

Experiments using HDPE with additive concentrations at and over the recommended level showed that in the case of Tinuvin P special care must be taken, since its recommended amount in the HDPE can result in additive concentrations near or even over the SML in milk. On the other hand, our results show that Irganox 3114 cannot reach the SML in milk or food simulant. Its low solubility correlates with its high log*P*. This draws attention to the fact that comparing the solubility of an additive with its SML may save migration experiments.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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