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A novel method to investigate the migration regularity of toxic substances from toys to saliva and sweat

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Abstract

Residual toxic substances found in toys are a serious threat to children's health; these substances may migrate into the body when toys are handled or sucked by children. However, the migration risk caused by toxic toys is difficult to estimate if the migration regularity of these substances is unknown. In this study, a method was established to investigate the migration regularity of toxic substances, specifically styrene, from acrylonitrile–butadiene–styrene toys to saliva and sweat. Two determination methods of residual amount and migration amount were initially developed and then utilized to obtain specific migration data by using a self-made migration device. Migration models were established according to Fick's second law; the models included a diffusion coefficient and a partition coefficient calculated on the basis of the obtained migration data. These models were then applied to predict the migration behavior of styrene in two real toys; afterward, predicted findings were subsequently compared with experimental data. The experimental data were consistent with the predicted findings and thus verified the validity of the migration models. Using this approach, we can determine the migration regularity of other toxic

substances in toys; the predictability of the risk caused by toxic toys is also a relevant factor to promote toy safety.

1 Introduction

Toys are important things for children; however, residual toxic substances found in plastic toys may migrate into the body through skin exposure, inhalation, and oral exposure when these toys are handled or sucked by children; as a consequence, children's health is severely harmed. Considering the risk posed by toxic toys to children, researchers should investigate the migration regularity of toxic substances from toys to the body. Acrylonitrile–butadiene–styrene (ABS) plastic exhibits numerous desirable properties, such as good impact and chemical resistance, high rigidity and toughness, and convenient coloring method and processing.[1, 2] As such, ABS is a widely used plastic in toy manufacturing.[3] Styrene is largely present in ABS toys and its content reaches >2,000 mg/kg.[4] It likely triggers eye and mucous membrane irritation and also increases the risk of central nervous system dysfunction, leukemia, or lung tumors during long-term exposure, as observed in previous toxicology studies.[5-8] Therefore, the International Agency on Cancer Research classified styrene as a Group 2B carcinogen, and the National Toxicology Program listed styrene in its Twelfth Report on Carcinogens as "reasonably anticipated to be a human carcinogen".[9] As a result, styrene has been strictly controlled by the international society. For example, 0.6 mg/kg in food and 0.75 mg/L in toys have been set as the specific migration limits of styrene by the European Commission Directive 92/39/EEC and The European Toy Safety Standard EN 71-9, respectively.[10, 11]

Studies on the safety of toys have mainly determined the residual or migration amounts of toxic substances. For example, Abe[4] detected the residual levels of 14 volatile substances in 59 ABS toys through headspace-gas chromatography/mass spectrometry (HS-GC/MS) and determined the amounts of these volatile substances that migrate from 10 toys to water at 40 ℃ for 30 min. Preliminary results revealed that only 4 substances migrating from toys to water via methanol are overestimated.

However, further studies on migration regularity have yet to be performed. Al-Natsheh[12] developed a GC/MS method to determine 8 phthalates and the migrated portions of plasticized polymeric toys and childcare articles and to survey the Jordanian market. Liquid-liquid extraction (LLE) has been applied to determine PAEs that migrate into artificial saliva at 37 ℃. Different substances, such as fragrance allergens,[13, 14] bisphenol A,[15] and polymeric additives,[16] have also been detected in plastic toys. The migration regularity and migration models of toxic substances in toys have yet to be determined, although these parameters have been explored in food contact materials.[17-20] According to Fick's second law, migration models can be established on the basis of specific migration experimental data. In migration models, two important parameters, namely, diffusion and partition coefficients, are effectively applied to examine and predict the migration behaviors of substances. Similar migration models can be used to examine these behaviors in toys. Nevertheless, models of food contact materials cannot be directly used to investigate plastic toys because toys exhibit different forms and migration conditions from food contact materials. If the migration regularity of toxic substances in toys is known, then questions can be easily clarified. For instance, the amount of toxic substances that migrate to the human body over a certain period and the maximum migration amount of toxic substances can be identified when the migration regularity is determined. Therefore, migration regularity should be identified to estimate the risk posed by toxic toys to children's health.

Using styrene in ABS toys, we aimed to establish a method to investigate the migration regularity of toxic substances from toys to the body. Two determination methods, namely, residual amount determination and migration amount determination, were initially developed and utilized to obtain migration data by using a self-made migration device. The migration models were then used to predict the migration behavior of toxic substances from two real toys by comparing the predicted values with the experimental data.

2 Experimental

Reagents and materials

Styrene (purity > 99.9%) was obtained from Dr. Ehrenstorfer (Germany). Stock solution of styrene (1000 mg/L) was prepared in hexane. Further dilutions were prepared as needed to series of working solution with hexane and stored in amber glass vials at 4 ℃. Acetone, methanol, dichloromethane, ethyl acetate, and hexane were of chromatographic grade (J.T. Baker, USA). Deionized water was obtained from a Milli-Q water purification system (Millipore, USA). Envi-carb SPE cartridges (500 mg, 6 mL) was purchased from Supelco (USA). SM2000 grinding machine and NTS-4000 water bath oscillator were offered by Retsch (Germany) and Eyela (Japan), respectively.

Simulated saliva and sweat were prepared in accordance with the standard of DIN53160 of EU[21]. Simulated saliva: 4.5 g NaCl, 0.3 g KCl, 0.3 g Na_2SO_4, 0.4 g NH_4Cl, 3.0 g lactic acid of 90%, and 0.2 g urea. Water was added until the volume reached 1000 mL. Simulated sweat: 4.2 g $NaHCO_3$, 0.5 g NaCl and 0.2 g K_2CO_3. Water was added until the volume reached 1000 mL.

The commercial ABS toys of different brands were obtained from supermarkets and malls in Beijing, China. Before analysis was conducted, the samples were maintained in sealed packages to avoid contamination.

Sample preparation and migration device

The residual amount was determined in accordance with the following procedures. The samples were cut into pieces with a size of <2 mm. Afterward, 0.1 g of the samples was placed in 20 mL glass vials and dissolved in 5 mL of acetone. After the solution was shaken slightly to achieve complete dissolution, 5 mL of methanol was slowly added, and the solution was vigorously shaken to re-precipitate the ABS polymer. The solution was set aside until two clear phases were observed; the supernatant was then transferred into a centrifuge tube. Afterward, the precipitate was washed with 3 mL of methanol. The combined solution in the centrifuge tube was centrifuged at 10,000 rpm and 4 ℃ for 5 min. The supernatant was allowed to pass

through an Envi-Carb SPE cartridge at a rate of 3 mL/min for clean-up, and the effluent was collected. The cartridge was eluted with 5 mL of hexane, and the effluent was collected and combined with the previous effluent. The combined solution was diluted with hexane to obtain a final volume of 20 mL; the solution was subsequently filtered through a 0.45 μm PTFE membrane. The resulting solution was subjected to GC-MS analysis.

The self-made migration device is illustrated in Fig. 1. We obtained the smooth side of the toy and cut round pieces with a diameter of 12 mm, a thickness of 1.3 mm, and a weight of 0.167 ± 0.026 g by using a round slicer with a pore in the center. Six round sample pieces were strung in a line with a stainless steel wire, and a glass bead was placed between samples to separate two samples from each other. The line was placed in a 40 mL customized colorimetric tube. The simulated saliva or sweat (20 mL) was placed in the tube and subsequently subjected to vibrations of 100 r/min in a water bath at 37 ℃ for 10 min–120 h to obtain a migration solution. Styrene was then subjected to LLE from the migration solution by using 3 mL of *n*-hexane for determination.

Analytical conditions

The extracts were analyzed using an Agilent 6890-5975 GC–MS system equipped with a 7683B autosampler. Separations were performed on a DB-624 capillary column (30 m×0.25 mm×1.4 μm) from Agilent Technologies (USA). Helium (purity > 99.999%) was used as a carrier gas at 1.0 mL/min. The split ratio was 30:1, and the injection volume was 1 μL. The temperatures of chromatographic inlet, transfer line, ion source, and quadrupole mass spectrometer were maintained at 260, 260, 230, and 150 ℃, respectively. The GC oven temperature was programmed from 60 ℃ (held for 1 min) to 220 ℃ (held for 6 min) at 20 ℃/min. The mass spectrometer was fitted with an EI source operated at 70 eV, and mass spectra were recorded in the range of m/z 40 to 200 amu in a full-scan acquisition mode. Styrene was quantified in the selected ion monitoring (SIM) mode set at m/z 104 (quantitative ion), 78, and 51 ions. The retention time of styrene was 6.158 min.

Migration models

The migration of substances from a polymer can be described as diffusion in accordance with Fick's second law: [22]

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} \qquad (1)$$

where C is the concentration of a migrant at time t and position x; D is the diffusion coefficient, which is considered constant during migration and is dependent only on temperature.

The amount of the migrant that transferred from the polymer to the simulant can be obtained using Eq. (1): [22, 23]

$$\frac{M_{F,t}}{M_{F,L}} = 2\left(\frac{Dt}{L_P^2}\right)^{0.5}\left\{\frac{1}{\pi^{0.5}} + 2\sum_{n=1}^{\infty}(-1)^i ierfc\left[\frac{nL_P}{(Dt)^{0.5}}\right]\right\} \qquad (2)$$

where $M_{F,t}$ (mg) is the mass of the migrant from the polymer to the simulant after time t; $M_{F,L}$ is the mass of the migrant from the polymer to the simulant for the entire migration period; and L_p (cm) is the thickness of the polymer.

Eq. (2) can be simplified as Eq. (3) if the simulant does not initially contain any migrant, that is, $M_{F,L} = M_{P,0}$, where $M_{P,0}$ is the initial amount of the migrant in the polymer, migration times are short, and $ierfc[nL_P/(Dt)^{0.5}] \rightarrow 0$: [24, 25]

$$\frac{M_{F,t}}{M_{P,0}} = \frac{2}{L_P}\left(\frac{Dt}{\pi}\right)^{0.5} \qquad (3)$$

Migration from polymer materials to simulants are mainly determined by diffusion coefficient (D) and partition coefficient ($K_{P/F}$). D is a measure of "how fast" a migrant travels, and this parameter can be obtained from Eq. (3); $K_{P/F}$ describes the relation between the concentrations in the polymer and in the simulant at equilibrium, that is, the amount of the migrant that transferred to the simulant from the polymer at equilibrium; this parameter can be calculated using Eq. (4): [21]

$$K_{P/F} = \frac{C_{P,\infty}}{C_{F,\infty}} = \frac{M_{P,0} - M_{F,\infty}}{M_{F,\infty}} \times \frac{V_F}{V_P} \qquad (4)$$

where $K_{P/F}$ is the partition coefficient of the migrant between a polymer and a simulant; $M_{F,\infty}$ is the mass of the migrant in the simulant at equilibrium; V_F is the volume of the simulant; and V_P is the volume of the polymer.

3 Results and discussion

Establishment of determination methods

For toxic substances, the determination methods of residual amount and migration amount should be initially established. Different methods, such as ultrasound, dissolution–precipitation, and headspace, can be performed to extract residual substances; LLE, solid-phase extraction (SPE), and headspace can be used to extract the migration amount. The extracts can be detected through GC-MS or LC-MS.

Styrene was extracted via a dissolution–precipitation method improved on the basis of our previous work[14] to determine the residual amount (See Supplementary Information for details). After the toy was dissolved in acetone and precipitated using methanol, the supernatant was centrifuged and passed through an Envi-carb SPE cartridge with functional graphitized carbon used as a sorbent to eliminate matrix co-extractants, such as dyes. Five solvents, namely, acetone, methanol, hexane, ethyl acetate, and dichloromethane, were evaluated as eluting solvents to achieve optimal recovery because graphitized carbon could also adsorb a portion of styrene. The optimum result was obtained with hexane (>95%), which was used in subsequent experiments. Dissolution–precipitation extraction was quantified using an external standard method. Linearity ranged from 10 mg/kg to 2000 mg/kg with a correlation coefficient of 0.9999. Multiple extraction and recovery studies were also conducted to determine the absolute extraction efficiency; conducting these experiments, we effectively avoided the underestimation of actual styrene content in a toy sample. The average recoveries spiked at two different levels ranged from 96.2% to 102.3% (RSD $\leq$ 6.1%). The results indicated that the proposed method is a reliable tool to determine residual styrene in ABS toys.

SPE and LLE were successively performed to determine the migration amount. Styrene standard solution (50 μL of 200 mg/L in methanol) was added to and completely mixed with the simulated saliva (20 mL) to prepare styrene aqueous solution, which was used in further recovery studies. Fig. 2 illustrates that the highest recovery of SPE (<75%), obtained by using Chromabond HR-P as the SPE cartridge and *n*-hexane as the elution solvent, was significantly less than that of LLE (>83%). These findings might be attributed to the volatility or strong retention of styrene in SPE cartridges. LLE was easily operated; thus this technique was suitable for the rapid processing of many samples. Recovery gradually decreased as solvent volume increases. *n*-Hexane (3 mL) was used because of high recovery and convenient sampling. An external standard method was also applied. Linearity ranged from 0.06 mg/kg to 15 mg/kg with a correlation coefficient of 0.9996. The average recoveries spiked at three different levels of styrene ranged from 82.3% to 93.2% (RSD ≤ 3.2%) and from 83.8% to 89.9% (RSD ≤ 4.0%) in saliva and sweat, respectively. This finding confirmed that this method can be used to determine the migration amount of styrene.

Acquisition of migration data

The residual amount determination method was used to analyze 18 commercially available ABS toys. A toy containing 1378 mg/kg styrene was selected as the migration sample. Migration experiments were performed at 37 ℃ to simulate normal body temperature, that is, actual contact temperature. Fig. 3 shows that changing patterns were almost consistent in the simulated saliva and sweat; these patterns indicated that the migration amount of styrene gradually increased as migration time was prolonged and eventually reached equilibrium within 120 h. The migration amount detected in saliva was slightly higher than that in sweat; this difference might be attributed to various components of saliva and sweat. The maximum migration amounts in saliva and sweat were 2.872 and 2.782 mg/kg, respectively; these values were higher than the migration limit of styrene in food (0.6 mg/kg). This finding implies that a considerable quantity of styrene can migrate

into the body; as a consequence, health hazards may occur when children place toys in their mouths for a prolonged period.

Establishment of the migration model

Migration from polymer materials to simulants is mainly determined by D and $K_{P/F}$. The diffusion coefficients of the process were obtained by calculating the slope between $M_{F,t}/M_{P,0}$ and $t^{1/2}$ according to Eq. (3), as shown in Fig. 4. The diffusion coefficients were 5.5752×10^{-14} $cm^2\ s^{-1}$ and 4.5405×10^{-14} $cm^2\ s^{-1}$ at a migration temperature of 37 ℃ in the simulated saliva and sweat, respectively. These diffusion coefficients were approximately lower than the reported ones. For example, Choi[17] reported that the diffusion coefficients of styrene in PS packaging were 3.61×10^{-13}, 9.45×10^{-13}, 3.49×10^{-12} $cm^2\ s^{-1}$ in *n*-hexane as a simulant at 10, 24, and 40 ℃ respectively. Paraskevopoulou[20] obtained the following diffusion coefficients of styrene in PS packaging: 9.08×10^{-11}, 2.21×10^{-10}, and 3.84×10^{-10} $cm^2\ s^{-1}$ in ethanol solution as a simulant at 25, 40, and 60 ℃, respectively. Different types of simulants and polymers at different temperatures yield various diffusion coefficients because of the interaction between a simulant and a polymer matrix. According to Eq. (4), the partition coefficients of the process were 10508.2 and 10848.8 in the simulated saliva and sweat, respectively.

On the basis of the diffusion and partition coefficients, we established the migration models of styrene from ABS toys to saliva and sweat according to Fick's second law. Using these migration models, we can easily predict the migration regularity and maximum migration amount of styrene when the initial styrene content in the toys are known. Furthermore, migration experiments need not be performed one by one; thus, the proposed method saves time and cost.

Practical application of the migration models

The established migration models were applied to estimate the migration behavior of styrene in two real ABS toys by comparing the predicted values with the experimental data. The initial contents of styrene in samples A and B were 1741 and 1348 mg/kg, respectively. These findings of Fig.5 exhibited the same increasing trend

as the migration time was extended; thus, the experimental values were consistent with the predicted values. Indeed, the migration models were valid. The predicted values were slightly larger than the experimental ones possibly because the toy surface was treated by a unique process; as a result, the migration of styrene was relatively difficult. The maximum amount of migration at equilibrium could be estimated on the basis of the partition coefficients, which were 10508.2 in saliva and 10848.8 in sweat. Therefore, the maximum amount of styrene migration from sample A to saliva and sweat were 3.145 and 3.047, respectively, and from sample B to saliva and sweat were 2.435 and 2.359, respectively.

4 Conclusions

In the present work, styrene in ABS toys was used to establish a novel method to investigate the migration regularity of toxic substances from toys to saliva and sweat. A self-made migration device was designed on the basis of the determination methods of residual and migration amounts to obtain migration data. Migration models with diffusion and partition coefficients were developed according to Fick's second law. The models were then used to estimate the migration regularity and maximum migration amount of styrene in real toys. Using this approach, we can determine the migration regularity of other toxic substances in toys; furthermore, the predictability of the risk caused by toxic toys is a relevant factor to promote toy safety.

Acknowledgements

This work was financially supported by the Quality Inspection Public Welfare Scientific Research Foundation (No. 201310057) and the Science Research Program of the Chinese Academy of Inspection and Quarantine (No. 2014JK017)

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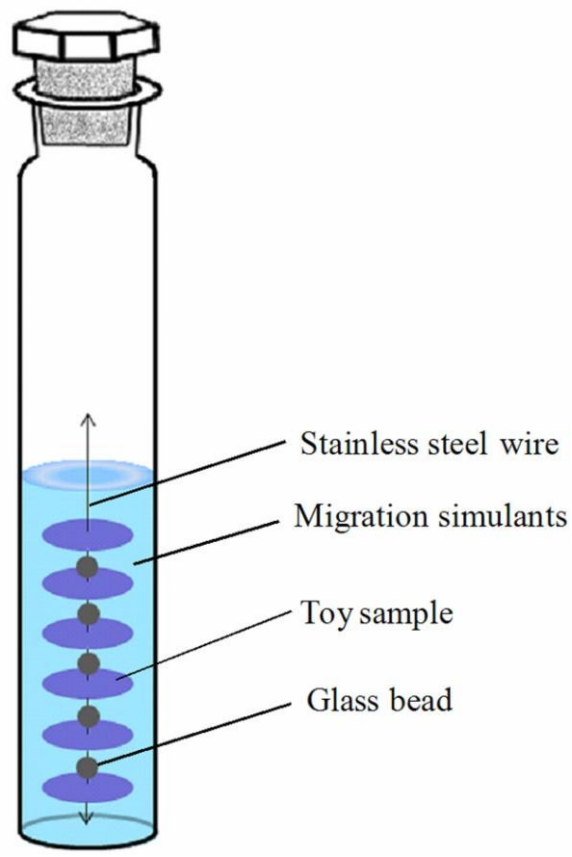
Fig. 1 Schematic of the self-made migration device

Fig. 2 Optimization of extraction conditions to determine the migration amount of styrene (n = 4)

Fig. 3 Effect of simulants on the migration of styrene (n = 3)

Fig. 4 $M_{F,t}/M_{p,0}$ versus $t^{0.5}$ of styrene from toys to simulants

Fig. 5 Migration: Experimental data and predicted values of migration models (n = 3)



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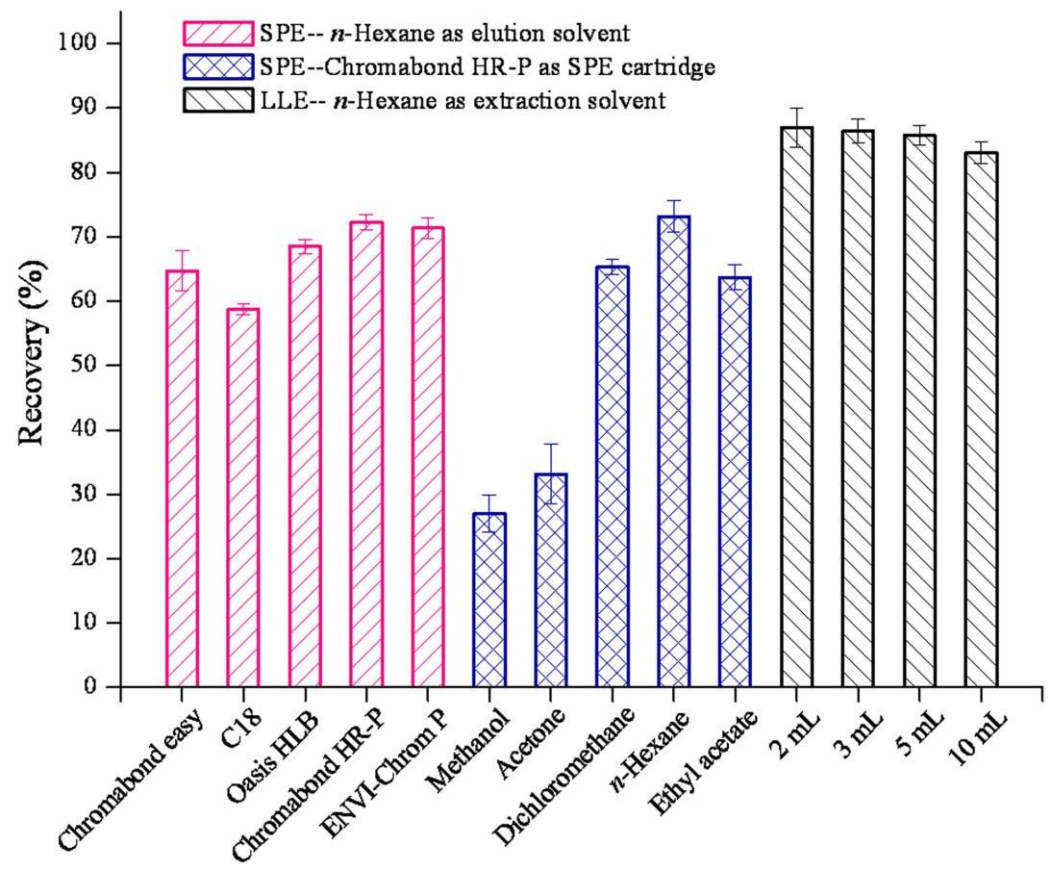

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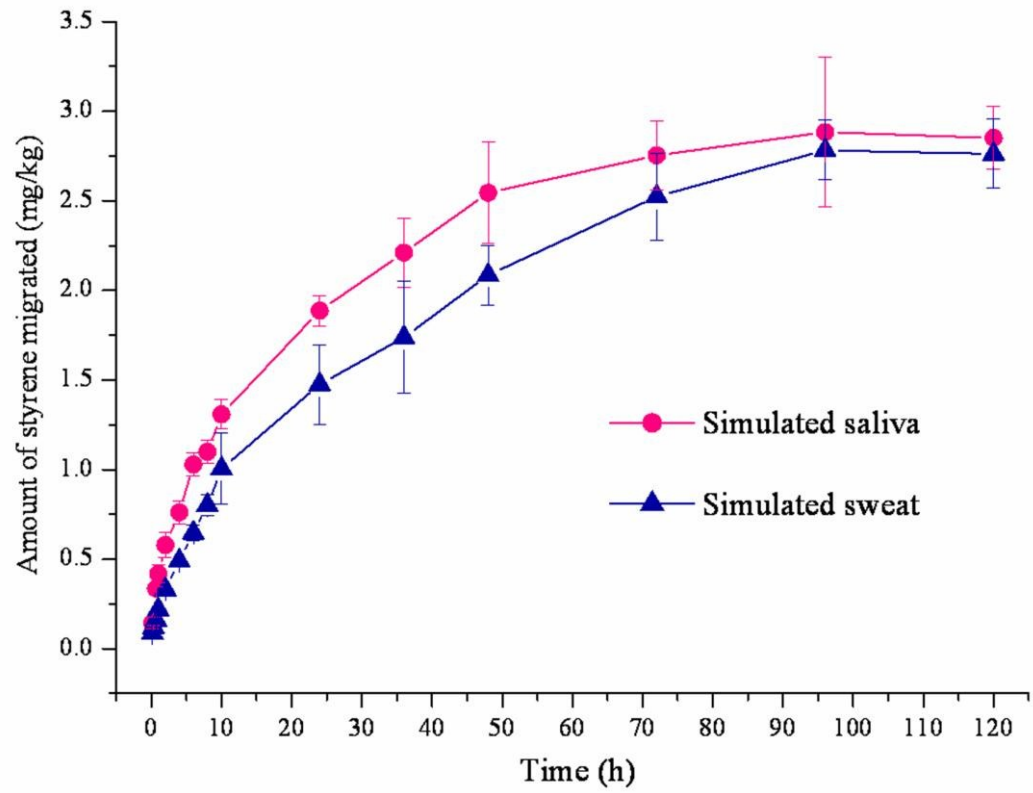


Fig. 3 Effect of simulants on the migration of styrene (n = 3)

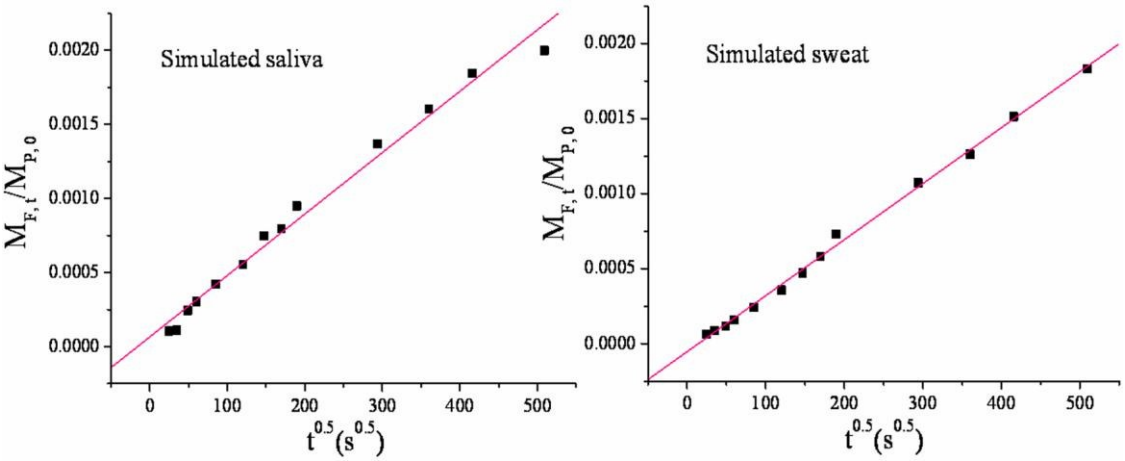


Fig. 4 $M_{F,t}/M_{p,0}$ versus $t^{0.5}$ of styrene from toys to simulants

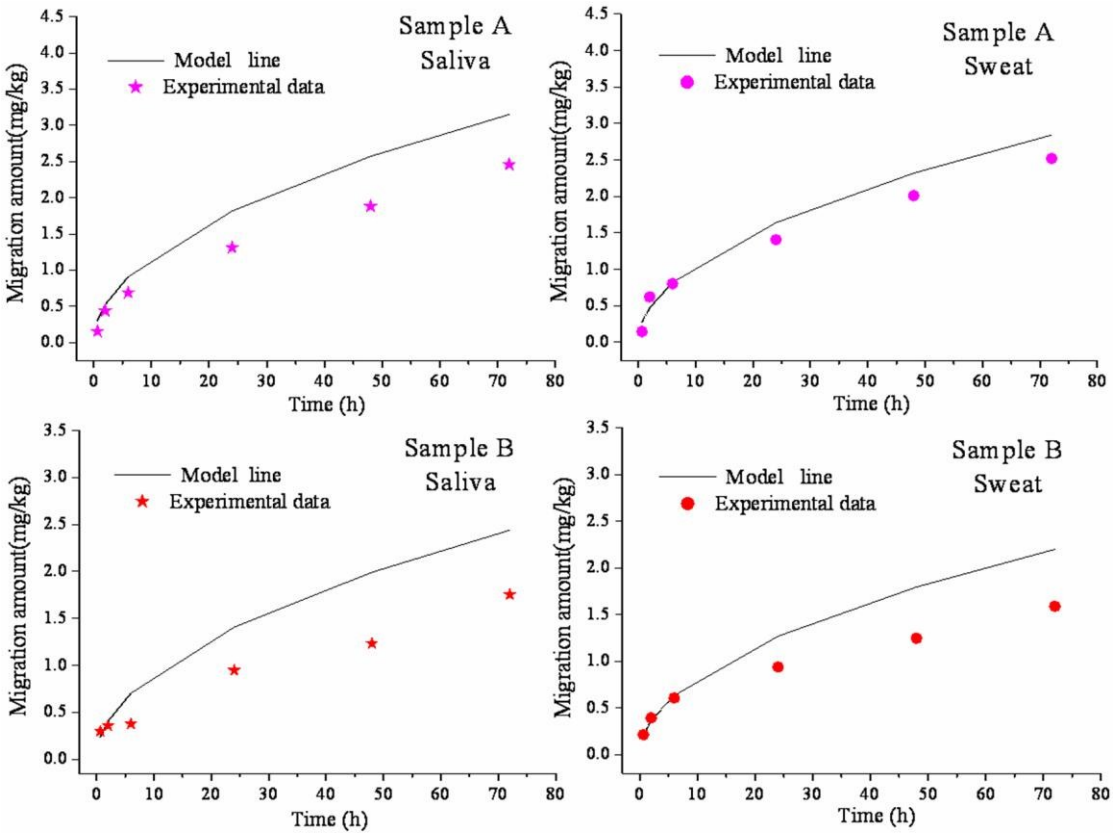
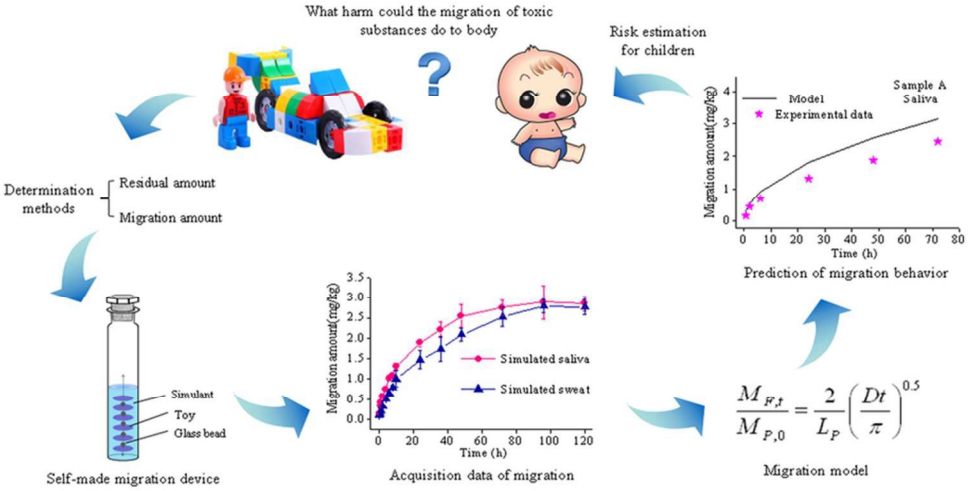


Fig. 5 Migration: Experimental data and predicted values of migration models (n = 3)



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