Children's exposure to polybrominated diphenyl ethers (PBDEs) through mouthing toys

Children's Exposure to Polybrominated Diphenyl Ethers (PBDEs) through Mouthing Toys

Alin C. Ionas[1,2#]*, Jocelyn Ulevicus[3#], Ana Ballesteros Gómez[2], Sicco H. Brandsma[2], Pim E.G. Leonards[2], Margot van de Bor[3], Adrian Covaci[1*]

[1]Toxicological Centre, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium

[2] Institute for Environmental Studies, VU University, de Boelelaan 1085, 1081 HV Amsterdam, The Netherlands

[3] Health and Life Sciences Department, VU University, de Boelelaan 1085, 1081 HV Amsterdam, The Netherlands

- shared first co-authorship

* - corresponding authors: alin.ionas@gmail.com; adrian.covaci@uantwerpen.be

Fax: +32-3-265-2722

Abstract

Polybrominated diphenyl ethers (PBDEs) have previously been detected in children toys, yet the risk of child exposure to these chemicals through the mouthing of toys or other items is still unknown. We aimed to expand on the current knowledge by investigating the impact of infants' mouthing activities on exposure to PBDEs present in toys. This was established by a leaching model for determining the amount PBDEs that can leach from toys into saliva in simulated conditions. The PBDE migration rate was at its highest for the 15 min low-exposure scenario incubations (198 pg/cm^2 × min) with the ERM EC-591 certified reference material (CRM) (0.17% *w/w* PBDEs). The leaching process was congener-dependent, since the percentage of lower brominated PBDE congeners that leached out was up to 4.5 times higher than for the heavier PBDEs. To study the scenario in which a child would mouth on a toy flame retarded with BDE 209 alone, a plastic item containing 7% BDE 209 (*w/w*) was also tested. The BDE 209 amounts leached out in only 15 min were higher than the amounts leached from the CRM after the 16 h incubation. For the Belgian population, the exposure scenario from mouthing on toys containing PBDEs in amounts similar to the REACH threshold was found to be lower than the exposure from mother's milk, but higher than the exposure through diet or even dust.

Keywords: Polybrominated diphenyl ethers; Mouthing; Plastics; Exposure Assessment; In Vitro; Saliva

1. Introduction

There is growing concern that some toys may contain harmful additives which, if absorbed into the body, may adversely impact long-term health (Stringer et al., 2000; Stapleton et al., 2011). It is well-documented that flame retardants (FRs) are present in indoor dust, and therefore available for human absorption through both dietary and non-dietary means, such that young children who spend a high amount of time playing with toys, on the ground in indoor areas, as well as using their hands and mouths to negotiate their environment, have a potentially high risk of exposure (Moya et al., 2004; Cohen Hubal et al., 2000; Xue et al., 2007). Thus, the mouthing of toys and other plastic items likely also contributes to FR exposure, especially as infants express a higher propensity for object mouthing than children of older ages, and may be in contact with the dust and chemicals present in toys (Xue et al., 2007; EPA, 2008). However, the data available on the process through which PBDEs migrate into children's saliva and body is scarce at best.

In a previous study (Ionas et al., 2014), a wide array of toys was examined for potentially harmful additives, including PBDEs. One of the main outcomes was that the most significant pathway of exposure was through mouthing, and the age group most at risk for this exposure was infants. These FRs, which are not covalently bonded to the matrix, have been proven to pose a risk of neurotoxicity and endocrine disruption (Lyche et al., 2015) and display a high potential for environmental leaching during their use, storage, disposal, as well as upcycle into newly manufactured goods (Ionas et al., 2014; Abbasi et al., 2015). This finding is of particular concern because early-life is critical in the physiological development of children and exposure to toxic chemicals at this stage can have lasting health effects.

To our knowledge, similar studies have been published mostly on phthalate esters, such as the ones of Niino et al. (Niino et al., 2002, 2001) and Könemann (Könemann, 1998). Only one publication (Chen et al., 2009) contained a brief, tentative *in vivo* study was done with 5 volunteers, which found highly variable migration rates among the volunteers and congeners. In the same study, polybrominated biphenyls (PBBs) were detected in 63% of the toy samples, with median values of 30 ng/g. These chemicals, which are very similar to the PBDEs in terms of physico-chemical properties and use, are restricted under the Restriction of Hazardous Substances Directive (RoHS) since 2003, but their use has started to be phased out since the 1970s. Therefore, it is likely that even if PBDEs have started to be phased out in many parts of the world, they will still be present in items made with recycled flame retarded materials for years to come.

The aim of this study was to provide an early exposure assessment by determining the leaching potential of PBDEs from children's toys into saliva in simulated conditions, taking into consideration the infant mouthing behaviour as well.

2. Experimental

2.1. Materials

n-Hexane, acetone, toluene, tetrahydrofuran and *iso*-octane were purchased from J.T. Baker (Deventer, the Netherlands) and dichloromethane (DCM) for residue analysis from Promochem (Wesel, Germany). Anhydrous sodium sulphate was obtained from Merck (Darmstadt, Germany). All solvents and reagents were at least of analytical grade. Isotopically labelled (^{13}C) BDE 209 and PBDE congeners were supplied by Wellington Laboratories. Fluorinated PBDE congeners (F-BDE 47 and F-BDE 183) were bought from Chiron (Trondheim, Norway). The ERM-EC591 CRM of BFRs in polypropylene (PBDEs: 17, 28, 47, 49, 66, 74, 75, 85, 97, 99, 100, 101, 118, 119, 138, 139, 153, 154, 155, 173, 180, 181, 182, 183, 190, 197, 204, 207, 208, 209 and BB-209) was purchased from IRMM (Geel, Belgium). Glass beads (G9268-100G, 425-600 µm) were acquired from Sigma Aldrich (St. Louis, MO, USA). Falcon™ conical centrifuge tubes (50 mL) were bought from Fisher Scientific (Waltham, MA, USA).

2.2. Simulation of mouthing/leaching into saliva

The procedure used was adapted from similar studies on phthalate esters (Könemann, 1998; Niino et al., 2001) which also included a comparison between the *in vitro* methodology and an *in vivo* assay (Niino et al., 2002), to serve as control. An Incubating Orbital shaker from VWR (Radnor, Pennsylvania, USA) was employed at a rotation speed of 250 rpm, and a temperature of 37 °C, to mimic *in vivo* conditions as much as possible.

The saliva simulant solution used was the one described in the British Standard BS 6684 (1987), with the following composition: 4.5 g NaCl, 0.3 g KCl, 0.3 g Na_2SO_4, 0.4 g NH_4Cl, 0.2 g urea, 3.0 g lactic acid, dissolved in 1000 mL MilliQ water (resistivity 18.2 MΩ·cm) and adjusted to pH 6.8. The chosen pH is derived from literature data (Könemann, 1998), to match the pH of infant and toddler saliva. A volume of 30 mL of saliva simulant was used per assay, and was added to a 50 mL Falcon™ conical centrifuge tube, along with the sample. Since the migration of chemicals in a solution occurs from the surface, the FR concentrations were normalised to surface area rather than weight. A 10 cm^2 total surface area was chosen, to correspond to the surface area of a child's open mouth, as this is the surface area typically

available for mouthing at any one time (Earls et al., 2003). The samples were cut in pieces shaped as rectangular parallelepipeds, to facilitate the calculation of their surface.

2.3. Samples

Real toy samples, as well as CRM samples, were run in triplicate for two exposure times (15 and 60 min, low and high exposure scenario, respectively). The concentrations of PBDE congeners in the ERM-EC591 CRM are one order of magnitude lower than what is required to impart flame retardancy. Such levels are representative of the threshold defined by the REACH directive (e.g., 0.1%). The two toy samples considered were analysed in a previous study (Ionas et al., 2014). They contained levels one order of magnitude lower than the CRM, and are representative for the scenario in which PBDEs are present only as a result of contamination during the manufacturing or recycling process. The constituent polymers were not specified on the samples. One toy was made of hard (brittle) plastic (toy car, made in China) and the other of softer (bendable) plastic (toy figurines, unknown country of production). Other plastic toy samples from the aforementioned study had similar concentrations as the ones chosen for this study or lower. A toy sample with high concentrations of PBDEs (percentage amounts) was not available for testing. The toy samples were included in the study as a proof-of-concept of the migration procedure and to monitor how well the used CRM mimics the real toy samples. To study the scenario in which a child would mouth on an item flame retarded exclusively with PBDEs (e.g. remote control, mobile phone, small toys with electronic components, etc.), a sample (TV-back panel) from another study (Gallen et al., 2014) containing 7% BDE 209 was tested with the same conditions.

2.4. Extraction

Saliva simulant (30 mL) underwent liquid-liquid extraction using 2 × 5 mL of a 1:1 *v/v* mixture of *n*-hexane and DCM. The organic phase was spiked with internal standards after phase separation, to ensure optimal solubility for the standards. Then the extract was passed over a cartridge containing anhydrous sodium sulphate, to retain all traces of water. The extract was then evaporated, reconstituted in 200 µL mixture of *iso*-octane and toluene (1:1, *v/v*) and transferred to injection vials.

2.5. Instrumental analysis

The identification and quantification of PBDEs was done on an Agilent GC 6890N (Agilent Technologies Netherlands BV, Amstelveen, the Netherlands) coupled to a 5975XL

MS with a chemical ionization source and equipped with a pulsed splitless inlet and an Agilent 7683 auto-sampler. F-BDE 47, F-BDE 183 and ^{13}C-BDE 209 were used as internal standards. Analyte separation was carried out on an Agilent J&W DB-5HT (15 m × 0.25 mm × 0.1 µm film thickness). One microliter was injected at 275 °C in the pulsed splitless mode (pulse pressure 15 psi kept for 1.5 min). The oven temperature was programmed from 90 °C, for 1.5 min, then raised with 25 °C/min to 190 °C, then raised with 6.75 °C/min to 310 °C which was kept for 4 min. Methane was used as moderating gas (purity 4.5). Helium (purity 5.9) was used as a carrier gas with a ramped flow. The initial flow was 1 mL/min (for 20 min), then ramp 20 mL/min to 2 mL/min. The following mass fragments were monitored: *m/z* 484.4/486.4 and *m/z* 494.4/496.4 for BDE 209 and of ^{13}C-BDE 209, respectively and *m/z* 79/81 for all other PBDEs. The temperatures of the interface, quadrupole and ion source were 300, 150 and 250 °C, respectively and the electron multiplier voltage was set at 1812 V.

2.6. QA/QC

Controls with the chosen migration media spiked with PBDE congeners were run for every step of the process to assess possible contamination or loss of analytes. Recoveries ranged between 78-86 %. Two procedural blanks were run with every batch of 8 samples analysed. PBDE amounts detected in the blanks varied between non-detected to 300 pg. The data was blank-corrected accordingly. The limits of quantification (LOQs), expressed in pg/cm^2 sample, were calculated by dividing the analyte levels detected in the blanks by the sample surface (10 cm^2) considered for the experiments (Table SI-3).

2.7. Exposure scenarios

A number of observational studies were taken as a reference for this study (Moya et al., 2004; Cohen Hubal et al., 2000; Xue et al., 2007; EPA, 2008; Könemann, 1998). Additionally, five parents were interviewed about the mouthing behaviour of their children. The focus was on mouthing of toys of different materials in addition to non-toy items. Both hard and soft plastic toys were most frequently targeted for mouthing. Foam and textile toys, as well as non-toy items (e.g. remote controls, pens, key chains, or cell phones) were only occasionally mouthed. Based on this information and the existing observational studies, two exposure scenarios were considered for plastic toys: a low exposure scenario (15 min mouthing time/day) and a high exposure scenario (or “favourite toy” scenario of 60 min mouthing time/day).

Children typically display mouthing behaviour all throughout their infancy and partly during their toddler age (up to 24 months). In rare cases, the child can still display mouthing behaviour even after the age of 24 months. This is an indicator of the development of a rare condition called "oral fixation" (Angelo, 2013). Children with this condition will mouth on items also at older ages, and if the items mouthed contain PBDEs, it can lead to increased exposure to these chemicals.

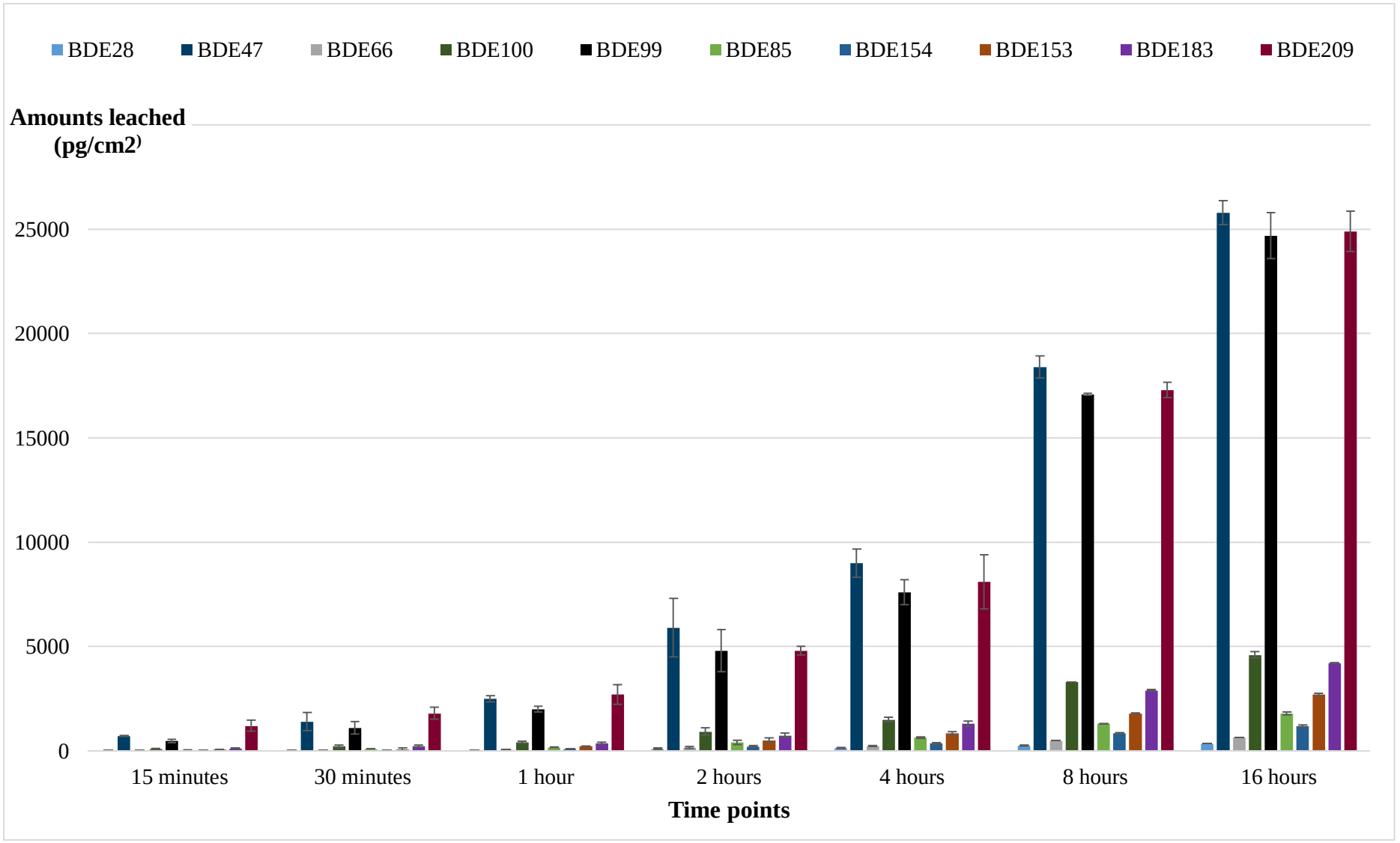
2.8. Method development

The mouthing behaviour of children was simulated through a step of incubation/shaking. First, a 24 h preliminary test was conducted using the ERM-EC591 CRM, which was chosen for this purpose because it contains PBDEs (0.17 % *w/w*) in amounts similar to the REACH threshold (0.1 % *w/w*). From this test, it was determined that the PBDE levels in the solution were very close to the limit of solubility in aqueous media. Three methods of incubation/shaking were then tested: uninterrupted shaking for 1 h (Niino et al., 2002), replenishing the artificial saliva solution after 30 min (Earls et al., 2003) (60 min total shaking time) and uninterrupted shaking for 60 min, with glass beads added (Könemann, 1998), meant to mimic chewing behaviour. The control against which these experimental conditions were tested was an incubation experiment using the same set-up and the same volume of real human saliva, collected by direct discharge, from nine volunteers of different age and gender and pooled together. Since a person can donate 2-4 mL saliva at one time and because 30 mL was required per replicate, this experiment was ran only once, to assess which set of conditions generates values closest to real saliva.

2.9. Kinetics and magnitude of the migration process

Additional experiments with different migration times (15 and 30 min, followed by 1, 2, 4, 8 and 16 h, all in triplicate) were done, in order to investigate the behaviour and migration rate of the analytes from the plastic matrix (Figure 1 and Table SI-1).

Figure 1: Amounts leached from EC-591 CRM at different time points (results in pg/cm^2) (n=3 replicates / time point)



3. Results and discussion

The method development showed that the addition of glass beads to the artificial saliva, to simulate chewing behaviour or its replenishment to better mimic the start of the digestive process, did not considerably increase the observed migration rates for PBDEs (Figure 2). All tested methods generated similar results. The real saliva control displayed a slightly higher migration rate for BDE 209, as compared to the other conditions, while adding the glass beads to the artificial saliva solution seemed to marginally favour the release of the lighter PBDE congeners from the matrix (Figure 2).

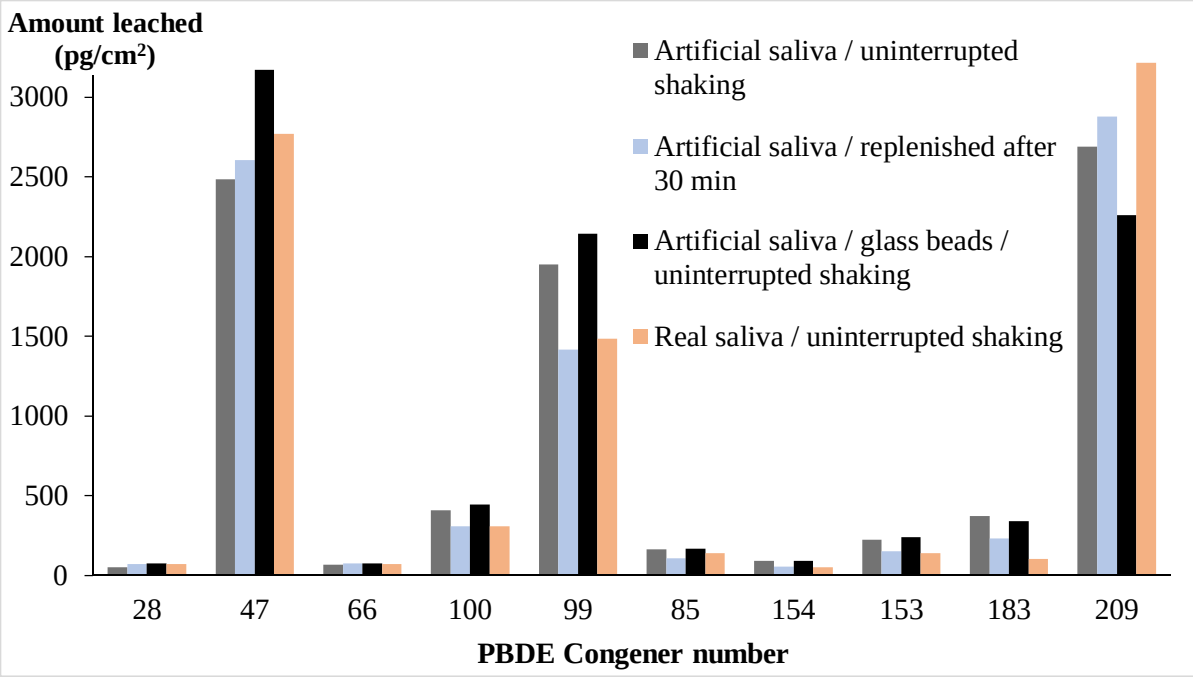


Figure 2: Comparison of different experimental conditions tested to simulate mouthing behaviour (incubation time: one hour, CRM used: ERM EC-591 containing 0.17% PBDEs; average sample mass corresponding to 10 cm^2 was 0.27 g)

The differences in the amounts of PBDEs migrated out of the CRM were low (Figure 2) and the procedure described by Niino et al. (Niino et al., 2002) produced data which correlated well with the data of the real saliva migration experiment (R^2=0.96, Figure SI-1). Therefore, this procedure was chosen for the purpose of our study.

3.1. Factors influencing the migration of PBDEs

Variables involved in the migration of PBDEs from the polymer were the sample surface loading, the replacement frequency of the saliva, and the physical-chemical properties of the compounds.

The kinetic leaching experiment showed that the migration rate was the greatest: 198 pg/(cm^2×min) during the first 15 min and the detected PBDEs levels were up to 1200 pg/cm^2 even for this time point which was the low exposure scenario. After 4 h, the migration rate was slowing down and reaching equilibrium with the water phase (Figure 3).

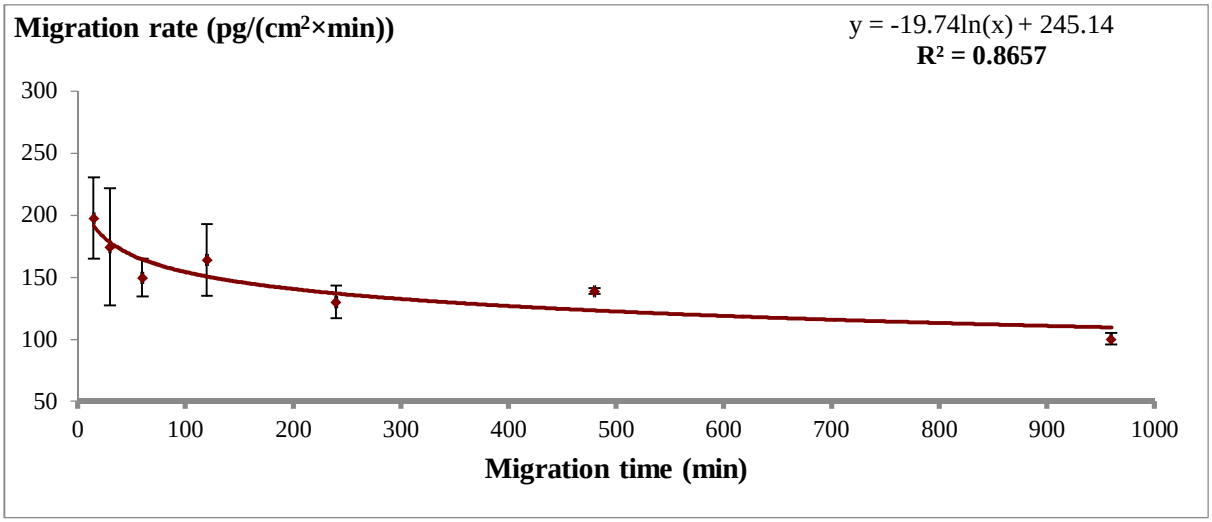


Figure 3: Migration rate of (total) PBDEs from the ERM EC-591 CRM (polypropylene matrix). Each point is an average of three experiments. The error bars represent the standard deviation for each migration time point.

However, considering the amounts which have migrated into the artificial saliva, signs of saturation started to show at the last time point (16 h) (Figure 4). The surface loading seems to be a more important exposure factor than solution saturation in the first hour of exposure.

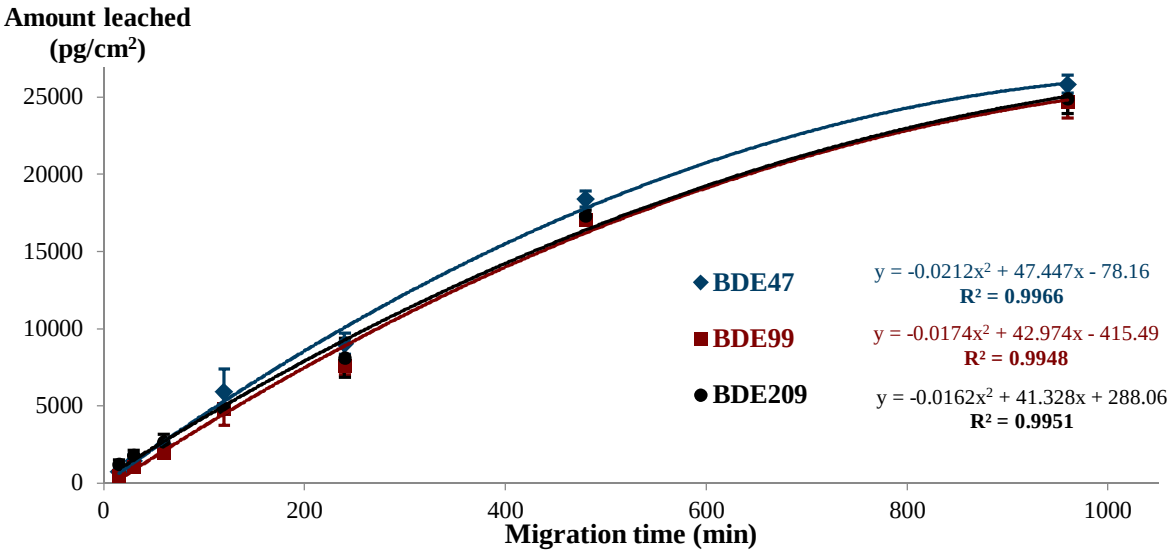


Figure 4: Profile of PBDE migration from the ERM EC-591 CRM (polynomial regression with the corresponding equations displayed, n=7). Each point is an average of three experiments. The error bars represent the standard deviation for each migration time point. For the migration profiles of the other congeners, see Figure 1 and Table SI-1.

In our closed system, the limit of solubility in aqueous media was a limitation to the migration process. However, in real life, the saliva from the mouth is continuously being swallowed into the digestive tract and is replaced by fresh saliva. As a consequence, the values generated by our model are likely slightly underestimated. But considering that the migration rate is only starting to considerably slow down after 4-8 h (at the PBDE concentrations of the considered CRM), this does not significantly influence the two exposure scenarios of 15 and 60 min.

Another important migration factor was the physical-chemical properties of the PBDE congeners. The surface to saliva leaching was congener-dependent, since the congeners with a lower molecular mass and lower octanol-water partition coefficient leached more readily than the heavier and more lipophilic congeners (Figure 5).

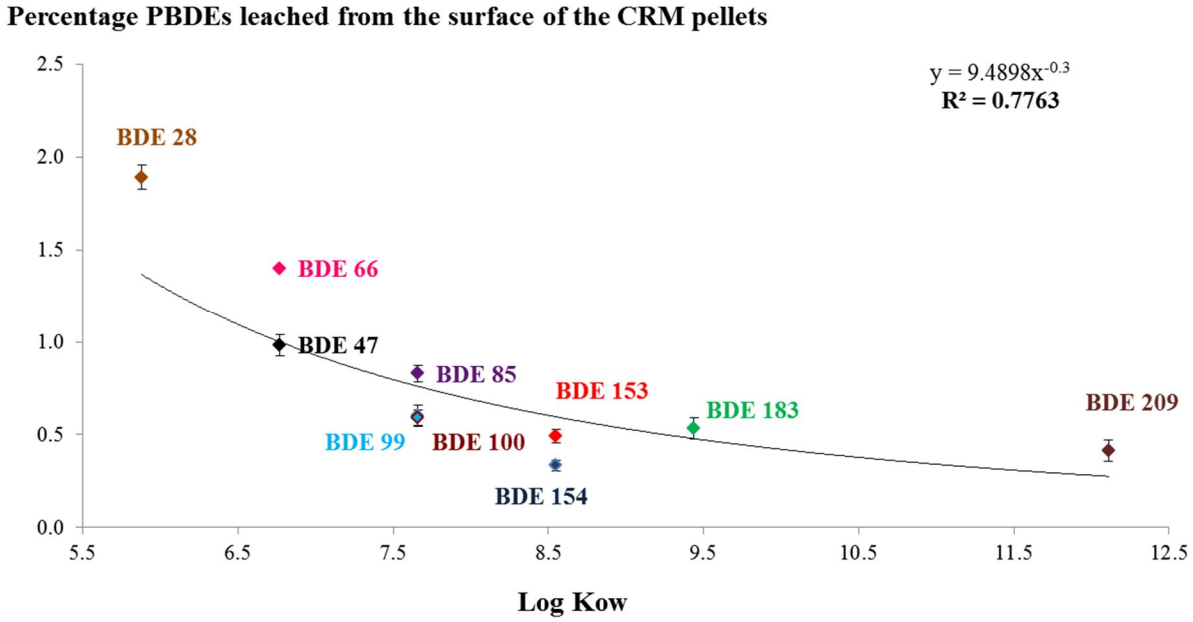


Figure 5: Influence of Log K_{ow} (calculated using the Syracuse Research Corporation EPI estimation program)(European Chemicals Bureau, 2002) on leaching (one hour shaking time with artificial saliva) (power regression, n=10). Each point is an average between three experiments. Information about how the point on the Y axis values were estimated is present in the Supporting Information section.

The lower congeners leached up to 2% from the CRM pellets, while ~0.5% of BDE 209 migrated into the aqueous solution. This indicates that the exposure to the lower congeners, which are more persistent, bioaccumulative and have been proven to cause adverse health effects (Lyche et al., 2015), is higher via this pathway than for BDE 209. The higher brominated PBDEs may undergo biotransformation into the body to lower brominated PBDEs (Noyes et al., 2011; Roberts et al., 2012) or other PBDE metabolites (Erratico et al., 2012; Meerts et al., 2001).

3.2. Influence of PBDE concentration on migration

By comparing the percentages of BDE 209 leached from the surface of the materials determined for the same time point, large differences between the materials can be observed (Figure 6). While both the CRM and the toy car sample leach around 0.3% during the 60 min leaching experiment, the toy figurines sample (made of softer plastic) leaches almost 3%, almost 10 times more.

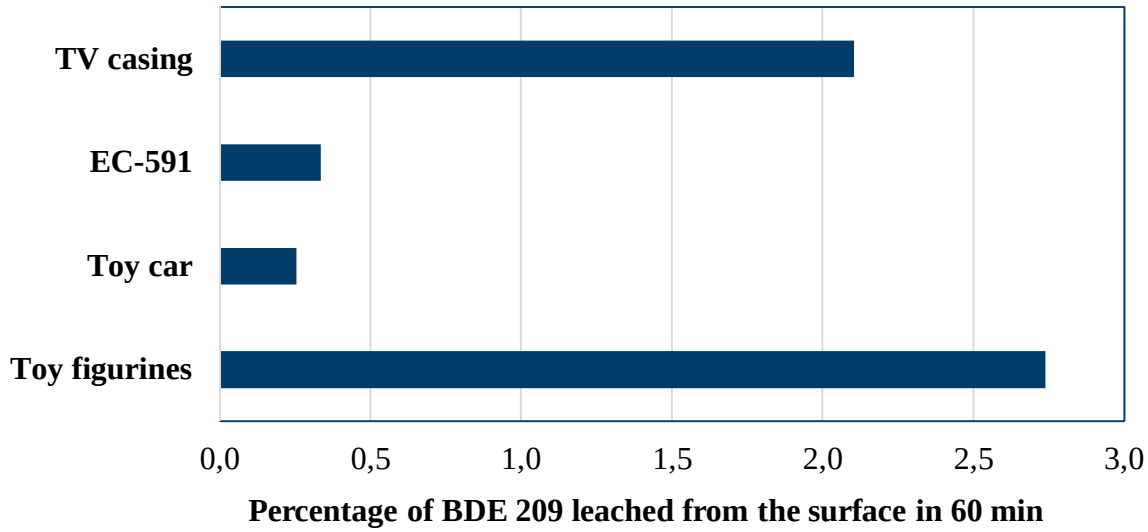


Figure 6: The percentage of BDE 209 leached after 60 min of simulated mouthing, from the surface of the samples. The amount of BDE 209 on the surface was estimated by dividing the amounts contained in the whole body of the sample by the surface-area-to-volume ratio.

The PBDE concentrations in the toy samples have been determined in a previous study (Ionas et al., 2014) to be similar (toy figurines 15 µg/g BDE 209, 19 µg/g in the toy car). However, the leaching rates varied between 410 and 50 pg/cm^2, respectively. The toy made out of a harder plastic (toy car) leached lower amounts of PBDEs than the one made out of a softer plastic (toy figurines), although the harder toy had higher levels of BDE 209. The same

phenomenon was noted during the preliminary tests with the EC-590 (polyethylene) and EC-591 (polypropylene). This could indicate that the type of material may also be factor in the migration process. The absolute amounts migrated from these toy samples were, as expected, lower than for the CRM (Table 1, Table SI-1).

The TV casing with BDE 209 levels one order of magnitude higher than the CRM (7% *w/w*), leached out higher amounts of BDE 209 in only 15 min than the CRM after 16 h incubation. Thus, if a child mouths on an item flame retarded with PBDEs, even in a low exposure scenario, he will get a much higher dose (one order of magnitude), as compared to a toy manufactured under the REACH directive.

3.3. Evaluation of children's exposure through mouthing

The safety of children's toys has been called into question as a number of (toxic) additives have been reported (Stapleton et al., 2011; Ionas et al., 2014; Chen et al., 2009). Currently, there is insufficient information in the literature about mouthing of toys as a route of infant exposure to FRs. The topic of this study is of special concern for exposure research, and should provide a springboard for future studies since young children are at risk of unnecessary PBDE exposure resulting from activity-driven, indoor contact with contaminated media and sources.

Early-life is as a particularly sensitive period for exposure to FRs, since the physiological characteristics of a young child are rapidly defining and therefore highly susceptible to influence. Possibly, exposure to some FRs in early-life may promote adverse endocrine-related activity (Jugan et al., 2010; Rudel and Perovich, 2009). Therefore, elucidating key sources, transport mechanisms, and exposure-dose relationships during critical stages of development is essential to promote long term health (Lyche et al., 2015).

Infant children are more likely to mouth toys than children of older ages, and receive a higher exposure to PBDEs *via* this pathway (Figure SI-2). However, infants also display mouthing behaviour with other items, including electronics, such as remote controls and mobile phones which fit easily into their mouths. When viewed from the perspective of an expanded exposure model, the mouthing of toys in combination with breast-feeding and the incidental ingestion of dust likely account for the overall daily dose. Although the simulated model presented herein theoretically examines exposure over typical time periods, the mouthing frequency of infants is intermittent and saliva actively reproducing, such that each new contact with a toy may lead to further uptake.

Table 1: Leaching levels (results in pg/cm^2) from toy samples) (n=3 replicates / time point) and from plastic from an item flame retarded with BDE 209 (n=1, due to low material availability). EC-591 CRM added for comparison. For levels in the material, see Table SI-2. For LOQs see Table SI-3.

Sample	Migration time	Analyte	BDE 28	BDE 47	BDE 66	BDE 100	BDE 99	BDE 85	BDE 154	BDE 153	BDE 183	BDE 209
Toy figurines (softer plastic)	15 min	*Mean*	<4	30	<0.5	<0.5	<1	<0.5	<1	<4	<0.5	<29
		SD	-	4	-	-	-	-	-	-	-	-
	60 min	*Mean*	<4	40	<0.5	<0.5	<1	<0.5	<1	<4	10	410
		SD	-	10	-	-	-	-	-	-	2	170
Toy car (hard plastic)	15 min	*Mean*	<4	<1	<0.5	<0.5	<1	<0.5	<1	<4	<0.5	50
		SD	-	-	-	-	-	-	-	-	-	15
	60 min	*Mean*	<4	<1	<0.5	<0.5	5	<0.5	<1	30	5	50
		SD	-	-	-	-	0*	-	-	10	1	25
EC-591 CRM (hard plastic, 0.17% PBDEs)	15 min	*Mean*	20	710	20	100	470	35	20	55	120	1200
		SD	2	25	4	10	80	5	4	15	5	260
	60 min	*Mean*	50	2500	65	410	2000	160	90	220	370	2700
		SD	2	150	0*	40	130	10	10	15	50	470
TV Casing (7% BDE 209)	15 min	Amount	<4	<1	<0.5	<0.5	<1	<0.5	<1	<4	170	46600
	60 min	Amount	<4	<1	<0.5	<0.5	<1	<0.5	<1	<4	470	152000

*Value lower than 0.5

Of additional consideration is that each time a toy (or object) is mouthed, and then either put on the floor, table, or elsewhere, the now saliva-saturated surface of the toy may absorb dust or other contaminated media from the child's environment. Apart from pacifiers, it is unlikely that a child will mouth any one object for long periods of time without intermittent opportunities for surface re-contamination. The qualification and quantification of this potential pathway can thus only lend itself useful toward elucidating the overall causal chain of exposure risk.

The current study has aimed to more accurately assess the exposure of infants to PBDEs through mouthing activities by clarifying the potential and rate of source to saliva migration in an experimental model. Our findings suggest that the migration/leaching of PBDEs into saliva is highly dependent on the physicochemical properties of the individual congener, and the type of material. The surface loading is a very significant factor to consider when elucidating exposure risk between a low-exposure (15 min mouthing time/day) and a high exposure scenario (or "favourite toy" scenario of one hour mouthing time/day), since the migration rate for the low exposure scenario is the highest. And if the item mouthed is flame retarded with PBDEs, it can lead to high exposure levels even in a short time. It is thus important for parents to be informed about what items typically contain these chemicals or other harmful additives, especially if the items are small enough so that they can be mouthed on by their children.

In order to put the obtained values in perspective with other exposure pathways, the experimental values were tentatively compared to data available for other exposure pathways (Table 2). For the Belgian population (Roosens et al., 2010), the exposure from mouthing on toys containing PBDEs in amounts similar to the REACH threshold (0.1% product) is lower than the exposure from mother's milk, but higher than the exposure through diet or even dust.

PBDE levels from Belgium, in the matrices listed in Table 2, are typically lower than in other parts of the world, such as the USA (Dodson et al., 2012). This is reflected in the average exposure levels from a similar study focusing on the American population (Johnson-Restrepo and Kannan, 2009), where the mouthing exposure is in the same range as the exposure through ingestion of dust, but still considerably lower than the exposure through maternal nursing.

Even higher contact times than our "favourite toy" scenario are reported in the literature, such as observed mouthing times of up to 171.5 min per day (Könemann, 1998).

Table 2: Exposure levels obtained in this study, (n=3 replicates) and average exposure levels derived from other literature studies, for infants (0-1 years)

Source	Exposure pathway	Σ PBDEs (ng/day)*
(Roosens et al., 2010)	Human milk	152.1
	Food	15.4
	Dust	8.2
	Indoor (house) air	0.4
(Johnson-Restrepo and Kannan, 2009)	Human milk	385.5
	Food	5.5
	Dust	33.5
	Indoor (house) air	3
Experimental data	**Mouthing on toys: Low exposure**	**24 ± 5**
	Mouthing on toys: High exposure	**67 ± 7**

**Because during this stage of development, children experience the fastest increase in body weight (up to 3 fold), it was preferred not to express the exposure depending on average weight*

Another important factor determining the leaching of PBDEs from plastic items during mouthing is the intensity of chewing behaviour. In the tentative *in vivo* study by Chen et al. (Chen et al., 2009), this is likely the cause of the considerable variability of the PBDE levels leached from toys. Differences in physicochemical properties of different matrices and different FRs have an impact on surface loadings, dust adherence and the whole set of processes leading to oral transfer (Ruby and Lowney, 2012; Stapleton et al., 2008; Stapleton et al., 2014; Webster et al., 2011; Weschler and Nazaroff, 2012). Chewing behaviour facilitates the leaching of chemicals from toys to saliva, in addition to potential ingestion of material microparticles.

3.4. Future Considerations

Additional studies are also needed for alternative FRs, such as phosphorus flame retardants (PFRs) and new BFRs containing more polar groups, such as –OH, =O or a triazine ring. Mouthing behaviour is an age-dependent activity and there is a wide variability of objects mouthed, including non-toy items. Therefore, a more comprehensive and integrated exposure assessment across infant groups, for additives in these items, *via* both dietary and non-dietary routes is needed. Additional areas of research may include: clarification on the rate of dust fractionation from object surfaces during the mouthing, licking, or sucking of objects; and a synthesis of data regarding source-to-skin or source-to-saliva migration patterns for both objects and dermal surfaces.

Acknowledgements

ACI and JU acknowledge the funding of a PhD scholarship from the European Union Seventh Framework Program (FP7/2007-2013) under grant agreement no 264600 (INFLAME project) and BOF DOCPRO1 - Research Council of the University of Antwerp (ACI). ABG also acknowledges funding by the Netherlands Organization for Scientific Research (NWO) (VENI2014-722.014.003).

References

Abbasi, G., Buser, A.M., Soehl, A., Murray, M.W., Diamond, M.L., 2015. Stocks and Flows of PBDEs in Products from Use to Waste in the U.S. and Canada from 1970 to 2020. Environ. Sci. Technol. 49, 1521–1528. doi:10.1021/es504007v

Angelo, F., 2013. Oral Fixation vs Oral Mouthing — Is There A Difference? Day2Day Parent.

Chen, S.-J., Ma, Y.-J., Wang, J., Chen, D., Luo, X.-J., Mai, B.-X., 2009. Brominated Flame Retardants in Children's Toys: Concentration, Composition, and Children's Exposure and Risk Assessment. Environ. Sci. Technol. 43, 4200–4206.

Cohen Hubal, E. a, Sheldon, L.S., Burke, J.M., McCurdy, T.R., Berry, M.R., Rigas, M.L., Zartarian, V.G., Freeman, N.C., 2000. Children's exposure assessment: a review of factors influencing Children's exposure, and the data available to characterize and assess that exposure. Environ. Health Perspect. 108, 475–486.

Dodson, R.E., Perovich, L.J., Covaci, A., Van den Eede, N., Ionas, A.C., Dirtu, A.C., Brody, J.G., Rudel, R. a, 2012. After the PBDE phase-out: a broad suite of flame retardants in repeat house dust samples from California. Environ. Sci. Technol. 46, 13056–66. doi:10.1021/es303879n

Earls, a O., Axford, I.P., Braybrook, J.H., 2003. Gas chromatography-mass spectrometry determination of the migration of phthalate plasticisers from polyvinyl chloride toys and childcare articles. J. Chromatogr. A 983, 237–46.

EPA, U., 2008. Chapter 5 - Ingestion of Soil and Dust Child - Specific Exposure Factors Handbook September 2008 Page Child - Specific Exposure Factors Handboo k Chapter 5 - Ingestion of Soil and Dust Child - Specific Exposure Factors Handbook September 2008. Exposure.

Erratico, C. a., Szeitz, A., Bandiera, S.M., 2012. Oxidative metabolism of BDE-99 by human liver microsomes: Predominant role of CYP2B6. Toxicol. Sci. 129, 280–292. doi:10.1093/toxsci/kfs215

European Chemicals Bureau, 2002. European Union Risk Assessment Report: deca-BDE.

Gallen, C., Banks, A., Brandsma, S., Baduel, C., Thai, P., Eaglesham, G., Heffernan, A., Leonards, P., Bainton, P., Mueller, J.F., 2014. Towards development of a rapid and

effective non-destructive testing strategy to identify brominated flame retardants in the plastics of consumer products. Sci. Total Environ. 491-492, 255–65. doi:10.1016/j.scitotenv.2014.01.074

Ionas, A.C., Dirtu, A.C., Anthonissen, T., Neels, H., Covaci, A., 2014. Downsides of the recycling process: Harmful organic chemicals in children's toys. Environ. Int. 65C, 54–62. doi:10.1016/j.envint.2013.12.019

Johnson-Restrepo, B., Kannan, K., 2009. An assessment of sources and pathways of human exposure to polybrominated diphenyl ethers in the United States. Chemosphere 76, 542–548. doi:10.1016/j.chemosphere.2009.02.068

Jugan, M.L., Levi, Y., Blondeau, J.P., 2010. Endocrine disruptors and thyroid hormone physiology. Biochem. Pharmacol. 79, 939–947. doi:10.1016/j.bcp.2009.11.006

Könemann, W.H., 1998. Phthalate release from soft PVC baby toys.

Lyche, J.L., Rosseland, C., Berge, G., Polder, A., 2015. Human health risk associated with brominated flame-retardants (BFRs). Environ. Int. 74, 170–180. doi:10.1016/j.envint.2014.09.006

Meerts, I. a T.M., Letcher, R.J., Hoving, S., Marsh, G., Bergman, Å., Lemmen, J.G., Van Der Burg, B., Brouwer, A., 2001. In vitro estrogenicity of polybrominated diphenyl ethers, hydroxylated PBDEs, and polybrominated bisphenol A compounds. Environ. Health Perspect. 109, 399–407. doi:10.1289/ehp.01109399

Moya, J., Bearer, C.F., Etzel, R. a, 2004. Various Life Stages. Pediatrics 113, 996–1006. doi:10.1542/peds.113.4.S1.996

Niino, T., Ishibashi, T., Itho, T., Sakai, S., Ishiwata, H., Yamada, T., Onodera, S., 2001. Monoester Formation by Hydrolysis of Dialkyl Phthalate Migrating from Polyvinyl Chloride Products in Human Saliva. J. Heal. Sci. 47, 318–322. doi:10.1248/jhs.47.318

Niino, T., Ishibashi, T., Itoh, T., Sakai, S., Ishiwata, H., Yamada, T., Onodera, S., 2002. Comparison of Diisononyl Phthalate Migration from Polyvinyl Chloride Products into Human Saliva in Vivo and into Saliva Simulant in Vitro. J. Heal. Sci. 48, 277–281. doi:10.1248/jhs.48.277

Noyes, P.D., Hinton, D.E., Stapleton, H.M., 2011. Accumulation and debromination of decabromodiphenyl Ether (BDE-209) in juvenile fathead minnows (Pimephales promelas) induces thyroid disruption and liver alterations. Toxicol. Sci. 122, 265–274. doi:10.1093/toxsci/kfr105

Roberts, S.C., Noyes, P.D., Gallagher, E.P., Stapleton, H.M., 2012. Species-Specific Differences and Structure-Activity Relationships in the Debromination of PBDE Congeners in Three Fish Species. Environ. Sci. Technol. 29, 997–1003. doi:10.1016/j.biotechadv.2011.08.021.Secreted

Roosens, L., Cornelis, C., D'Hollander, W., Bervoets, L., Reynders, H., Van Campenhout, K.,

Van Den Heuvel, R., Neels, H., Covaci, A., 2010. Exposure of the Flemish population to brominated flame retardants: model and risk assessment. Environ. Int. 36, 368–76. doi:10.1016/j.envint.2010.02.005

Ruby, M. V., Lowney, Y.W., 2012. Selective soil particle adherence to hands: Implications for understanding oral exposure to soil contaminants. Environ. Sci. Technol. 46, 12759–12771. doi:10.1021/es302473q

Rudel, R. a, Perovich, L.J., 2009. Endocrine disrupting chemicals in indoor and outdoor air. Atmos. Environ. (1994). 43, 170–181. doi:10.1016/j.atmosenv.2008.09.025

Stapleton, H.M., Klosterhaus, S., Keller, A., Ferguson, P.L., van Bergen, S., Cooper, E., Webster, T.F., Blum, A., 2011. Identification of flame retardants in polyurethane foam collected from baby products. Environ. Sci. Technol. 45, 5323–31. doi:10.1021/es2007462

Stapleton, H.M., Misenheimer, J., Hoffman, K., Webster, T.F., 2014. Flame retardant associations between children's handwipes and house dust. Chemosphere 116, 54–60. doi:10.1016/j.chemosphere.2013.12.100

Stapleton, M.H., Kelly, S.M., Allen, J.G., Mcclean, M.D., Webster, T.F., 2008. Measurement of polybrominated diphenyl ethers on hand wipes: Estimating exposure from hand-to-mouth contact. Environ. Sci. Technol. 42, 3329–3334. doi:10.1021/es7029625

Stringer, R., Labunska, I., Santillo, D., Johnston, P., Siddorn, J., Stephenson, a, 2000. Concentrations of phthalate esters and identification of other additives in PVC children's toys. Environ. Sci. Pollut. Res. Int. 7, 27–36. doi:10.1065/espr199910.007

Webster, T., Stapleton, H., McClean, M., 2011. Exposure to Polybrominated Diphenyl Ethers in Indoor Environments. Epidemiology 22, S117. doi:10.1097/01.ede.0000392027.72377.2c

Weschler, C.J., Nazaroff, W.W., 2012. SVOC exposure indoors: Fresh look at dermal pathways. Indoor Air 22, 356–377. doi:10.1111/j.1600-0668.2012.00772.x

Xue, J., Zartarian, V., Moya, J., Freeman, N., Beamer, P., Black, K., Tulve, N., Shalat, S., 2007. A meta-analysis of children's hand-to-mouth frequency data for estimating nondietary ingestion exposure. Risk Anal. 27, 411–420. doi:10.1111/j.1539-6924.2007.00893.x