

ANALYSIS OF HARMFUL ORGANIC CHEMICALS IN CHILDREN'S TOYS

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Introduction

An integral part of a child's developmental process is to learn about the world around him by playing. At a young age, this is how our infants spend most of their time. Nowadays, as our society has developed technologically, a very wide array of toys are produced. However, some of these toys may contain harmful chemicals. Phthalates are added to plastic toys to increase flexibility and durability. These compounds have been shown to cause damage to the liver, kidneys and in some cases to the reproductive tract. Some phthalates are endocrine disruptors¹ and play a role in the development of obesity². Also, they might be increasing the incidence of allergies and even asthma³. Polybrominated diphenyl ethers (PBDEs) are also added to some products, toys included, as flame retardants (FRs). This is especially true for the toys with a production date before the 1st of June 2007, when the REACH ("Registration, Evaluation, Authorisation and Restriction of Chemicals") Directive entered into force. These compounds have been proven to have thyroid disrupting properties⁴, as they are similar in structure to human endogenous hormones and thus bind competitively to the thyroid hormone receptors⁵. In the last decade, since the use of PBDEs has slowly declined, organophosphates are added more and more to a variety of materials to impart flame retardancy or as plasticisers⁶. But these compounds also pose a serious health concern: the chlorinated organophosphates are neurotoxic and carcinogenic^{7,8}, the aryl organophosphates are reproductive toxicants and increase the incidence of allergies⁹.

The children typically spend a high amount of time playing with these toys so they get a high exposure to these compounds, considerably more than the adults. The aim of this study is, therefore, to identify classes of harmful organic chemicals that the children may be exposed to while playing, present in a selected batch of toys. Depending on the available standards, some of the identified compounds were further quantified, while for other chemicals quasi-quantitative assessments were given.

Materials and methods

Samples. A number of 64 samples were kindly donated by a few parents of children of different ages or collected from a recycling park in Antwerp, Belgium during a period of two months. The toys were chosen as to represent the main exposure pathways to these compounds: inhalation, mouthing, dermal contact and oral ingestion. Of special significance is the exposure through mouthing because it allows very young children to get a high exposure to potentially harmful compounds, at an age when their organisms are very vulnerable to external stimuli. When collecting the samples, information about the year and country of production was collected.

Materials. All solvents used during analysis were of analytical or pesticide grade. *n*-Hexane was purchased from Acros Organics (Geel, Belgium). Acetone, ethanol, methanol, toluene and *iso*-octane were purchased from Merck (Darmstadt, Germany).

Quantification. Standards of BDE 28, 47, 66, 85, 99, 100, 153, 154, 183 and 209 were purchased from Wellington Laboratories (Guelph, ON, Canada). BDE 77 was obtained from AccuStandard Inc. (New Haven, CT, USA). Standards of tri-isobutyl phosphate (TiBP), tri-*n*-butyl phosphate (TnBP), triphenyl phosphate (TPhP), tris(2-chloroethyl) phosphate (TCEP), ethyl-hexyl-diphenyl phosphate (EHDPP), triscresyl phosphate (TCP, mixture of 4 isomers) and tris(1,3-dichloropropyl) phosphate (TDCPP, mixture of 2 isomers) were purchased from Chiron AS (Trondheim, Norway). Tris(2-butoxyethyl) phosphate (TBEP) were purchased from Sigma Aldrich. Tris(1-chloro-2-propyl) phosphate (TCPP, mixture of 3 isomers) was purchased from Pfaltz & Bauer (Waterbury, CT, USA). Purity of analytical standards was >98%, except for TBEP (>94%). Standards of BFRs (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) in polymeric matrices (polyethylene: ERM-EC590 and polypropylene: ERM-EC591) were purchased from IRMM (Geel, Belgium). Modified nylon centrifugal filters with 0.2 µm and 0.45 µm pore size were bought from VWR.

Screening for harmful phthalates. In the absence of adequate standards for quantifying these compounds, a quasi-quantitative screening approach was decided upon. The extracts, diluted 50×, were injected in full-scan

mode in a gas chromatograph coupled to a mass spectrometer and the compounds were identified by comparing the respective mass spectra to the Wiley Registry 9th/NIST 2008 spectral library. The match with the library spectra was over 85%, unless the chromatogram was overloaded, in which case a spectrum taken at the start or end of the peak was compared to the spectral library. Further, the identified compounds were divided into two categories based on the abundance of the ion $m/z = 149$: a) compounds with high abundance (peaks often overloaded even after 50x dilution and over a value of 7.5 out of a maximum of 8 million counts) and b) compounds with lower relative abundance (above the levels found in blanks, but under 7.5 million counts).

Extraction of the analytes. As extraction, just one method was insufficient due to the wide array of materials contained in toys. In consequence, the toys were divided in 5 categories according to the constituent materials: foam and textile toys, hard plastic toys, soft plastic toys, rubber toys and wooden toys. In all cases a combination of ultrasound assisted extraction (UAE) and vortexing was employed.

The sample conditioning and preparation consisted of two main steps: the samples were pre-cleaned with a purified water impregnated tissue as to remove any outside contamination and then were submitted to one or two size reduction steps. The soft plastic, foam and textiles, wood and rubber samples were easily cut with pliers and scissors to pieces with a diameter of 2 mm or less. The hard plastics could not be reduced in size (pieces with a diameter >3 mm were obtained) as much due to the rigidity of the matrix so they were submitted to an additional size reduction step. A Mixer Mill MM400 from RETSCH (Haan, North Rhine-Westphalia, Germany) was employed for this purpose. The samples along with one 25 mm diameter metal ball were added to the metal grinding jars (50 mL volume), submerged in liquid nitrogen until they reached the temperature of the surrounding liquid (-196 °C) and then ground at a vibrational frequency of 30 Hz for 3 min. The resulting particles typically had a diameter in the range of 10 μ m. The rationale behind trying to get the samples in pieces as small as possible is to increase the contact surface with the solvent and improve the extraction.

The solvents used in this process needed to provide an efficient extraction and at the same time, not dissolve the matrix, as it would be detrimental to the GC column. For the plastic samples, both hard and soft, a mixture of dichloromethane and acetone was used (1:1, v/v). For the wooden samples, a mixture of ethanol and hexane (4:1, v/v) was more adequate in this case. Wood is comprised out of three major constituents: cellulose, hemicellulose and lignin. Small molecular solvents with high polarity (e.g. H_2O) are very efficient at distancing the biopolymeric chains, thus allowing the small molecular compounds to be extracted. Yet, water is not such a good choice as extraction solvent, because of its high boiling point. As a substitute, ethanol was chosen. The solvent is reasonably polar and it also has a hydroxyl group which can disrupt the hydrogen bonds from the biopolymeric chains contained in wood. Hexane was also added to elute the non-polar compounds present. At this particular volume ratio, the two solvents form a positive azeotropic mixture with a boiling point of about 59 °C (as opposite to 78.4 °C for ethanol), which allows for an increased analysis throughput. For the rubber samples a mixture of acetone and *n*-hexane was used (3:2, v/v). In these particular proportions, these two solvents also form a positive azeotropic mixture with a boiling point of around 50 °C.

The extraction process consisted of consecutive steps of vortexing (1 min) and ultrasonication (15 min) with 4-6 mL of the chosen solvent mixture. This cycle of vortexing and ultrasonication was repeated 3 times. The samples were left in solvent overnight to insure high recoveries. No destructive clean-up method was applied to insure that no analytes of interest are degraded.

Instrumental analysis. The extracts obtained were injected on a GC-MS system (Agilent 6890 GC coupled to an Agilent 5973 MS), operated in electron capture negative ionization (ECNI) mode. The GC system was equipped with a programmable-temperature vaporizer inlet (PTV) which was run in the pulsed splitless mode. One μ L of extract was injected on an Agilent J&W DB-5 column (15 m \times 0.25 mm \times 0.10 μ m). The GC temperature program was 90 °C, hold 1.25 min, ramp 10 °C/min to 310 °C, hold 12 min. Helium (purity 5.9) was used as a carrier gas with a ramped flow. Methane was used as moderating gas (purity 4.5). The initial flow was 1 mL/min (for 20 min), then ramp 20 mL/min to 2 mL/min. The ion source, quadrupole and interface temperatures were set at 250, 150 and 300 °C, respectively and the electron multiplier voltage was at 2200 V.

All extracts were also injected in an identical GC-MS system operated in the electron ionisation (EI) mode. The PTV was run in the pulsed splitless mode. One μ L of extract was injected on a SGE HT-8 column (25 m \times 0.22 mm \times 0.25 μ m). The GC temperature program was 90 °C, hold 1.50 min, ramp 10 °C/min to 310 °C, hold 20 min. Helium (purity 5.9) was used as a carrier gas with a constant flow (1 mL/min). The ion source, quadrupole and interface temperatures were set at 230, 150 and 300 °C, respectively and the electron multiplier voltage was at 2200 V.

Results and discussion

Development and optimisation. To assess the efficiency of our analytical method, the ERM-EC590 and ERM-EC591 reference materials for BFRs in polymeric matrices were used. They were extracted using the method for hard plastics. The size of the plastic beads (diameter approximately 0.3 mm) was not reduced in any way and they were extracted as they were. The recoveries obtained at first were mediocre (Table 1).

Table 1. PBDE recoveries under different experimental conditions on the analysis of two reference materials for BFRs in polymeric matrices

Reference material	Compounds	Recovery (%)			
		15 min sonication/ no milling	2h sonication/ no milling	15 min sonication/milling	2h sonication/ milling/toluene
ERM-EC590	Lower PBDEs	14	17	61	64
	BDE-209	11	12	23	53
ERM-EC591	Lower PBDEs	4	5	69	55
	BDE-209	2	2	53	44

The reference material plastic beads were submitted to 3 cycles of 15 min sonication and alternatively to 2 cycles of 15 min and one larger cycle of 2h. The recovery for the latter was 20% higher compared to the recovery for the standard extraction. The next step in increasing the recoveries was to add a second size reduction step for the hard plastic samples. For this, a RETSCH Mixer Mill MM400 was used. The samples were frozen with liquid nitrogen to make them more brittle and then ground into fine pellets. The matrices of the two reference materials, low-density polyethylene and polypropylene, are rather soft so pellets were obtained rather than a fine powder. But the harder plastic samples were successfully ground to a fine powder. The grinding process is more efficient with a larger sized metal ball (25 mm diameter) than with several smaller ones (5 mm diameter). When re-analysing the now finely ground reference materials, a 4-fold and 2-fold increase in recovery for lower PBDE and BDE-209 respectively was observed for the ERM-EC590. For the ERM-EC591, the difference was even greater: 17-fold for the lower PBDEs and 26-fold for BDE-209 (Table 1).

The next parameter to be improved was the extraction solvent. For nonpolar compounds that contain phenyl rings, toluene alone or in a mixture with other solvents, is often used¹⁰. For wooden toy samples, several solvent mixtures were tested: *n*-hexane/ethanol, *n*-hexane/methanol, *n*-hexane/ethanol/water and *n*-hexane/ethanol/*iso*-propanol in different ratios. The best combination as extraction efficiency and throughput proved to be *n*-hexane/ethanol (1:5, v/v).

Presence of harmful phthalates. Six of the phthalate compounds were restricted to a mass percentage of 0.1% by the European Commission through the regulation no. 1907/2006 (REACH). These compounds are: dibutyl phthalate (DBP), benzil butyl phthalate (BBP), dioctyl phthalate (DNOP), bis (2-ethylhexyl) phthalate (DEHP), di-isononyl phthalate (DINP) and di-isodecyl phthalate (DIDP).

As no quantitation was possible, the compounds were divided into two categories as mentioned above. An overview of our estimation on their presence in analysed toy samples is presented in Table 2. Therefore, it seems clear that in some samples they are present in significantly high amounts, especially considering the fact that these compounds are not covalently bound to the polymeric chains in the plastics.

Table 2. Estimation of phthalate esters detection frequencies (DF, %) and their abundance in analysed toys

Analyte / Relative abundance	DBP (DF)	BBP (DF)	DEHP (DF)	DNOP (DF)	DINP (DF)	DIDP (DF)
High	24	0	32	0	10	2
Low	6	14	6	5	3	0

Presence of FR compounds. The analytes quantified in analysed samples were PBDEs and OPFRs. The PBDEs are restricted by the regulation no. 1907/2006 (REACH) of the European Commission to a maximum mass percentage of 0.1%. However, the overall concentrations for the FRs analysed were low (Table 3) and this translates to a lower exposure for the children using these toys. The highest PBDE concentration is an order of magnitude lower than the threshold set by the REACH regulation. Similar considerations cannot be entirely applied to the OPFRs because the REACH directive does not restrict most of these compounds. The only phosphate esters it does restrict are the tris (2,3-dibromopropyl)phosphate (TDBPP) which was not detected in

this study and TCEP which was found in concentrations 5 orders of magnitude lower than the REACH threshold concentration.

Of the PBDEs present, BDE-209 was measured at the highest concentrations. Three samples have concentrations noticeably higher than the others. Of those 3, 2 originate from China and 1 has an unknown production country; 2 were produced before 2007 (with concentrations of 142800 and 14500 ng/g, respectively), when the REACH directive went into force and 1 after this date (concentration: 19100 ng/g sample). The highest concentration of BDE-209 encountered (142800 ng/g) came from a toy that contained a foam inner core and a textile cover. Surprisingly, most of the BDE-209 was in the textile material rather than in the foam. A possible explanation for the presence of PBDEs in levels insufficient to impart flame retardancy is that recycled materials that contained these FRs were used in the production of the toys.

Table 3. Overview of the detection frequencies (%) and concentrations (median, 25th and 75th percentile, ng/g) measured in toy samples.

Class	Analyte	Detection frequency (%)	Analyte concentration (ng/g)		
			median	25 th percentile	75 th percentile
PBDEs	BDE 28	2	<LOQ	<LOQ	<LOQ
	BDE 47	11	12	<LOQ	18
	BDE 66	2	<LOQ	<LOQ	<LOQ
	BDE 100	3	<LOQ	<LOQ	<LOQ
	BDE 99	6	<LOQ	<LOQ	25
	BDE 85	5	<LOQ	<LOQ	1
	BDE 154	17	24	19	30
	BDE 153	14	9	4	52
	BDE 183	11	14	<LOQ	33
	BDE 209	16	340	87	7561
OPFRs	TnPP	6	<LOQ	<LOQ	<LOQ
	TiBP	40	1127	831	3469
	TnBP	8	<LOQ	<LOQ	<LOQ
	TCEP	13	<LOQ	<LOQ	70
	Σ TCP	30	109	12	140
	TBEP	6	<LOQ	<LOQ	<LOQ
	TPhP	41	1608	1324	2074
	EHDPP	29	48	<LOQ	128
	Σ TTP	6	<LOQ	<LOQ	<LOQ

Future perspectives. Although the concentrations encountered do not seem to be of serious concern, more samples need to be included into this study to be able to fill the knowledge gaps regarding the exposure that our children are getting from toys, in their early life. Also, the number of companies mass producing toys worldwide is enormous and their circulation has become global. As such, a larger amount of samples is required to get an idea about the possible contamination arising from toys.

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