Phthalate in children's toys and childcare articles in Croatia

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Children's toys are made of artificial material often softened by phthalates. These are synthetic compounds added to PVC as plasticizers for the purpose of improving its elasticity and flexibility. Phthalates can endanger the health of children exposed to their effect by inducing reproductive, hormonal and developmental disorders. The goal of the present study was to determine phthalate presence in children's toys and childcare articles from different sources sold in different areas in Croatia in 2012 and 2013. Diisononyl phthalate, di(2-ethylhexyl) phthalate (DEHP), dibutyl phthalate, diisodecyl phthalate, di-*n*-octyl phthalate and butylbenzyl phthalate were included in the analysis. About 60% of the toys analysed contained plastic; up to 20% had detectible levels of phthalates, 94–96% containing toxic DEHP. Over 60% contained DEHP at concentrations more than 10 times than that permitted, mostly dolls and toy animals. The percentage of toys containing phthalates was higher in 2013 in comparison to 2012. We discuss the availability and similarities in composition of phthalates by comparing our results with reports from other countries.

Keywords: Childcare articles, children's toys, chromatography, phthalates.

CHILDREN'S toys are one of the most important and highly controlled products of common use in the European Union (EU). Toys in the marketplace should be in compliance with the applicable legislation in the EU for the protection of consumer health and safety, as well as the environment[1]. However, the strict requirements do not prevent the manufacture and sale of toys which do not meet the set standards.

Children's toys are defined as products that are designed or intended for use by children up to 14 years of age. Childcare articles are products intended for sleep facilitation, relaxation, hygiene, feeding or sucking by the children[1]. Toys and childcare articles made of polyvinyl chloride (PVC) usually contain softeners or plasticizers which enable flexibility. Plasticizers that are mostly used for the improvement of elasticity in PVC are phthalates (Figure 1) or diesters of ortho-phthalic acid (di-alkyl or alkyl aryl esters 1,2-benzenedicarboxylic acid)[2–5]. At a global level, 6 million tonnes of softeners are used per year, including 1 million used in Europe, 80% of which includes phthalates[6]. Phthalates are a serious threat to the environment and human health[6]. They do not form covalent bonds and thus easily migrate when in contact with lipophilic matter[6,7]. After phthalate intake, monoesters and oxidative metabolites are formed during biotransformation[8]. For example, after ingestion of DEHP, 30 metabolites are formed, namely mono-(2-ethyl-5-hexyl) phthalate (MEHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) and mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP). MEHP is primarily formed by DEHP hydrolysis in the gastrointestinal tract, which is then absorbed, while MEOHP, MEHHP and MECPP are formed by liver oxidative metabolism of MEHP and are present in concentrations 3–5 times greater than MEHP in urine[9,10]. Human exposure to phthalates occurs through food, mother's milk, by inhaling, chewing plastic and through skin contact[11]. Toys and childcare articles that can be placed in the mouth, even if not intended for that purpose, present a health risk for children if they contain phthalates[12,13]. Determination of the allowed legislative levels is based on risk estimation and exposure dose extrapolations; it is not a simple process. The guidelines for allowed estimated dose exposure to individual phthalate types are explained in detail by CSTEE (European Scientific Committee on Toxicity, Ecotoxicity and Environment)[2]. By Commission Regulation (EU) No. 143/2011, the EU has categorized phthalates as reproductive toxic compounds of category 1B and regulated the maximal allowed amounts of these toxic chemicals in toys and childcare article[12,14,15]. Toxicological reports show a connection between phthalates and breast cancer[16]. Phthalates might potentiate their adverse effects combined with other chemicals[17]. They are responsible for changes in brain function during the development of boys by influencing testosterone levels and contribute to obesity in children[18,19]. High exposure to phthalates is also associated with the use of medical products containing DEHP or DBP[20]. Animal toxicity

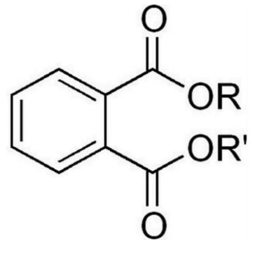

Figure 1. General structure of phthalates, *R* and *R′* are the same or different alkyl or aryl groups.

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Table 1. Samples of toys and childcare articles sold in Republic of Croatia collected for analysis of phthalate content

Sample	Total *N* of samples	Toys		Articles (bottles etc.)	
		N Toys	Analysed for phthalate content	*N* Articles	Analysed for phthalate content*
All sampled child articles	555	518	255	37	37
2012	336	301	98	35	35
2013	219	217	157	2	2

*In childcare articles, phthalate was below detection limit.

Table 2. Products containing plastic shown by product type in joint analysis of all samples (total) and separate analysis by year of import (2012 and 2013)

Toys	Total *N*	*N* 2012	*N* 2013	Products containing phthalates above detection limit (% of *N* per product type)		
				% of Total *N*	% of *N* 2012	% of *N* 2013
Dolls	94	39	55	24	28	22
Animals	68	21	47	9	14	6
Balls	18	12	6	22	17	33
Cars	26	8	18	0	0	0
Tools, shovels buckets	21	8	13	0	0	0
Arms	9	6	3	0	0	0
Make-up set	3	1	2	0	0	0
Others	16	3	13	0	0	0
Articles						
Soothers	22	20	2	0	0	0
Bottles	5	5	0	0	0	0
Chewing soothers	10	10	0	0	0	0
Total *N* samples analysed	292	133	159			

studies indicate that DEHP causes hepatotoxicity through peroxisome proliferation, DINP causes appearance of liver neoplasms, while DEHP and BBP modify rat breeding, causing pronounced testicular and sperm damage; such adverse endocrine effects were observed even in offspring of exposed pregnant females[2,21–24]. Studies showed that DEHP is one of the most toxic phthalates with accidental poisoning recording lethal DEHP concentrations 0.3 to 1.0 mg/kg (ref. 4). Attempts have been made to regulate and replace DEHP by DINP[7]. However, the margins of safety are lower for DINP and DEHP than for DNOP, DIDP, DBP and BBP[2]. Thus, exposure to DINP is also a cause for concern. There is also a synergistic interaction between more phthalate types that can be present in children's toys and multiple sources of child exposure[2]. Thus monitoring the levels of various phthalates and the analysis of their source is important. The present study is an analysis of phthalates in child products during a period of the adaptation of the legislative framework for phthalate control prior to the accession of the Republic of Croatia to the EU. The earlier legislation was significantly different regarding the values of prohibited and/or restricted allowable phthalate concentrations and did not prescribe limits for certain phthalates. We show that the status of phthalate levels was not satisfactory, although prior to the entry of Croatia to the EU, the higher EU standards started to be applied. In particular, the study shows that despite the introduction of controls, there was an increase in the number of toys softened by prohibited phthalates recorded between two consecutive years. By analysis of particular types of phthalates we wanted to find out whether those like DEHP whose adverse effects are well known, could still appear in the even with strict laws of the EU.

Samples of toys and childcare articles ($N = 555$) were collected from the markets in the Republic of Croatia in the years 2012 and 2013. Table 1 shows the number of samples collected in 2012 and 2013, and the number of analysed samples. Samples that contained plastic materials were chosen for phthalate analysis. Tables 2 and 3 give the product type and country of origin. Analysis included DEHP, CAS No. 117-81-7/EC No. 204-211-0; DBP, CAS No. 84-74-2/EC No. 201-557-4; BBP, CAS No. 85-68-7/EC No. 201-622-7; DINP, CAS No. 28553-12-0, 68515-48-0/EC No. 249-079-5 and 271-090-9; DIDP, CAS No. 26761-40-0 and 68515-49-1/EC No. 247-977-1 and 271-091-4, and DNOP, CAS No. 117-84-0/EC No 204-214-7.

Phthalates in toys and childcare articles were determined by GC-MS, operated in selected ion monitoring

(SIM). GC-MS was chosen because it is highly specific and widely available, whereas SIM provides higher sensitivity. The most abundant ion formed in the mass spectrometer is called the base peak. In the mass spectra of phthalates (Figure 2), the base peak is indicated at the *m*/*z* value of 149. Identification and quantification of phthalates used the extracted ion *m*/*z* 149, for each of the phthalates tested, except for DNOP and DINP, because of co-elution of both. For DNOP, *m*/*z* value of 279 was used for quantification, while for DINP *m*/*z* it was 293. Confirmation of presence was monitored by the following qualifier ions: *m*/*z* 149 (DNOP), *m*/*z* 223 (DBP), *m*/*z* 149 (DINP), *m*/*z* 307(DIDP), and *m*/*z* 206 (BBP). Method for determination of phthalates in toys and childcare articles is based on extraction of sample with dichloromethane on Soxtherm, followed by GC-MS analysis of the resulting extracts. Determination of phthalates was performed on Shimadzu GC-MS QP 2010, using electron ionization (EI) mode. Separation was performed with a SPB-5 (5% diphenyl, 95% dimethyl siloxane) capillary GC column, 60 m, 30 mm ID, with film thickness of 0.25 μm. The column was held at 40°C for 5 min, ramped at 10°C/min to 280°C and finally held for 21 min. The gas chromatograph was operated in split/splitless injection mode at a temperature of 280°C. The method has been validated according to the HRN EN 14372 : 2004 norm, with some modifications regarding extraction time and quantity of analysed sample. Satisfied recovery was achieved with extraction time of 4 h instead of 6 h, and with 1 g of sample taken into the procedure instead of 2 g. This improvement, shorter extraction time and smaller sample amount lead to better efficacy, proven by proficiency testing[25,26]. In order to construct a calibration curve, regression of peak area on concentration was undertaken using the external standard calibration method. The assay linearity was studied by injection of five different standard concentrations in the range 0.5–15 $\mu g\ ml^{-1}$ for DBP, BBP, DEHP and DNOP, and 1.0–20 $\mu g\ ml^{-1}$ for DINP and DIDP. All correlation coefficients were > 0.990. Limit of detection (LOD) and limit of quantification (LOQ) were established by analysing samples with added analytes in different concentrations considering signal-to-noise (S/N) ratio 1 : 3 for LOD and 1 : 10 for LOQ. LOD was 0.005% for DBP, BBP, DEHP and DNOP, and 0.01% for DINP and DIDP, while LOQ was 0.01% and 0.02% respectively. Recovery was determined by analysing samples spiked at five different concentration levels. Each level has been prepared and analysed in duplicate. Recovery range was from 90% for DINP to 98% for BBP. Measurement precision was examined by injecting seven replicate injections of standard (RSD < 5%). Method precision was examined by analysing spiked sample in triplicate (RSD < 10%).

Table 3. Country of origin of analysed products

Country of origin	Total *N*	Products containing phthalates above detection limit (N)
China	272	47
Germany	14	0
UK	1	0
Italy	2	0
France	1	0
USA	1	0
Macedonia	1	1

In statistical analysis, the descriptive statistics is shown by percentage ratios and differences in percentage between the different years of sampling were tested with chi-square test (χ^2) with the level of significant difference of $P \leq 0.05$ between the years. Concentrations of individual types of phthalates from the total analysed sample are shown in mean values and by standard deviation, but because of the large range of data, median value is shown together with minimal and maximal values. Concentration range is shown graphically as a distribution of percentage ratio for individual percentage concentration of DEHP. A correlation between percentage concentration of DEHP and other phthalates was made. However, as no statistically significant correlation was found, the analysis is not shown.

From the total of 555 collected samples intended for use among children, around 60% contained plastic materials (Figure 3 *a*). Also 40% of the samples collected in 2012 and 80% of the samples collected in 2013 contained plastic materials (Figure 3 *a*; χ^2, $P \leq 0.05$). The products that contained plastic were chosen for phthalate analysis (Table 1). Figure 3 *b* shows that 16% of all samples contained detectable levels of phthalate. When analysing the products that contained phthalates per year, 20% of the samples collected in 2013 and only 12% of the samples collected in 2012 contained phthalates. Although in 2013 the phthalate percentage was indicatively higher, statistical analysis (χ^2) showed that there was no significant difference between the years. All of the products that contained phthalates were toys. None of the childcare articles contained phthalates (Table 2). From the total analysed toys ($N = 255$), dolls were the most abundant type (36% of analysed samples), followed by animals (26%), cars (10%) and tools (8%), whereas other types were present to a lesser extent (Table 2). Phthalates were found in 24% of dolls, 22% of balls and 9% of cars and they were not found in other types of toys (Table 2). Most of the analysed products (93.4%) were manufactured in China; therefore, most of the products with detectible levels of phthalates were from China (Table 3). Figure 3 *c* shows the percentage distribution of individual phthalate type amongst the positive samples. Forbidden DEHP was present in 95.8% of positive samples, DINP was present in 41.7% of positive samples and DBP was present in 16.7% of positive samples. DIDP and DNOP were found in only one sample (thus the n.a. mark in Table 4 for min,

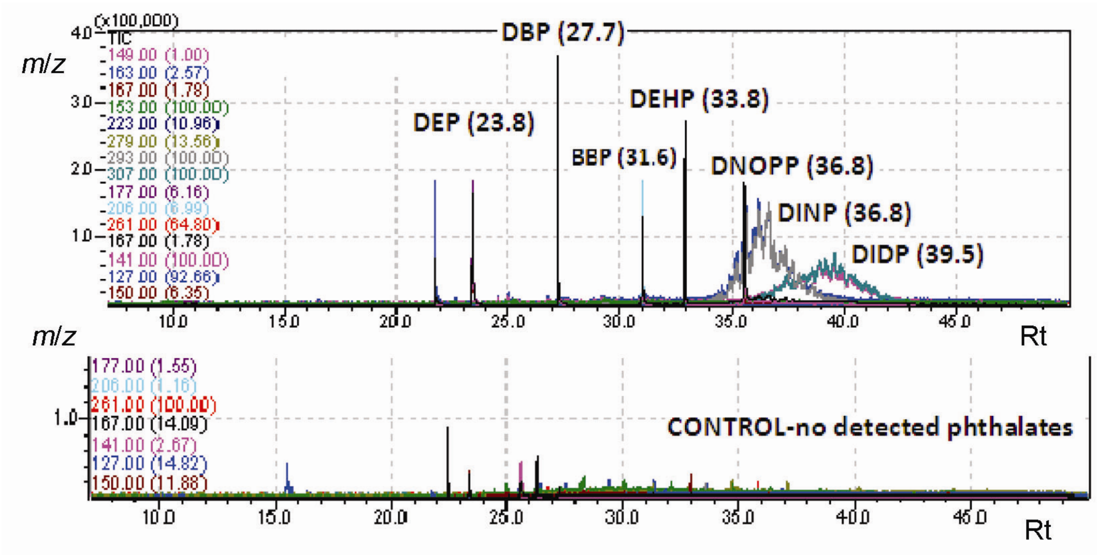

Figure 2. (Top) Chromatogram of GC-MS (sample inoculated by external standard), of six phthalate types analysed; retention time given in parenthesis. (Bottom), Sample without phthalates.

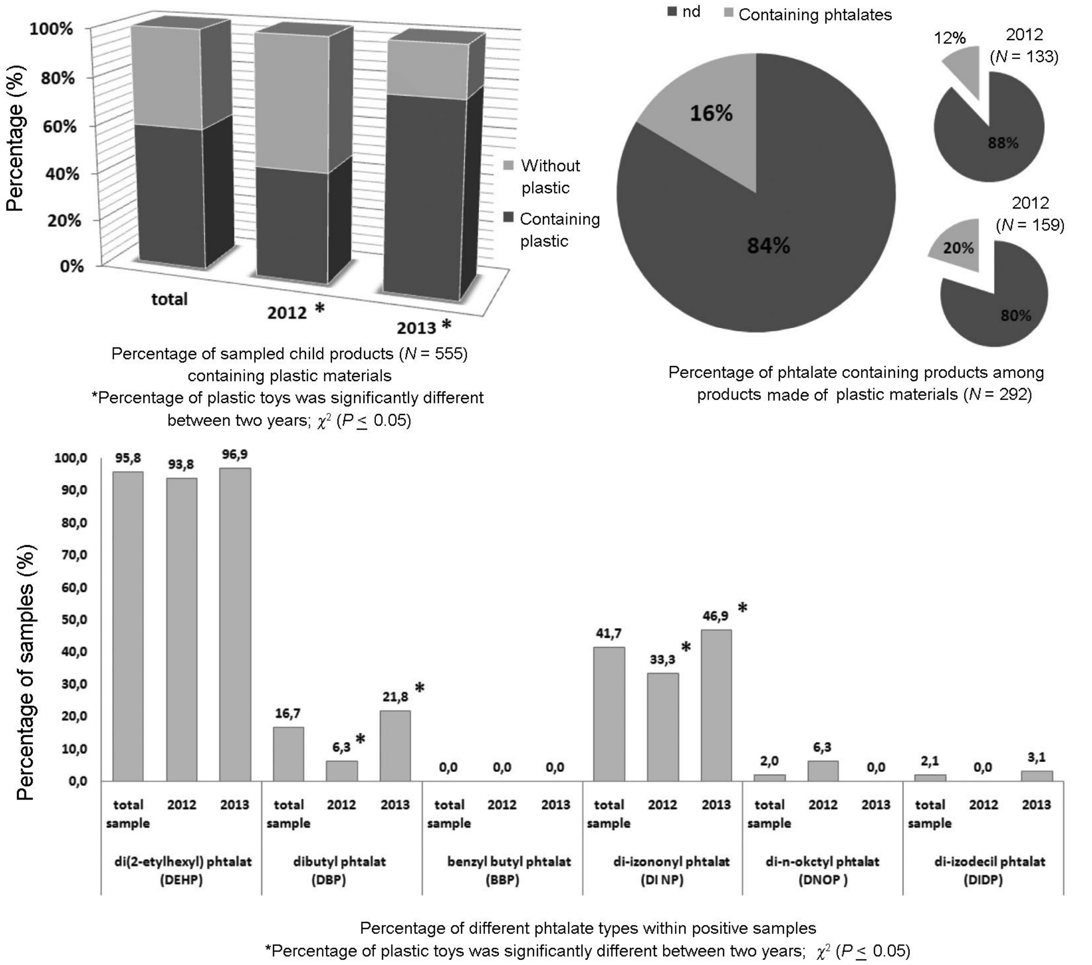

Figure 3. ***a***, Percentage of plastic toys and articles in joint analysis of all samples (total) and separate analysis by year of import (2012 and 2013). ***b***, Percentage of products positive for phthalate content above detection limit in the joint analysis of all samples (total) and separate analysis by year of import (2012 and 2013). ***c***, Individual phthalate type distribution (%) of positive child products of all samples (total) and separate analysis by year of import (2012 and 2013).

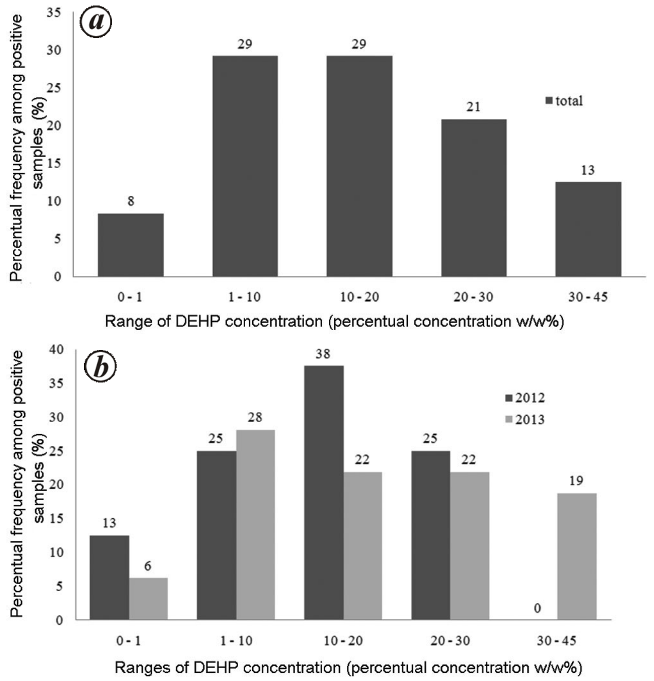

Figure 4. ***a***, Percentage of products containg different DEHP concentrations (0–40% (w/w)). ***b***, The percentage (%) of products containg different DEHP concentrations (0–40% (w/w)) analysed separately by year of import (2012 and 2013).

max and SD), and none of the samples contained BBP. Figure 4 shows products (expressed as percentage of phthalate positive products) containing DEHP expressed as concentration percentage weight (w/w) range (0–1%, 1–10%, 10–20%, 20–30% and 30–45%). Results show that over 60% of the positive samples contained more than 10% (w/w) of DEHP. Among the products collected in 2012 none contained over 30% (w/w) of DEHP, while among products collected in 2013, 19% contained high concentrations of DEHP (between 30 and 45% (w/w)). Correlation analysis did not show any statistically significant connection; thus it is not presented here.

The results of the present study show that phthalates were, present to a great extent, in the market, at the time when the Croatian legislative and control was adopting and introducing the legislative of the EU. The EU limited the use of DEHP, DBP and BBP in children's toys and childcare articles to a maximum percentage concentration of 0.1% (w/w) and restricted the use of DINP, DIDP and DNOP in toys and childcare articles which can be placed in the mouth. It was expected that the importers and vendors in Croatia would reduce the presence of toys containing the phthalates due to the strict EU regulation. However the results reveal the opposite. Out of 60% of the toys in the market that contain plastic, approximately 12–20% had detectible levels of phthalate.

In similar EU studies, conducted in Germany, Austria and Switzerland, higher presence of phthalates (27%) in the analysed samples has been reported, with 8% being articles for children[27]. However, in the case of childcare articles designed for chewing (bottles, baby soother, etc.) for children under 3 years of age, phthalate was not recorded in our study. We found that among positive samples majority contained DEHP (96%; Figure 3 *c*). Over 60% contained DEHP in concentrations more than 10 times greater than that permitted (Figure 4); mostly dolls and toy animals, with an average value more than 100 times greater than the allowed percentage concentration of 0.1% (w/w). Similar results were obtained in a study conducted in India, which also reported DEHP in 96% of products made in China, Taiwan, Thailand and India[28,29]. The maximal detected DEHP concentration was 45% (w/w), which is 450 times greater than that allowed (Table 4). Since DEHP synthesis in 1993, toxicological studies have shown its toxic effects on the liver, induction of testicular atrophy[30,31], on androgynous signalling pathway[32], premature mammary growth in females[33]; and it has also been added to a list of carcinogens according to Annex I of the Council Directive 67/548 (refs 34, 35).

The second most commonly detected phthalate above the highest permissible value was DINP found in 41.7% of the samples with concentration ranging from 0.02% to 40% (w/w) (Figure 3 *c* and Table 4). The second most common phthalate in toys and childcare articles in the Indian market was also DINP (42% of the samples)[28], ranging from 0.1% to 16.2% (w/w). Even though Indian and Croatian studies detected DINP as the second most abundant phthalate type, other studies have shown showed DINP as the most abundant in child products, followed by DEHP, DIOP and DIDP[2,27–29]. Samples of dolls from a study conducted by Biedermann-Brem *et al.*[27] showed that DINCH (diisononyl-cyclohexane-1,2-dicarboxylate) was the most abundant phthalate in dolls and articles for children. In the present study (Figure 3 *c* and Table 4), the third most common phthalate was DBP, followed by DIDP and DNOP, with 2% of positive samples, maximal concentration being 0.53% (w/w) and 0.08% (w/w) respectively. None of the samples contained BBP. DBP, DNOP and BBP were detected in relatively low concentrations, while DBP and BBP were present in less than 1% of the samples. Therefore it is considered that they are by-products of technological procedure and not intentionally added[2,27–29]. The literature revealed that China manufactured the largest number of such toys[2,27–29,35].

From the present study we have gained important data for risk assessment of exposure of children to phthalates from toys in 2012 and 2013, prior to the introduction of legislative of the EU. Based on these data it could be estimated that there is a high possibility that among 20 toys bought in 2012 and 2013, especially plastic dolls, four could contain DEHP while 2–3 would have high probability of containing DEHP in concentrations 100–400 times greater than that allowed. Thus, we cannot

Table 4. Detected phthalates and their concentration expressed as per cent weight unit (w/w%) found in the analysed products

Phtalate type	CAS no.	LOD% (w/w)		Min (w/w%)	Max (w/w%)	Median (w/w%)	Average (w/w%)	SD
Bis(2-ethylhexyl) phthalate	117-81-7	≤0.005	Total sample	0.31	45.00	13.00	15.99	11.48
			2012	1.15	26.00	12.00	11.89	7.50
			2013	0.31	45.00	17.00	17.59	12.90
Dibutyl phthalate	84-74-2	≤0.005	Total sample	0.02	5.30	0.23	0.86	1.60
			2012	0.02	0.26	0.01	0.04	0.07
			2013	0.23	5.30	0.60	1.76	2.06
Benzyl butyl phthalate	85-68-7	≤0.005	Total sample	n.d.	n.d.	n.d.	n.d.	n.d.
			2012	n.d.	n.d.	n.d.	n.d.	n.d.
			2013	n.d.	n.d.	n.d.	n.d.	n.d.
Di-'isononyl' phthalate	68515-48-0	≤0.01	Total sample	0.02	40.00	0.60	0.00	8.97
			2012	0.02	40.00	0.01	4.16	10.79
			2013	0.19	2.41	0.60	0.76	0.63
Di-*n*-octyl phthalate	117-84-0	≤0.005	Total sample	0.08	0.08	n.a.	n.a.	n.a.
			2012	0.08	0.53	n.a.	n.a.	n.a.
			2013	n.d.	n.d.	n.d.	n.d.	n.d.
Di-'isodecyl' phthalate	26761-40-0	≤0.01	Total sample	0.53	0.53	n.a.	n.a.	n.a.
			2012	n.d.	n.d.	n.d.	n.d.	n.d.
			2013	0.53	0.53	0.53	n.a.	n.a.

LOD, Limit of detection = 0.1%; n.d., Not detected (less than LOD); n.a., Not applicable.

totally disregard or neglect the risk of exposure, especially considering that some toys are used for a number of consecutive years by children. On the basis of this information further studies on exposure are needed. Nevertheless, the percentage of 12–20% of products containing phthalates was expected to be lower since their maximal residue levels were regulated by law and restricted because of their toxicity.

We conclude that phthalates are still being used in the manufacture of toys and other articles endangering the health of children. Considering the fact that the global toy market is worth US$ 105 billon[28], it is clear that the issue exceeds health standards of individual countries. Hence, studies on the harmful effects of phthalates and their presence in objects of common use, as well as exposure to phthalates of risk groups should be done in all countries with and legislative control the countries of manufacture in order to minimize this global health problem, especially considering the health of children.

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Biogeochemistry of shallow lake sediments: a case study from Verlorenvlei, South Africa

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Studying the biogeochemistry of shallow lake sediments, especially the source of sedimentary organic matter (OM), is challenging because of the low preservation of OM in shallow lake sediments. Here we report the source of sedimentary OM in a shallow freshwater lake, Verlorenvlei, in South Africa using a number of biogeochemical proxies. Elemental carbon and nitrogen ratio (C/N), and stable C and N isotopes (δ^{13}C and δ^{15}N) indicate algal source of the sedimentary OM. Total organic and inorganic C, different phosphorus fractions, δ^{13}C and δ^{15}N values indicate repetitive presence of non-N-fixing cyanobacteria under moderate N-limited conditions. Cyanobacterial population in Verlorenvlei is likely influenced by the availability of dissolved inorganic C. Cyanobacterial proliferation in the lake has ceased with accelerated N input as recorded at the top of the core.

Keywords: Carbon, cyanobacteria, nitrogen, organic phosphorus, shallow lakes, stable isotopes.

PRIMARY production is a major biotic process in the lakes. In-lake primary production responds quickly under stresses induced by anthropogenic activities such as external nutrients (nitrogen and phosphorus) input from

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