

# High Performance Liquid Chromatographic (HPLC) Method for Comparison of Levels of some Phthalate Esters in Children's Toys and Their Health Implications.

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## ABSTRACT

The phthalate esters contents of children toys were analyzed using High Performance Liquid Chromatography (HPLC). Understanding the distribution of ubiquitous phthalate esters in plastic products were necessary because of their subtle toxicity to humans. The phthalate esters were obtained using liquid-liquid extraction procedure, after which the extracts were cleaned up using solid phase clean up technique, and finally analyzed by HPLC. Results revealed that the toy samples contained high concentrations of phthalate esters. For dimethyl-, diethyl-, diphenyl-, and dibutyl-phthalates, baby suckers toy mean values ( $\mu\text{g/g}$ ) of  $44.56 \pm 1.14$ ,  $3.29 \pm 0.03$ ,  $225.73 \pm 3.16$ , and  $47.94 \pm 0.62$  were recorded while truck toys have mean values ( $\mu\text{g/g}$ ) of  $278.81 \pm 2.84$ ,  $175.32 \pm 2.00$ ,  $41.01 \pm 1.23$ , and  $89.20 \pm 1.21$ , respectively. A soft colorful rabbit with carrot toy showed mean values ( $\mu\text{g/g}$ ) of ND,  $217.79 \pm 1.39$ ,  $60.86 \pm 1.38$ , and  $1625.25 \pm 25.3$ . Mean values ( $\mu\text{g/g}$ ) of ND,  $387.50 \pm 3.54$ ,  $7.84 \pm 0.06$ , ND and  $1.19 \pm 0.01$ ,  $0.32 \pm 0.03$ ,  $48.18 \pm 0.23$ ,  $82.47 \pm 3.71$  were recorded for a Barbie-type girl doll toy and teether and rattle toy, respectively.

On percentage basis, the amounts of these phthalate esters as determined in these toys were much higher than the stipulated EU limit of 0.1% (w/w) in toys. The percentage concentration for dibutyl-phthalate ranges between 0.1 – 14.17% and between 0.1 – 1.00% for diphenyl-phthalate. Conversely, diethyl-phthalate percentage concentrations ranged between 0.1 – 5.06 %, and that of dimethyl-phthalate ranges between 0.1 – 63.34%, respectively. The study is noteworthy and significant because most toys used by children (0 – 3 years) are usually intended to be placed in the mouth which could lead to the leaching of these chemicals into their blood

stream, which in turn, poses a health risk to infants.

(Keywords: children's toys, plastic products, phthalate esters, soxhlet determination, HPLC, chromatography)

## INTRODUCTION

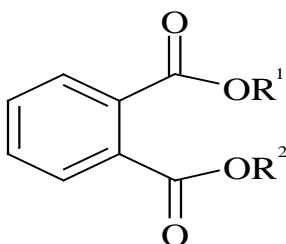
There has been a growing interest and concern for the study of the impact of man-made chemicals on wildlife, humans, and the environment. These studies have suggested that some synthetic and naturally occurring substances in the environment may affect the normal functioning of the endocrine system.

One of the major classes of environmental substances is the endocrine disrupting chemicals (EDCs), which interfere with the function of the endocrine system. In recent years, these endocrine disrupting chemicals have become one of the most important topics of research in the field of environmental sciences (Olowu et al., 2010; Colon et al., 2000). EDCs are refers to as exogenous substances that cause adverse effect in organisms or its progeny, consequent to changes in endocrine function. They are ubiquitous in the environment due to their large number of uses in the industrial, residential and agricultural applications (Olowu et al., 2010).

In wildlife, alterations in sexual reproductive behavior have been reported in areas of contamination with EDCs. Ironically, phthalate esters are multi-functional and one of the most widely used groups of industrial chemicals and have been indicted as Endocrine Disrupting Chemical based on recent epidemiological studies (Casajuana and Lacorte, 2004; Ying et al., 2002).

The manufacture of plastic products often includes additives which are intended to modify the physical properties of the polymer. Common additives are plasticizers, antioxidants, and flame retardants. Most often phthalate esters are mainly used as plasticizers (substances added to plastics to increase their flexibility, transparency, durability, and longevity). They are primarily used to soften polyvinyl chloride (PVC). Recently phthalates are being faced out of many products in the United States and European Union over health concerns.

Phthalates are dialkyl, or alkyl aryl esters of 1,2 – dibenzenedicarboxylic acid (phthalic acid) with general structure shown below, where R<sup>1</sup> and R<sup>2</sup> can be various combinations of straight and branched alkyl chain or aryl group.



**Figure 1:** General Structure of the Phthalates.

Phthalates are among the most widely used industrial chemicals in existence. Apart from being used primarily as plasticizers, to impart flexibility, workability, and durability to polymers, they are also utilized in products such as adhesives, inks, cosmetics, munitions, industrial and lubricating oil, as well as solvents in perfumes and paints, and additives in hair-sprays and insect repellent (Shanker et al., 1985; Ling et al., 2007; Huang et al., 2008). To provide flexibility phthalate esters are not chemically bonded to resin and therefore are easily released (Yuan et al., 2008). The most commonly used phthalate plasticizers are di-2 ethylhexyl phthalate (DEHP) and di-n-butyl phthalate (DBP) with the others used only for selective applications (ATSDR, 2006).

Phthalates in soft PVC products are not tightly bound to the plastic, but are present as mobile components of the plastic matrix. Loss of phthalates by volatilization over time from soft PVC has long been recognized (Cadogan et al., 1993, UK Department of the Environment, 1991). Exposure to phthalates is therefore twofold. Humans may be exposed to background levels of

contamination in air, food and water, but may also be exposed through direct contact with soft PVC products, particularly those intended as high contact products, such as children's toys and teethingers.

Although less persistent in the body and environment than many other synthetic organic compounds, the continued release of large quantities of these chemicals to the open environment ensures that concentrations and exposures remain substantial. Moreover, recent research suggests that phthalates may persist in human body tissues for longer periods than previously assumed (Dirven et al., 1993).

There has been growing interest in the unusual ubiquity of phthalates in the environment (Fatoki and Vernon, 1990; Fatoki and Ogunfowokan, 1993a) and their wide range of toxicological characteristics (Blount et al., 2000). Phthalates have possible tetraaromatic and carcinogenic effects (Blount et al., 2000; Tomita et al., 1982; Huff and Kluwe, 1984). DEHP has been included in Class B2 (probable human carcinogens) (Alatrisme-Mondragon, et al., 2003). They are also suspected endocrine disruptors (Fredricsson et al., 1993).

A study by Colon et al. (2000), in which Puerto Rican girls with premature breast development had higher levels of blood phthalates compared to other girls, suggested an association between phthalate exposure and abnormal reproductive development. Testicular toxicity of phthalates has been documented. Studies have indicated that these chemicals may affect sperm cells, sperm mobility, cryptorchidism, and hypospadias, in laboratory animals (Imajima et al., 1993; Poon et al., 1997; Arcadi et al., 1998).

One of the main routes of exposure to phthalates is via water use as these chemicals find their way into the water system through effluent discharges and leaching from waste dumps, and through several diffuse sources (WHO, 2003). Also it has been established that toys are also good sources of phthalate contamination in humans. The objectives of this study therefore is to determine the level of phthalate esters in children's toys, to provide an information that most of the children toys on sale globally have phthalates as well as to provide data for routine monitoring of imported toys from the Asian continent.

## **MATERIALS AND METHODS**

### **Sample Materials**

Twenty new toy samples for children (0-3 years) such as teether (teething rings), pacifiers, vinyl dolls, toy rabbits, baby suckers, plastic trucks and Barbie-style girl dolls of major brands were purchased randomly from organized and unorganized sectors at Dosunmu Market, Lagos Island, Lagos State, in April 2010.

### **Preparation of Sample Materials**

All sample bottles and glassware used in the preparation of sample extracts were pre-cleaned and washed thoroughly with detergents and soaked in chromic acid then rinsed severally with distilled water, after which they were oven dried for 2 hrs at 105°C, before being finally rinsed with acetone.

### **Materials and Reagent Purification**

The materials and reagents employed in the experiment include: anhydrous sodium sulphate ( $\text{Na}_2\text{SO}_4$ ), dichloromethane (DCM,  $\text{CH}_2\text{Cl}_2$ ), ethyl acetate ( $\text{CH}_3\text{COOC}_2\text{H}_5$ ), n-hexane ( $\text{C}_6\text{H}_{14}$ ), and acetonitrile ( $\text{CH}_2\text{CN}$ ). All the reagents were doubly distilled to ensure the purity of the solvent before they were used for extraction and cleanup experiment. The acetonitrile used were of HPLC grade.  $\text{Na}_2\text{SO}_4$ , silica gel (60 – 120 mesh), aluminum foil and glass wool were placed in the muffle furnace at 450°C for 4 hrs, to remove any organic contaminants (Fatoki and Ogunfowokan, 1993ab, Ogunfowokan et al., 2006). The  $\text{Na}_2\text{SO}_4$  and glass wool were further treated (soaked and washed) with DCM and then dried in the oven at 105°C to eliminate any background contamination from the salt and glass wool.

### **Sample Preparation and Extraction**

All soft sections of the PVC toys were cut into pieces of less than 2 mm<sup>2</sup> with scissors and razor blades and the plastic toys were later pulverized into plastic powder. About 10 g each of the pulverized component were wrapped properly with 12.5 mm Whatman filter paper before Soxhlet extraction.

The weighed PVC samples were Soxhlet extracted with 100 ml dichloromethane (DCM) for

7 hours at 60°C (Rastogi, 1998). 90 ml of the extract was concentrated under vacuum using a rotary evaporator at 30°C.

### **Clean – up and Separation**

A glass chromatographic column (2.0 cm X 20 cm) was packed with about 5 g of activated silica gel (60 – 120 mesh). The column was then tapped to settle the silica gel for efficient column packing. Then about 1cm of  $\text{Na}_2\text{SO}_4$  was added to the top of the silica gel and the chromatographic column was then pre-eluted with 20 mL of n-hexane with rate of about 2 ml/min. The eluate was then discarded just prior to the exposure of the sodium sulphate layer. About 2 mL of the sample extract was quantitatively transferred into the column and additional 2 mL of hexane was then used to complete the transfer of the sample down the column, then about 40 mL of hexane was then used to elute the column for non-polar (hydrocarbons) substituent. This fraction was also discarded.

40 mL of ethyl acetate was then used to elute the column for phthalate esters prior to the exposure of the  $\text{Na}_2\text{SO}_4$  layer. The eluate was then concentrated to 1 mL over a thermostated water bath at 30°C. The eluate was then kept in the fume hood to dry at room temperature; about 1 mL of acetonitrile was then added to the concentrate for TLC and HPLC analysis.

For the TLC, ethyl acetate – hexane mixture (80:20 v/v) were employed as developing solvent. While the phthalate contents of the extracts were determined by comparing their retardation factors with that of standard phthalates after development and identification of various spots in an iodine tank and also observed under the UV lamp to ensure that all the separated organic components are visible for measurements.

### **Instrumental Analysis**

An AKTA™ Basic 10/100 HPLC system (Amersham Pharmacia biotech), coupled with UV detector transmitting at 254 nm was used. The flow rate was 1.0 ml/min. Chromatographic separation was carried out using a thermostated capillary column C<sub>18</sub> (S<sub>5</sub> ODS2 250 cm X 3.0 mm) at ambient temperature. 20 µL was used as the injection volume and separation was performed under isocratic elution condition using acetonitrile

and water (90:10) as mobile phase (Yuwatini et al., 2006). Under this condition separation lasted for 12 min.

### **Response Factor and Quality Assurance Study**

Preparation of sampling materials, sampling procedures, reagents and materials, extraction and analytical procedures were carefully controlled to maintain good quality assurance. Quality assurance study was carried out in terms of recoveries of phthalate ester in order to ascertain the efficiency of the extraction and analytical procedures since no certified reference material was available.

$$\text{Response Factor (RF)} = \frac{\text{Peak Area of Phthalate Esters}}{\text{Peak Area of Internal Standard}}$$

### **Recovery Studies**

The recovery study was carried out to assess the efficiency of the methodology. The study was carried out by extracting a pre-extracted PVC toy sample with 10 ml of mixture of DMP, DEP, DBP, DPhP and Internal standard at the concentration of 1000 mg/L.

### **Percentage Amount of Phthalate Ester Present in Sample**

The Percentage phthalate present in each plasticized toy sample was calculated using the formula:

$$\% \text{ Phthalate } (w/w) = \frac{C \times V \times D}{W \times 1000} \times 100$$

where C= Concentration of phthalate in HPLC sample (in µg/g)

V = Total volume of DCM used in the extraction method

D = Dilution factor of phthalate extraction method

W = Weight of sample collected (in mg)  
(US-CPSC, 2009)

### **Quantification of Phthalate Esters in Toy Samples**

The method of quantification adopted for this work is the method adopted by Fatoki and Noma, 2002, which was the use of Response Factor (Rf).

The response factor (Rf) for DMP, DEP, DBP and DPhP were determined based on these approach.

Concentration of each phthalate ester in the extract was determined from the expression that follows:

$$\begin{aligned} \text{Response Factor (RF)} &= \frac{\text{Peak Area of Phthalate Esters}}{\text{Peak Area of Internal Standard}} \\ &= \frac{\text{Conc. of phthalate ester in the extract}}{\text{Conc. of Internal Standard}} \end{aligned}$$

Since 100% of the phthalates were not recovered, the value obtained from the above expression has to be corrected using the value of percentage recovery.

$$\begin{aligned} \text{Corrected concentration value} &= \frac{\text{Conc. of PEs in sample}}{\% \text{ Recovery for the PE}} \\ &\quad \times 100 \% \end{aligned}$$

The values obtained from this expression were finally recorded as the real concentrations of the phthalate esters.

## **RESULTS AND DISCUSSION**

This study was targeted at plasticized PVC toys and so does not cover the entire range of toys on sale. Nevertheless, some trends were evident. Although the majority of the toys bought were made wholly or partly of PVC, the plastic was only infrequently with the packaging. All inflatable toys (soft) were composed of phthalates – plasticized PVC.

Contrary to initial expectations, DEHP was not the most commonly used phthalate in the toys analyzed. This contrasts with a report prepared

for the US Consumer Product Safety Commission in 1983 (Hanson, 1983), in which DEHP was the only phthalate identified in eleven of fourteen children's products analyzed. DEHP was also the focus of the study by Meek and Chan in 1994 (Meek & Chan, 1994).

The data from this investigation do not allow estimation of the dose to which a child playing with any toy will be exposed. It is well recognized that DINP and other phthalates leach from PVC to the air, into liquid solvents, and on to the skin. The dose received by an individual child playing with a certain toy will depend on many factors, including phthalate composition and concentration, toy design and purpose, exposure time, method of play, and degree of mouthing and chewing activity.

A number of different methods are under development for the estimation of leaching rate and there may be limited comparability between methods (as reported by Marin et al., 1998). That different leaching tests may yield significantly different results has its being recognized for some time (PINDAR et al., 1993). Any estimates of

dose are, therefore, likely to remain highly subjective. Some experts in this field are of the opinion that leaching tests are useful and could form a reliable basis for regulation. However, the validity of these tests has not been universally accepted.

In the present study, 20 new toy samples for children 0 – 3 years such as teething, vinyl rabbit doll, Barbie-type girl doll, and rattles manufactured in China were analyzed for 4 phthalate esters – Dimethyl phthalate (DMP), Diethyl phthalate (DEP), Diphenyl phthalate (DPhP) and Dibutyl phthalate (DBP) using Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC) results shown in Tables 2 – 4.

The separation of the phthalates standard from the TLC plates with their retardation factor ( $R_f$ ) were DMP – 0.42, DEP – 0.58, DPhP – 0.66, DBP – 0.93. One or more of the phthalates mainly DPhP, DBP, DEP and DMP were detected due to their retardation factor ( $R_f$ ) in number of prominence, as compared to the retardation factor of the standards.

**Table 1:** Retardation Factor of Mixtures of Phthalate Standards.

Standards	Retardation Factor ( $R_f$ )
Dimethyl Phthalate (DMP)	0.42
Diethyl Phthalate (DEP)	0.58
Diphenyl Phthalate (DPhP)	0.66
Butyl Benzoate (Internal Standard)	0.71
Dibutyl Phthalate (DBP)	0.93

**Table 2:** Values for Response Factor, Retention Time, %Recovery and LOD of Phthalate Esters.

Phthalate Esters	Response Factor (RF)	Retention time (Minutes)	Percentage Recovery (%)	Limit of Detection (LOD)
DMP	1.41	3.13	12.83	0.70
DEP	1.29	3.33	74.70	0.80
DPhP	3.41	3.71	25.77	0.30
DBP	1.27	4.18	89.23	0.80

**Table 3:** Group Mean Levels of Phthalate Esters in Toy Samples and Blank.

Group	DMP( $\mu\text{g/g}$ )	DEP( $\mu\text{g/g}$ )	DP <sub>h</sub> P( $\mu\text{g/g}$ )	DBP( $\mu\text{g/g}$ )
Blank	0.02 $\pm$ 0.01	0.61 $\pm$ 0.07	36.02 $\pm$ 0.08	ND
SK <sub>(1-3)</sub>	44.56 $\pm$ 1.14	3.29 $\pm$ 0.03	225.73 $\pm$ 3.16	47.94 $\pm$ 0.62
PVC <sub>(T1-T5)</sub>	278.81 $\pm$ 2.84	175.32 $\pm$ 2.00	41.01 $\pm$ 1.23	89.20 $\pm$ 1.21
PVC <sub>(3-6)</sub>	ND	217.79 $\pm$ 1.39	60.86 $\pm$ 1.38	1625.25 $\pm$ 25.3
PVC <sub>(D1&amp;DB)</sub>	ND	387.50 $\pm$ 3.54	7.84 $\pm$ 0.06	ND
T <sub>11</sub> , T <sub>TH</sub> & T <sub>TW</sub>	1.19 $\pm$ 0.01	0.32 $\pm$ 0.03	48.18 $\pm$ 0.23	82.47 $\pm$ 3.71

**Table 4:** Percentage Amount of Phthalate Esters in Toy Samples.

Sample	DMP%(w/w)	DEP%(w/w)	DP <sub>h</sub> P%(w/w)	DBP%(w/w)
SK <sub>1</sub>	ND	0.004	0.02	0.13
Sk <sub>2</sub>	0.07	0.05	0.07	0.60
SK <sub>3</sub>	0.6	ND	3.30	ND
PVC <sub>T1</sub>	1.84	ND	1.00	1.90
PVC <sub>T2</sub>	0.05	0.09	0.08	0.38
PVC <sub>T3</sub> (A)	ND	0.11	0.14	0.39
PVC <sub>T3</sub> (B)	ND	ND	ND	0.02
PVC <sub>T4</sub>	0.27	ND	ND	ND
PVC <sub>T5</sub>	63.3	5.06	ND	ND
PVC <sub>3</sub>	ND	0.38	ND	11.7
PVC <sub>4</sub>	ND	ND	0.13	1.64
PVC <sub>5</sub>	ND	2.71	0.54	ND
PVC <sub>6</sub>	ND	1.27	0.55	14.2
PVC <sub>D1</sub>	ND	3.88	0.08	ND
PVC <sub>DB</sub>	ND	ND	ND	ND
T <sub>11</sub>	0.0002	0.003	0.18	ND
T <sub>TH</sub>	ND	ND	0.54	1.21
T <sub>TW</sub>	0.02	ND	0.005	ND

Isocratic elution was used and the phthalate esters were eluted from the HPLC column in the order Dimethyl phthalate (DMP), Diethyl phthalate (DEP), Diphenyl phthalate and Dibutyl phthalate. The values obtained are in agreement with the trend obtained in some past work on phthalate determination in children toys using HPLC (Go'mez-Hens and Aguilar-Caball0s, 2003; Fatoki and Noma 2002; Ogunfowokan et al., 2006).

It has been seen from past work on phthalates in environmental media that using GC-MS, retention time of phthalate esters were seen to be greater than the retention time of phthalate esters in HPLC-UV<sub>254nm</sub>. This was justified from the retention time of the phthalate esters determined by HPLC in this study. This fact shows that the determination of phthalate esters using HPLC

give more ease of detection and faster analysis time than GC for routine analysis of large number of samples.

Since low molecular weight phthalate esters elute from the column at a lower retention time before the higher molecular weight phthalate esters as recorded in past works were confirmed by the result in this work according to their volatility.

HPLC can also be coupled with MS to give the molecular weight (MW) of identified phthalate esters during analysis, as UV or MS are reliable and preferred analytical detectors.

The percentage recoveries obtained from the spiking experiments were 12.83%, 74.70%, 25.77% and 89.23% for DMP, DEP, DP<sub>h</sub>P, and DBP, respectively. The range for the percentage

recovery is comparable to percentage recoveries of phthalate esters obtained in earlier studies with percentage recoveries range of; 43.36 - 71.5% (Ogunfowokan et al., 2006;), 23.78 - 85.5% (Fatoki and Ogunfowokan, 1993a;), 50 - 105% (Vitali, 1997;) and 11.97 - 36.92% obtained by Marin, and his co-workers in 1998. This fact therefore shows the validity of the recovery study values obtained, to confirm the efficiency of the analytical procedure adopted for this work.

The Response Factor (RF) of the detector as determined using the standards of phthalate esters were 1.41, 1.29, 3.41 and 1.27 for DMP, DEP, DPhP, and DBP, respectively.

Limits of detection were obtained for the phthalates esters and this was calculated as twice the standard deviation of peak areas of respective phthalate esters for 5 runs on the HPLC machine. The values obtained for the limit of detection (LOD) was 0.70, 0.80, 0.30, and 0.80 for DMP, DEP, DPhP, and DBP. This very low value obtained for LOD confirms the high sensitivity of the Instrumental analytical procedure adopted for this work.

Three out of four Phthalate esters were determined in the blank DMP, DEP, and DPhP with DPhP being the most prominent, and this was corrected for in the sample for background contamination, this in relation confirm the ubiquitous nature of phthalate esters in the environment, as this could be as a result of phthalate ester that adhere within the Soxhlet extractor.

The results of quantitative analysis of the phthalate esters in the toy samples are presented in Tables 2 – 4. The levels of phthalate esters in this study showed that most children's toys contains phthalate ester above the EU limit of phthalate of 0.1% of the plasticized part of the toy which is also in agreement with Johnson et al., 2010.

Phthalates were detected in all the categories of toy samples however; some of the phthalate content exceeded the EU limit of 0.1 % (w/w). DBP was present in high concentrations at levels from < 0.1 - 14.17%, DPhP was detected at levels from < 0.1 - 1.00%, DEP was detected at levels from < 0.1 - 5.06%, and DMP was detected at levels from < 0.1 - 63.34%. The identity of the phthalate detected in the TLC experiment was

confirmed by HPLC from the results discussed above.

T<sub>W</sub> is the purified water sample present in the teether and said to be safe for drinking if the water by chance enters the mouth of a child chewing the toy. But sample water also show the presence of DMP and DPhP which are lower than the EU limit for phthalate esters, this is just a confirmation that phthalate esters are not covalently bonded to the sample matrix in which they are present, they leach out of plastic matrix into the environment if the condition for their movement is favored (i.e. the right temperature and the solvent into which they are leached to) thereby revealing the ubiquitous nature of phthalate esters in the environment at large (Fatoki and Vernon, 1990; Fatoki and Ogunfowokan, 1993a).

DEHP and DINP, the known isomeric phthalate esters, are usually present in most soft squeezable toys, and from previous work done DINP and DEHP was noted and they are usually found in high concentrations in many toys especially DINP which has been restricted in toys are usually present in the Chinese made toys (Johnson et al., 2010), but for this work the standards were not available for their quantification in the samples examined, because the chromatograms show the presence of other phthalate esters with prominent peaks which is one of the limitations examined in this research work.

## CONCLUSION

Exposure to phthalates is two-folds: Humans may be exposed to background levels of contamination in air, food, and water, but may also be exposed through direct contact with soft PVC products, particularly those intended as high contact products, such as children's toys and teethers. Although less persistent in the body and environment than many other synthetic organic compounds, the continued release of large quantities of these chemicals to the open environment ensures that concentrations and exposures remain substantial. Moreover, recent research suggests that phthalates may persist in human body tissues for longer periods than previously assumed (Dirven et al., 1993).

Young children (below 3years) suck and chew on toys, and they extract and ingest certain

quantities of the plasticizers. Some phthalates are suspected to cause allergy, asthma, and affect the kidneys and liver, and may also cause testicular damage. DEHP, BBP, and DBP are restricted for all toys; DINP, DIDP, and DnOP are restricted only in toys that can be taken into the mouth. The restriction states that the amount of phthalates may not be greater than 0.1% mass percent of the plasticized part of the toy. Since phthalate has been indicted as an endocrine disrupting chemical with their ubiquity as an environmental contaminant cannot be over emphasized. This led to the need for their monitoring in environmental media. It is on this note that the study focused on the determination of phthalates esters in children's toys.

The results obtained from this study showed high levels of phthalate esters for children's toys (soft PVC toys) precisely DBP. One or more of the phthalate esters were detected in high significant level in children's toy for ages (0 – 3years), this pose a lot of health risk to the human population in terms of child training and weaning a child as most of this toy are intended to be put inside the mouth.

There are no regulations for phthalates in toys in Nigeria. Children may be at higher risk of adverse effect of phthalates because of anticipated higher exposure during a time of developmental and physiological immaturity. Phthalates have been banned in EU, United States, and Canada with several other countries, from toys intended for mouthing hence from this result is important that Nigeria government should propose regulations for phthalates in toys in Nigeria.

## REFERENCES

- Alastriste-Mondragon, F., R. Iranpurb, and B.K. Ahringa. 2003. "Toxicity of Di-(2-ethylhexyl) Phthalate on the Anaerobic Digestion of Wastewater Sludge". *Water Res.* 37:1260-1269.
- Arcadi, F.A., C. Costa, C. Imperatore, A. Marchese, A. Rapidisarda, M. Salemi, G.R. Trimarchi, and G. Costa. 1998. "Oral Toxicity of Bis (2-ethylhexyl) Phthalate during Pregnancy and Suckling in Long-Evans Rat". *Food Chem. Toxicol.* 36:963-974.
- ATSDR. 2006. Agency for Toxic Substances and Disease Registry. US Department of Health and Human Services: Atlanta, GA.
- Blount, B.C., M.J. Silva, S.P. Caucill, L.L. Needham, J.L. Pirkle, E.J. Sampson, G.W. Lucier, R.J. Jackson, and J.W. Brock. 2000. "Levels of Seven Urinary Phthalate Metabolites in a Human Reference Population". *Environ. Health Perspect.* 108(10): 979-982.
- Cadogan, D.F., M. Papez, A.C. Poppe, D.M. Pugh, and J. Scheubel. 1993. "An Assessment of the Release, Occurrence, and Possible Effects of Plasticisers in the Environment". In: *PVC 93: The Future*. The Institute of Materials. 260-274.
- Casajuana, N. and S. Lacorte. 2004. "New Methodology for the Determination of Phthalate Esters, Bisphenol A, Bisphenol A Diglycidyl Ether, and Nonylphenol in Commercial Whole Milk Samples". *Journal of Agriculture and Food Chemistry.* 52: 3702-3707.
- Colon, I., D. Caro, J. Carlos, J. Bourdony, and O. Rossario. 2000. "Identification of Phthalate Esters in the Serum of Young Puerto Rican Girls". *Environ. Health Perspect.* 108 (9):895-900.
- Dirven, H.A.A.M., P.H.H. Van Den Broek, A.M.M. Arends, H.H. Nordkamp, A.J.G.M. De Lepper, P.T.H. Henderson, and F.J. Jongeneelen. 1993. "Metabolites of the Plasticizer Di (2-Ethylhexyl)Phthalate in Urine Samples of Workers in Polyvinylchloride Processing Industries". *Int. Arch. Occup. Environ. Health.* 64:549-554.
- Fatoki, O.S. and A. Noma. 2002. "Solid Phase Extraction Method for Selective Determination of Phthalate Esters in the Aquatic Environment". *Water Air Soil Pollut.* 140:85-98.
- Fatoki, O.S. and A.O. Ogunfowokan. 1993a. "Determination of Phthalate Esters Plasticizers in the Aquatic Environment of South Western Nigeria". *Environ. Int.* 19:619-623.
- Fatoki, O.S. and A.O. Ogunfowokan. 1993b. "Procedural Clean-up Technique for the Determination of Phthalate Esters in an Aquatic Environment". *Int. J. Environ. Stud.* 44:237-243.
- Fatoki, O.S. and F. Vernon. 1990. "Phthalate Esters in the Rivers of Greater Manchester Area, UK". *Sci. Total Environ.* 95:227-232.
- Fredricsson, B., L. Muller, A. Pousette, and R. Westerholm. 1993. "Human Sperm Mobility is Affected by Plasticizers and Diesel Particle Extracts". *Pharmacol. Toxicol.* 72:128-133.
- Go´mez-Hens, A. and M.P. Aguilar-Caballós. 2003. "Social and Economic Interest in the Control of Phthalic Acid Esters". *Trends in Anal. Chem.* 22 (11):847 – 857.



15. Hanson, R.L. 1983. "Phthalate Ester Migration from Polyvinyl Chloride Consumer Products. Phase 1 Final Report". US Consumer Product Safety Commission: Washington, D.C.
16. Huang, P.C., C.J. Tien, Y.M. Sun, C.Y. Hsieh, and C.C. Lee. 2008. "Occurrence of Phthalates in Sediment and Biota: Relationship to Aquatic Factors and the Biota-Sediment Accumulation Factor". *Chemosphere*. 73:539-544.
17. Huff, J.E. and W.M. Kluwe. 1984. "Phthalate Ester Carcinogenicity in F344/N Rats and B6C3F Mice". *Prog. Clin. Biol. Res.* 141:137-154.
18. Imajima, T., T. Shono, O. Zakaria, and S. Suita. 1993. "Prenatal Phthalate Cause Cryptorchidism Post-natally by inducing Trans Abdominal Ascent of the Testis in Fetal Rats". *J. Pediatr. Surg.* 32:18-21.
19. Johnson, S., N. Saikia, and R. Sahu. 2010. "Phthalate in Toys". Centre for Science and Environment, Tughlakabad Institutional Area: New Delhi, India. [www.Cseindia.org](http://www.Cseindia.org).
20. Ling, W., C. Gui-Bin, H. Ya-Qi, W. Bin Ya-Wei, and S. Da-Zhong. 2007. "Cloud Point Extraction Coupled with HPLC-UV for the Determination of Phthalate Esters in Environmental Water Samples". *J. Environ. Sci.* 19:874-878.
21. Marin, M.L., J. Lopez, A. Sanchez, J. Villaplana, and A. Jimenez. 1998. "Analysis of Potentially Toxic Phthalate Plasticizers used in Toy Manufacturing". *Bull. Environ. Contam. Toxicol.* 60:68-73.
22. Meek, M.E. and P.K.L. Chan. 1994. "Bis(2-Ethylhexyl)Phthalate: Evaluation of Risks to Health from Environmental Exposure in Canada". *Environ. Carcinog. Ecotoxicol. Rev. C.* 12:179-194.
23. Olowu, R.A., O. Arotiba, S.N. Mailu, T.T. Waryo, P. Baker, and E. Iwuoha. 2010. "Electrochemical Aptasensor for Endocrine Disrupting 17 $\beta$ -Estradiol Based on a Poly(3,4-ethylenedioxythiophene)-Gold Nanocomposite Platform". *Sensors*. 10:9872-9890.
24. Pindar, A., V. Barwick, and M. Cody. 1993. "Release of Phthalate Plasticizers from Toys and other Child-Care Products". Paper presented at the European Consumer Safety Association (ECOSA) Conference on International Product Safety Research: Amsterdam, The Netherlands.
25. Poon, R., P. Lecavaliert, R. Mueller, V. Valli, B. Procter, and I. Chu I. 1997. "Subchronic Oral Toxicity of Di-n-Octyl Phthalate and Di (2-Ethylhexyl) Phthalate in Plastic Toys". *Chromatographia*. 47:724-726.
26. Rastogi, S.C. 1998. "Gas Chromatographic Analysis of Phthalate Esters in Plastic Toys". *Chromatographia*. 47:724 – 726.
27. Shanker, R.C., C. Ramakrichma, and P. Seth. 1985. "Degradation of Some Phthalic Acid Esters in Soil". *Environ. Pollut.* 39:1-5.
28. Tomita, I., Y. Nakamura, N. Aoki, and N. Inni. 1982. "Mutagenic/Carcinogenic Potential of DEHP and MEHP". *Environ. Health Perspect.* 14:119-125.
29. UK Department of Environment. 1991. "Environmental Hazard Assessment: Di(2-Ethylhexyl) Phthalate". Report TSD/2: Department of the Environment, Toxic Substances Division: London, UK. 52
30. United States Consumer Product Safety Commission (US-CPSC). 2009. "Standard Operating Procedure for Determination of Phthalates. CPSC-CH-C1001-09.2". Directorate for Laboratory Sciences, Division of Chemistry, USCPSC: Gaithersburg, MD.
31. Vitali, M., V. Leoni, S. Chiavarini, and C. Creminini. 1993. "Determination of 2-Ethyl-1-Hexanol as Contaminant in Drinking Water". *J. Assoc. off. Anal. Chem. Int* : 76:1133-1137.
32. WHO (World Health Organization). 2003. "State of the Art Report. Health Risks in Aquifer Recharged Using Reclaimed Water". Water, Sanitation and Health. Protection and Human Environment. SDE/ WSH/03.08. WHO: Geneva, Switzerland.
33. Ying, G.G., B. Williams, and R. Kookana. 2002. "Environmental Fate of Alkyl Phenols and Alkylphenol Ethoxylates: A Review". *Environ. Int.* 28:215-226.
34. Yuan, B., Z. Li, and N. Graham. 2008. "Aqueous Oxidation of Dimethyl Phthalate in a Fe(VI)-TiO<sub>2</sub>-UV Reaction System". *Water Res.* 42:1413-1420.
35. Yuwatini, E., N. Hata, and S. Taguchi. 2006. "Behavior of Di- (2-ethylhexyl) Phthalate Discharged from Domestic Waste Water into Aquatic Environment". *J. Environ. Monitor.* 8:191 – 196.

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