DETERMINATION OF PHTHALATES IN SELECTED LOOM BANDS MARKETED IN KEPALA BATAS

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By

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LIST OF SYMBOLS AND ABBREVIATIONS

$^{\circ}C$	=	degree Celcius
%	=	percentage
µg/mL	=	Microgram per milliliter
h	=	hour
g	=	gram
min	=	minute
ml	=	milliliter
mM	=	miliMolar
nm	=	nanometer
g	=	Gravity force
w/v	=	weight per volume
v/v	=	volume per volume
AgNPs	=	Silver nanoparticles
IC_{50}	=	Inhibitory Concentration at 50%

ABSTRAK

Tiga phthalate esters (PEs) yang terdiri daripada benzilbutil phthalate (BBP), dibutil phthalate (DBP) dan dietil heksil phthalate (DEHP) telah dihadkan oleh Kesatuan Eropah kepada kurang daripada 0.1 peratus bagi semua alat permainan yang diimport. PEs dilaporkan akan menyebabkan kesan buruk kepada kesihatan manusia dan disyaki menjadi pengacau estrogen. Kini, terdapat satu penciptaan baru dalam alat permainan yang dikenali sebagai 'loom band'. United Kingdom telah mengharamkan 'loom bands' daripada pasaran kerana telah dikesan dengan kepekatan phthalate yang sangat tinggi. Walaubagaimanapun, masih belum ada apa-apa data berkenaan kepekatan phthalate yang terkandung dalam produk 'loom band's yang dipasarkan di Malaysia. Dalam kajian ini, tujuh sampel 'loom bands' telah diperolehi daripada kedai- kedai yang terdapat di Kepala Batas, Pulau Pinang. Kromatografi gas-spektrometri jisim (GC-MS) telah digunakan untuk menganalisa kandungan phthalate di dalam sampel. Lima jenis rujukan phthalate telah digunakan iaitu BBP, DBP, DEHP, dimetil phthalate dan dietil phthalate (DEP).Sampel diesktrak dengan berpandukan kaedah yang telah diperkenalkan oleh Suruhanjaya Keselamatan Produk Pengguna (CPSC). Sampel dilarutkan dengan pelarut yang dikenali sebagai tetrahidrofuran. Kajian ini mendapati bahawa tiga daripada tujuh sampel berjaya dikesan mengandungi kandungan BBP dan DEHP pada aras yang boleh diterima. Kepekatan BBP dan DEHP adalah dalam julat 0.15- 0.2 μg/mL. Spesis organik yang lain iaitu butilated hidroksitoluen juga dikesan dalam sampel. Kehadiran phthalate di dalam sampel dengan peratusan yang sangat rendah adalah disyaki disebabkan oleh proses pencemaran.

ABSTRACT

Three phthalates esters (PEs); benzyl butyl phthalate (BBP), dibutyl phthalate (DBP), and diethylhexyl phthalate (DEHP) had been regulated by European Union to a limit of less than 0.1% in all imported toys and articles. PEs were reported to cause adverse effects on human health and suspected to be an estrogen disruptor. There are new invention of toys have been introduced known as loom band. United Kingdom had banned the loom bands from the market due to high level of phthalate concentration been detected. However, there is no data available yet about the concentration of phthalates inside the loom bands that is marketed in Malaysia. In this study, seven (7) samples of loom bands were obtained from markets in Kepala Batas, Pulau Pinang. Gas chromatography– Mass Spectrometry (GC-MS) was used to analyse the PEs content in the sample. Five (5) types of phthalate esters of BBP, DBP, DEHP, dimethyl phthalate (DMP) and diethyl phthalate (DEP) were used as the reference standard. The samples were extracted in accordance to the method that was established by Consumer Product Safety Commission (CPSC). Loom bands were dissolved in tetrahydrofuran. Three out of seven samples was found to contain an acceptable level of BBP and DEHP at a percentage of 0.02 %. The concentration BBP and DEHP were in the ranged of 0.15- 0.2 µg/mL. An organic species known as butylated hyroxytoluene was detected in the sample. The presence of phthalate inside the samples with a low percentage was suspected due to the contamination process.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Phthalates or chemically known as Phthalates Esters (PEs) always being added to the plastics based products like building materials, medical devices and children's toys, to soften, provide flexibility and durability (USEPA,2007; Ozer and Gucer, 2012). In the Regulation of European Union (EU) No 1907/2006 (REACH) and USA CPSIA, six representative class of phthalates compound have been banned. Di(2-ethylhexyl)phthalate (DEHP), dibutyl phthalate (DBP), benzyl butyl phthalate (BBP), di-n-octyl phthalate (DNOP), di-iso-nonylphthalate (DINP) and diiso-decyl phthalate (DIDP) were permanently banned in any amount greater than 0.1 % in children's toys and certain child care articles which children may put in the mouth (Huang *et al*., 2011).

Phthalates have the potential to be released from their components as they are not chemically bound to the plastics Due to the concern of harmful effects of phthalates on human health several studies have been done. Reprotoxicity, carcinogenesis, cardiotoxicity, hepatotoxicity and nephrotoxicity are the examples of phthalates toxicity that may involve. Studies on animals have shown that phthalates may also have influenced the immune and allergic response. Although effects observed in rodents regarding immune toxicity may not be relevant to humans based on much lower human exposure and on the route of human exposure, one of the main potential toxic effects of some phthalates experimentally observed concerns the human endocrine system (Gimeno *et al*., 2014).

Loom band is actually a colourful rubber band that is weaved together forming a bracelet. Among children and adults, it has been a nationwide craze popular. Tests were carried out by Birmingham Assay Office (an independent laboratory in United Kingdom) and auspices of Swiss consumer group in Switzerland found that, the loom band contained about 20 to 50% of phthalates concentration (Mcteague, 2014). Before becoming a worldwide phenomenon, the loom band was first created in the United States, under the brand Rainbow Loom (Ng, 2013).

However, due to popularity, they are now come with many different brands. Rainbow Loom is a brand that has been tested to comply with any ASTM, CPSIA, EU or Japan's toy safety standards (Ng, 2013). Consumer Association of Penang (CAP) reported, most of the loom bands marketed in Malaysia are identical to the loom bands (the counterfiet one) being sold in United Kingdom which have been banned (Idris, 2014). Recently, many studies have been done in determining the possible effect of phthalate exposure to human. Heudorf *et al*., (2007) found that phthalates are easily migrated or evaporated into indoor air and atmosphere, foodstuff, due to its characteristic that is not chemically bound to PVC. Regarding to Ozer & Ucer (2012), children could be exposed to phthalates through ingestion, by dermal contact or both.

Schetller (2005) also stated that human exposure to phthalates can occur as a result of direct contact with sweat. Here, inspite of the long time usage of children and adult with the loom band as they wear as a bracelet, there is no data available yet about the concentration of phthalates inside the loom band sold in Malaysia.

1.2 Research objective

1) To identify the types of phthalates esters present in Loom Bands marketed in Kepala Batas, Pulau Pinang.

2) To quantitate the concentration of phthalates in selected Loom Bands marketed in Kepala Batas, Pulau Pinang.

CHAPTER 2

LITERATURE REVIEW

2.1 Phthalate esters (PEs)

Phthalates esters (PEs) are groups of diesters of phthalic acid (dialkyl or alkyl of 1, 2- benzenedicarboxylic acid). Oily, colourless, odourless liquids that do not evaporate readily are classified as their properties in general. Phthalates were used as plasticisers since 1920 and remain as the largest class of plasticizers in the 21st century (Rahman & Brazel (2004). They are often used in the manufacture of plastics, including polyvinyl chloride plastics (PVC) and found abundantly in products such as toys, vinyl flooring, herbal pill coating, and plastic shower curtains. Phthalates are believed to prolong the durability and increase flexibility of plastics. Statistically, several million tonnes of phthalates are used per year all over the world in the production of soft polyvinyl chloride (PVC) and other plastics (Wormuth *et al.*, 2006).

In addition, phthalate also been used in a certain cosmetic products, such as nail polish, perfumes, skin moisturisers and shampoos to increase penetration and maintain the scent and/or colour. The molecular weights influence the uses of many kinds of phthalates. Bis (2-ethylhexyl) phthalate (DEHP), (DiDP) and (DiNP) are high molecular weight phthalates that often used in construction materials, clothing and children's toys. Meanwhile, relatively low molecular weight phthalates, dibutyl phthalate (DBP), diethyl phthalate (DEP), dimethyl phthalate (DMP), tend to be used as solvents and in adhesives, waxes, inks, cosmetics, insecticides, and pharmaceuticals industry.

Di/bis-2-ethylhexyl phthalate (DEHP), diisononyl phthalate (DINP), and diisodecyl phthalate (DIDP) are the commonly used phthalates as it's made up of more than 75% of the total European phthalate consumption of more than 1 million tons in 2003. Dimethyl phthalate (DMP), diethyl phthalate (DEP), diisobutyl phthalate (DiBP), di-n-butyl phthalate (DnBP), and butylbenzyl phthalate (BBP) are the other examples of phthalates that were used in common.

The physiochemical characteristics of phthalates may depend on the chemical structures. The general chemical structure of phthalates (R and R' = $C_nH_{2n+1)}$ is shown in Figure 2.1.

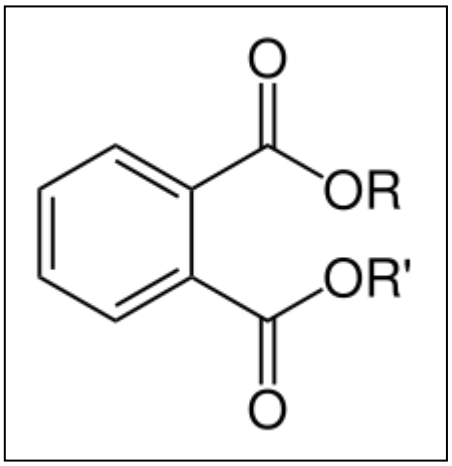

Figure 2.1. General chemical structure of phthalates. R and R' = C_nH_{2n+1}; n = 4-15

The widespread use of phthalates which around 50 years making it to become ubiquitous in the environment (Cancer & Environment 2007). Lipophilic characteristic of phthalates influences their leaching to the environment. PEs easily released and migrate from the products into the environment as they are not chemically bounded to polymers (Cancer & Environment 2007).

Rogers (2008) reported that, PEs is identified as endocrine disrupting chemicals (EDCs) and shown to cause reproductive and developmental toxicity in animals study and also associated with reproductive and abnormalities in humans and wildlife. With respect to their endocrine disrupting potential, phthalates such as BBP, DBP and DIBP have been found to elicit estrogenic responses *in vitro* assays. It is possible that phthalates are a contributory factor to endocrine-mediated adverse effects observed in wildlife and humans over the past few decades (Amiridou & Voutsa, 2011).

2.1.1 Subcategories of phthalate esters

The subcategories are divided based on their physicochemical and toxicological properties which consist of; low molecular weight phthalates, transitional phthalates, and high molecular weight phthalates.

Low molecular weight phthalates are produced from alcohols with straight-chain carbon backbones (less than C3). They are commonly used as solvents or in cellulose acetate polymers. Compared to transitional and higher molecular weight phthalates they have a higher aqueous solubility. Instead of having a high aquatic toxicity, they also have lower mammalian toxicity potential than do the transitional phthalates (Chemicals & Srs, 2010).

Transitional phthalates are produced from alcohols with straight- chain carbon backbones of C4- C6. They were used in common as a solvent and also plasticizers for PVC. These phthalates have greater mammalian toxicity potential, particularly with regard to reproductive and developmental effects, compared to either the low or high molecular weight phthalate categories (Chemicals & Srs, 2010).

High molecular weight phthalates are produced from alcohols with straight-chain carbon backbones of >C7 or a ring structure. These subcategories are the one that play major role of plasticizers to various plastic products. Most of the plasticizers are from these subcategories. However, compared to others, they are more harmful as have been reported with a few biological effects to human and environment (Chemicals & Srs, 2010).

Common commercial phthalate esters are liquids at ambient temperatures and usually having a boiling point ranging from 230°C to 486°C. However, for the higher molecular weight phthalate esters, the boiling points must be determined at reduced pressure. The high boiling point and low melting point will affect the usage of these phthalate esters as plasticizers (Stales *et al*. 1997).

2. 1. 2 Endocrine disruptor chemicals (EDCs)

Due to its potentiality to cause variety of adverse effects towards human endocrine system, phthalates have been described as an endocrine disrupting chemicals. The Environmental Protection Agency (EPA) defines endocrine disrupting chemicals as "External agents that will disturbing the hormone that is responsible for the maintenance, homeostasis, reproduction, development and behaviour by altering the synthesis, secretion, transport, binding, action or excretion of natural hormones in the body (Barlow *et al*., 2007). Phthalates were claimed to cause an adverse effect in young children and developing foetuses mainly to their endocrine and reproductive development. Antiandrogenic activity *in vitro, in vivo* and animal model as well as estrogenic actions strongly proved this possibility. According to USEPA (2011), phthalates can mimic or inhibit the natural hormone, hence change the hormone levels

besides affect in the hormone functions. Foetuses, infants and young children will responses quickly for any changes in the hormone concentration because they have a very low susceptibility to substances that may affect their growth and development. Congenital adrenal hyperplasia masculinise is one of the response usually shown by male foetus due to the increase of androgens (Sathyanarayana, 2008).

2. 1. 3 Metabolism of phthalate esters

Diester phthalates usually hydrolyzed into monoester phthalates in the intestine and parenchyma cells, where PEs are converted to their metabolites. Phthalates are quickly excreted from the body in the form of urine and faeces as its having a short half-life, between hours to several days (Hauser & Calafat, 2005). Metabolism process of phthalate esters would be depending on the structure of parent diester compounds.

The polar, short- branched low molecular weight compounds (e.g. DEP and DMP) are mainly excreted in form of hydrolytic monoesters. High molecular weight compounds (e.g. DEHP) usually undergo a few biotransformations process including enzymatic oxidation and hydroxylation of long alkyl chains into hydrophilic monoesters before being excreted in urine and faeces. Mono-(2-ethylhexyl) phthalate (MEHP) is the example of DEHP metabolite that has been shown to cause teratogenicity effect in mice and rats (USEPA, 2007). Initially, MEHP was the only metabolite measured in the exposure involving DEHP, but further research showed that mono-2-ethyl-5-oxohexyl phthalate (MEOHP) and mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP) were predominant metabolites, and recently other DEHP metabolites mono-2-ethyl-5-carboxypentyl phthalate (MECPP) and mono-2-carboxymethylhexyl phthalate also been discovered. Due to this, further research should be done.

In addition, the bioavailability of phthalate compounds depends on the route of exposure. Sathyanarayana (2008) reported that exposure through ingestion or dermal transfer resulted in large bioavailability of monoesters and other oxidative metabolites. In the Third National Report on Human Exposure to Environmental Chemicals, the Centers for Disease Control and Prevention's (CDC) reported that concentration of several phthalate metabolites have been detected in urine of majority Americans. One of the metabolites is MEHP (Meeker *et al*., 2009).

Table 2.1 shows the most commonly used of phthalates with their metabolites (Barlow *et al*., 2007).

Phthalate esters		Metabolites
Butyl benzyl phthalate	BBP	Mono benzyl phthalate (MBP)
Di-n- butyl phthalate	DBP	Mono-n- butyl phthalate (MBP)
Di- (2- ethylhexyl) phthalate	DEHP	Mono- (2-ethylhexyl) phthalate (MEHP)
Diethyl phthalate	DEP	Mono ethyl phthalate (MEP)
Dimethyl phthalate	DMP	-
Di- isobutyl phthalate	DiBP	-
Di- isodecyl phthalate	DiDP	-
Di-n- octyl phthalate	DNOP	-

2. 1. 4 Route and Source of exposure

Phthalates usually enter the environment via phthalate-containing products and during industrial production, use, and disposal. Exposure of people to phthalates always been considered through the ingestion route usually by eating or drinking contaminated food and water (Kamrin, 2009; Swan, 2008). However, other absorption pathways, like dermal, inhalation, may also play an important role (Bornehag & Nanberg, 2010).

2. 1. 5 Specific toxicities of phthalates

Findings in animal investigation shown that phthalates are toxic for several body systems. Long term exposure to low doses of these substances showed reproductive toxicity in rodents. Studies in the most widely used phthalates, DEHP found to cause carcinogenicity effect to the liver of rodents and mice (Cao, 2008). Meanwhile, the effects are depending on the amount of dose and duration of exposure. High incidences of death and malformations have been reported in 7 to 8 days exposure, but fewer effects in another day's exposure (Tomita *et al*., 1986). It is proved from several studies of *in vitro* and *in vivo* effects of phthalates, epidemiological studies that have been done and also from evidences shown by human in other clinical studies.

2. 1. 5. 1 Reproductive system

Antiandrogenic or estrogenic effects in humans are the most common phthalates reproductive toxicity was observed. Study done from Harvard School of Public Health proved the exposure to harm the health of human reproductive system. The decreased of sperm production on male reproductive health have been reported in recent animal studies. According to Duty *et al* (2003), men who had detected with the presence of phthalate metabolites (e.g. monobutyl phthalate or monobenzyl phthalate) in their urine tended to have a low number of sperms. The higher the concentrations the lower the sperm numbers.

The concentration of phthalate metabolites also have been detected upon diagnosis in the urine samples of pregnant women that having a male babies with a short anogenital distance (AGD). Prenatal urinary levels of phthalate metabolites were inversely related to AGD. *In-vivo* toxicity study done by British in recent showed that DBP phthalate and its metabolite (MBP) tend to suppress the steroidogenesis by fetal-type Leydig cells in primates and rodents (Hallmark *et al*, 2007). Meanwhile, DEHP exposures in adult rats result in hypoestrogenic anovulatory cycles and polycystic ovaries (Lovekamp-Swan & Davis, 2003).

2.1. 5. 2 Phthalates and carcinogenesis

Phthalates may be responsible for cancer in humans. Hepatocellular adenoma or carcinoma in mice and rats of both sexes has been observed in the ingestion cases of DEHP. Several studies suggested that daily intake of DEHP causes pancreatic and liver tumours in rats (Ito *et al*., 2007, Selenskas *et al.,* 1995). Phthalates also were believed to increase the risk of pancreatic cancer as observed in a case control study conducted among workers in a plastic industry in United States (Selenskas *et al*., 1995). The carcinogenicity of phthalates was reported for the first time in the nineties. Positive correlations between the concentrations of DEHP in urine and risk of developing breast cancer have been reported in a recent epidemiological survey in Mexico (Lopez-Carrillo *et al*., 2010).

2.1. 5. 3 Asthma

Asthma is described as a disease that involving chronic inflammatory of the airways (Pelaia *et al.,* 2008, 2009). Main pathological feature of asthma is airway remodelling which are closely related with the severity. Besides airways inflammation, hyper reactivity, mucus hyper secretion and reversible airway obstruction were also included as the main clinical features of this disease (Murphy and O'Byrne, 2010). In fact, certain phthalates plasticizers have been related to pathogenesis of asthma since the recent years. Jaakkola & Knight (2008) reported that the existence relationship between exposure to phthalates and asthma and contribution to airway remodelling had been proved by many studies, in addition of epidemiological studies. The release of phthalates from PVC materials also increases the risk of allergies as shown by many toxicological studies (Jaakkola & Knight, 2008).

An epidemiology study done by Swedish found that children who exposed to certain phthalates esters like BBP, DEHP and DBP via house dust developed the symptoms of asthma and allergy. From the cohort, of 10,852 children, 198 cases were selected with persistent allergic symptoms and 202 controls without allergic symptoms (Bornehag *et al* 2005).

The same result whereby the exposure to phthalate from PVC products involved in the development of asthma and allergies were also shown by other study from Birmingham. Exposure to high level of phthalates from PVC products can cause murine immune response to be modulated to a co-allergen. Heated PVC fumes possibly contribute to development of asthma in adults (Jaakkola & Knight, 2008). However, Kimber & Deaman (2010) reported, the whole involvement of phthalates in asthma is still far from being fully understood.

2.1. 5. 4 Autism

Autism or also known as autism spectrum disorders (ASDs) is a group of complex neurodevelopmental disorders. In clinical, this disorder is still poorly understood, constantly increasing and refractory to drug treatment Weintraub (2011). Weintraub (2011) mentioned that an increasing frequency of ASDs has been reported last 20 years ago. Although the aetiology is still unknown, but it is believed to result from disturbance of normal neurobiological mechanisms primarily in the prenatal period (Nelson, 1991). The solely "genetic hypothesis" cannot become the evidence for the cause of ASDs as the prevalence of this disorder has exponentially grown in the last two decades, although strong genetic components are widely recognised in ASDs's patient is widely recognised (Anney *et al*., 2011).

Testa *et al* (2012) suggested that there is an involvement of environmental factors that contribute to the ASDs. Phthalates have the potential to interfere with neurological development as it is one of the ubiquitous environmental contaminants. Thus, become potential risk factors for the pathogenesis of ASDs. The relationship between phthalates and ASDs had been studied among 48 children with ASDs (male: 36, female: 12, mean age: 11 years). This study was conducted to evaluate concentrations of primary and secondary metabolites of DEHP in the 48 children. A diagnostic method, based on the determination of urinary concentrations of metabolites of DEHP (MEHP, 6-OH-MEHP, 5-OH-MEHPe 5-oxo-MEHP) by HPLC–ESI-MS was applied to urine samples. From this study, as compared to control group, significant increases of 5-OH-MEHP and 5-oxo-MEHP metabolites were detected in patients with ASD (Testa *et al*., 2012).

These findings primarily demonstrated the relationship of phthalate exposure with ASDs, suggesting a key role for these environmental contaminants in the pathogenesis of autism probably due to a potential neurotoxic action of these substances. Recent research has shown that exposure to DEHP begins during foetal life; in fact DEHP is present in breast milk. The presence of phthalates has been well documented even in baby food. DEHP is rapidly absorbed and metabolised to MEHP in gastrointestinal tract of children (after oral ingestion) compared to adults before it is excreted after conjugation with glucuronic acid. In this subject the capacity of glucuronide conjugation is reduced (Sjoberg *et al*., 1991). This concluded that children will have a high potential risk of adverse effects of phthalates exposure compared to the other groups of the population.

2.2 Phthalates in toys

Because of their potential health effects especially on reproductive development, phthalates have been under concerned. It is reported that, from the total weight of toys phthalates make up of 10%–40%. Di(2-ethylhexyl)phthalate (DEHP), dibutyl phthalate (DBP), diisononylphthalate (DINP), diisodecyl phthalate (DIDP), benzyl butyl phthalate (BBP) and dinoctyl phthalate (DNOP) are the most commonly used phthalates (Sathyanarayana, 2008).

The use of DBP, DEHP, BBP from children's toys and childcare articles, and DINP, DIDP and DNOP from items that children are likely to put in their mouths had been banned by The European Union (EU). Restriction on six phthalate esters in toys and childcare products meant to be placed in the mouths of children under the age of three with a maximum concentration (total phthalate content) not exceeding 0.1% (w/w) has been implemented by the European Union in December 1999. The phthalate esters of interest includes dibutyl phthalate (DBP), benzyl butyl phthalate (BBP), bis(2-ethylhexyl)phthalate (DEHP), dinoctyl phthalate (DNOP), diisononyl phthalate (DINP) and diisodecyl phthalate (DIDP).

Chinese regulation GB/T 22048-2008 was issued in June 2008 and came into effect in May 2009 to improve the supervision of these harmful compounds as China is one of the major exporters of toys and children's products in the world. In August 2008, US Congress enacted the Consumer Product Safety Improvement Act (CPSIA 2008) had been enacted by US congress which established a 0.1% (w/w) limit for the six phthalate esters (Yun Zou, 2013).

2.2.1 Regulation of phthalates in toys

The banned of phthalates had been occurred in several countries such as European nations, Argentina, Fiji and Mexico followed by Austria, Japan, Denmark, Finland, France, Germany, Greece, Norway, and Sweden (banned the use of phthalates in manufacturing soft vinyl toys) (Saikia *et al*. 2010).

2.2.1.1 European Union

Since 1999 the European Union has prohibited the use of phthalates in children's toys. An adopted measure prohibit the sale of toys and childcare articles intended to be placed in the mouth by children under three years of age made of soft PVC containing one or more of the substances - DINP, DEHP, DBP, DIDP, DNOP, and BBP have been introduced by The Council Directive (1999/815/EC). Importing these products is also prohibited. An amendment on the approximation of the laws, regulations and administrative provisions of the Member States relating to restrictions on the marketing and use of certain dangerous substances during preparations of the toys, has been sent by Directive 2005/84/EC to Council Directive 76/769/EEC. This amendment resulted in the extends ban on 6 phthalates (DINP, DEHP, DBP, DIDP DNOP, BBP) for toys manufactures involving children under age of three. 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 of Europian Union strictly stated that the amount of phthalates may not be greater than 0.1% mass percent of the plasticized part of the toy. Any toys as stated before, having concentrations of phthalates greater than the limit mentioned above could not be placed on the market (Saikia *et al.* 2010).

2.2.1.2 Denmark

In Denmark, the ban of phthalates is according to Statutory Order no. 786. On 11 July 2006, their Ministry of Environment also mentioned that the phthalates DEHP, DBP, BBP DINP, DIDP and DNOP shall be banned from use as substances or as constituents in chemical products in an amount exceeding 0.1% by mass of plasticised material in toys and childcare articles. Sale and import of these toys and childcare articles shall also be banned. Other phthalates than the six as mentioned, also need to be banned from use as substances or as constituents in chemical products or components hereof in concentrations exceeding 0.05% by mass, if are found in toys for children (0-3 years old) which are they tend to intend, or normally can be expected, to be placed in the mouth, including in particular dummies, bibs, jewellery as well as bathing articles etc. shall be banned (Saikia *et al*. 2010)

2.2.1.3 United States of America (USA)

In the USA, DEHP has been listed in Proposition 65 (edition September 2003) as being potentially carcinogenic and reproductive hazards by the California Environmental Protection Agency (Cal/EPA). Starting in 2009, three types of phthalates have permanently restricted by the federal government of the California on February 10, 2009 in children's toys sold in California (Saikia *et al*. 2010).

Children's products that contain certain concentrations of phthalates also have been banned by the Consumer Product Safety Improvement Act (CPSIA) in the section 108. This interim ban was applicable on February 9, 2009. Childcare products which declared as products for children under age of three) and children's toys (toys produced for children under age twelve) are considered as hazardous materials that need to be banned if found to contain concentrations of DEHP, BBP, and DBP exceed 0.1%. In the

USA, DINP, DNOP, and DIDP was placed on a provisional ban unless there is a future study of safety if the amount of phthalates are exceeded the allowable limits (Saikia *et a*l. 2010).

2.2.1.4 China

Since early in the year 2007, China's toy industry is regulated by China Compulsory Certification (CCC) from the nation's Certification and Accreditation Administration (CNCA). Every toy in China will be subjected to inspection and certification review. Chinese toys must meet the criteria set by International standard ISO8124.1:2002 which itself is modelled on the EU's standard for toys (EN71 Safety of Toys - Safety aspects related to mechanical and physical properties) (Saikia *et al.* 2010).

2.2.2 Regulation of phthalates children's toys in Malaysia

So far, there is no any specific restriction of phthalates in Malaysia yet as done by some other countries all over the world. Developed countries like United States of America and Canada banned the use of DEHP, BBP, and DBP, DINP, DIDP and DNOP in an amount greater than 0.1 % mass percent by weight of the toys. The banned phthalates as mentioned must be withdrawn from the toy's content before marketed. Malaysian Government Ministry of Domestic Trade, Cooperatives and Consumerism (MDTCC) are the government body that responsible to control the safety of toys and prevent any possible risks of adverse effect to the consumers in Malaysia. Consumer Protection (Safety Standard for toys) Regulations 2009 and Consumer Protection (Certificate of Approval and Conformity Mark of Safety Standards) Regulations 2009 are the two of consumer protection regulations published by MDTCC, applicable in 2010. Under these regulations, all toys (any goods designed or intended for use in play by children below 14 years old) and the counterpart of the non- toy items (fashion jewellery for children, slingshots) must be tested to comply with the Malaysian toy safety standards (shown in Table 2.1), supplied with Certificate of Approval, labelled with Conformity Mark (Figure 2.1) with approval number (Pentadbiran &Persekutuan, 2014).

Figure 2.2: Conformity mark that need to be labelled for each toy as an approval

Table 2.2 : List of Malaysian Toy Safety Standards

No	Standard	Specification
1	MS 1SO 8124-1: 2001	Safety of toys- Part 1: Safety aspects related to mechanical and physical properties
2	MS ISO 8124-2: 1999	Safety of toys – Part 2: Flammability
3	MS ISO 8124-3:2002	Safety of toys – Part 3: Migration of certain elements
4	MS 1774-Part 4:1998	Safety of toys – Part 4: Experimental sets for chemistry
5	MS 1774-Part 5:1998	Safety of toys- Part 5: Chemical toys (sets) other than experimental sets
6	MS 1774-Part 6:1998	Safety of toys – Part 6: Graphical symbol for age warning labelling
7	MS 1725:2004	Safety of electric toys

In details, these regulations required the product owner to test their product first, from an authorised laboratory for the particular toys and confirmed to the prescribed safety standard for toys before being marketed. Any suppliers or advertisers of the toys also need to make sure that the toys comply with prescribed safety standards for toys. Only products that have a test report are allowed to be sold in Malaysia. It is applicable to all toys, either imported, new or existing products (BVCPS, 2010).

2.2.3 Issues of loom bands

Loom bands are actually elastic bands that are woven together become a bracelet. Rainbow Loom is the first loom band brand have been created, in the United States of America. Its popularity had increased very quickly and become a worldwide phenomenon. According to Martin (2013), due to their popularity, there are now many different brands can be found in the market. Loom bands are classified as a toy in most of the countries. Each country having their own regulation on toys and having a specific restriction of phthalate, whereby many countries had allowed the toys to contain only 0.1 % of phthalates, prohibited any exceed. Birmingham Assay Office (an independent laboratory in United Kingdom) had tested and reported that many brands of loom bands in the market were found to contain a higher content of phthalates, from up to 40 % - 50 % phthalates. United Kingdom is one of the countries that already banned loom bands from being marketed due to high levels of phthalates detected inside. The cheaper, counterfeit loom band products were noted in common to have a high level of phthalates (Mcteague, 2014).

2.2.3.1 Original licensed product (Rainbow Loom Band)

Rainbow Loom Band was created in 2011 by a man with a degree holder in Mechanical Engineering from Wichita State University, Cheong Choon Ng. He is a Malaysian Immigrant of Chinese descent who came to the United States in 1991 to pursue the study before settling down there, in Novi, Michigan after finishing the study (Martin, 2014). Rainbow Loom Band was claimed as the original licensed products among loom bands products. It has been extensively tested to comply with any ASTM, CPSIA, EU or Japan's toy safety standards and is certified as being phthalate- free (Them, 2013). Them (2013) reported counterfeit products are quite similar to the original product.

2.2.3.2 Issues in Malaysia

Nevertheless, while the United Kingdom has banned loom bands because of the high level of phthalates in copycat loom bands. There is no safety hazard risk involving loom bands. In Malaysia, it is unnecessary to ban loom bands as it was not claimed as a toy instead of accessories or costume jewellery. Therefore, the regulation would not be under the list of Malaysian Toy Safety Standards. Consumer Association of Penang (CAP) reported, most of the loom bands marketed in Malaysia are identical to the loom bands (the counterfiet one) being sold in United Kingdom which have been banned (Idris, 2014). Thus, the concerns were only on the child's hat are possible to chew and suck the loom bands. However, the migration of phthalates from the loom bands through the dermal contact of the adults that wearing it as a bracelet also needs to be concerned.

CHAPTER 3

MATERIALS AND METHODS

3.1 Reagents and materials

Phthalate esters standards, dimethyl phthalate (DMP), dibutyl phthalate (DBP), bis(2 –ethylhexyl) phthalate (DEHP), benzyl butyl phthalate (BBP), diethyl phthalate (DEP) were purchased from Sigma- Aldrich (Laborchemikalien GmbH, Seelze, Germany). Benzyl benzoate (BB) used as internal standard was purchased from Fisher Chemical (Bishop Meadow Road, Loughborough,United Kingdom). The tetrahydrofuran (THF), cyclohexane, hexane, urea, lactic acid were also purchased from Fisher Chemical (Bishop Meadow Road, Loughborough,United Kingdom).

3.2 Instrumentations

For the phthalates analysis, the Agilent 7890A gas chromatograph coupled to inert XL EI/CI mass-selective detector (Agilent technologies, Stevens Creek Blvd, Santa Clara, USA) was used. Compounds were separated on a HP- 5MS column 5% Phenyl methyl Siloxane (30 m; diameter 0.25 mm; film thickness 0.25 μm) from Agilent Technologies (Stevens Creek Blvd, Santa Clara, USA). Rotary shaker (Zhejiang,China) was used to agitate the sample extract in order to allow the sample to dissolve properly in the solvent.

3.3 Sample collection

Samples of loom bands with different brands were purchased randomly from various markets and groceries in Kepala Batas, Pulau Pinang. The focus on the sampling is towards the counterfeit products. There were 7 samples collected in related with the different types of brands and each sample was labelled as A, B, C, D, E, F and G.

3.4 Sample preparation and extraction

The samples were cut into pieces of about 2mm length. This is to ensure the extraction efficiency. The samples were extracted in accordance to test method CPSC-CH-C1001-09.3, prescribed by the CPSC (Anon,2010). Approximately 0.5 g of the samples were taken and added with 5 mL of tetrahydrofuran (THF). To ensure a complete dissolution with the extractant solvent the mixture was agitated about 30 minutes using rotary shaker. Agitation above 2 hours longer was necessary for incomplete dissolved samples.

For a complete dissolved samples, 10 mL of hexane were added and incubate quietly for 5 minutes to allow the samples becomes precipitate. Using polytetrafluoroethylene (PTFE) filter (0.45 μm), the samples were then been filtered. For sample that was not completely dissolved, it must be filtered first, before adding 10 mL of hexane and followed the next step as complete dissolved samples. 0.2 mL of internal standard (benzylbenzoate) were spiked into 0.3 mL of tetrahydrofuran- hexane mixture and topped up with cyclohexane until reach a volume of 1.5 mL.