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DI-ETHYLHEXYL PHTHALATE (DEHP) CONCENTRATIONS IN CHILDREN'S TOYS MARKETED IN MALAYSIA AND ITS POTENTIAL HEALTH RISKS

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BY

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ABSTRACT

DI-ETHYLHEXYL PHTHALATE CONCENTRATIONS IN CHILDREN'S TOYS MARKETED IN MALAYSIA AND ITS POTENTIAL HEALTH RISKS

AMIRA FARHANA AMARUDDIN

Introduction: These days, phthalates are used extensively as plastic additive in manufacturing plastic children's toys. In Malaysia, many small-scale stores sell various types of children's toys that are inexpensive and affordable where the safety of the toys remain uncertain. Furthermore, there is no specific regulation administered in Malaysia to monitor the permissible concentration of phthalates in children's toys as done by other countries. DEHP is the most concern phthalate compound that commonly used in plastic products including children's toys due to adverse effects observed. **Objectives:** The purpose of this study is to determine the concentrations of DEHP, to assess associations between DEHP concentrations with the toys' properties and measure its potential health risks. **Methodology:** Thirty children's toys obtained from the night markets were analyzed with DEHP concentrations using high-performance liquid chromatography (HPLC). The surface of the samples were scraped off and weighed for 0.5 g. The samples were then extracted by shaking with methanol for 1 hour, sonicated using an ultrasonic for 30 minutes, filtered and transferred to a schott bottle for analysis. The potential health risk associated with DEHP exposure by ingestion route based on child's behavior of mouthing was calculated and IBM SPSS Statistics was used to analyze data. **Results and Discussion:** The results of the study showed that the DEHP concentration in toys was in the range of 0.3 mg/g – 2556.76 mg/g. DEHP concentrations were significantly associated with the country where the toys were made. HQ results of more than 1 were observed only in toy sample 03, 05 and 11, indicated that there were potential health risks due to DEHP exposure in children's toys **Conclusion:** In conclusion, DEHP concentrations in children's toys should be under scrutiny as it established a potential health risk in some toys containing DEHP. A more representative study accounted for larger samples and a variety of phthalate compounds to be analyzed are required in future studies.

Keywords: Di-ethylhexyl phthalate, children's toys, potential health risks

ABSTRAK

KONSENTRASI 'DI-ETHYLHEXYL PHTHALATE' DALAM MAINAN KANAK-KANAK YANG DIPASARKAN DI MALAYSIA DAN POTENSI RISIKO KESIHATAN

AMIRA FARHANA AMARUDDIN

Pengenalan: 'Phthalates' kini digunakan secara meluas sebagai aditif dalam plastik dalam pembuatan mainan kanak-kanak. Di Malaysia, banyak kedai berskala kecil yang menjual pelbagai jenis mainan kanak-kanak yang murah dan berpatutan di mana mainan tersebut tidak menentu keselamatannya. Selain itu, tiada undang-undang tertentu yang ditadbir di Malaysia bagi memantau jumlah 'phthalates' yang dibenarkan dalam mainan kanak-kanak seperti yang dilakukan di negara-negara lain. DEHP adalah kompaun 'phthalate' yang paling kerap digunakan dalam produk plastic termasuk dalam mainan kanak-kanak. **Objektif:** Tujuan kajian ini adalah untuk menentukan jumlah DEHP dalam mainan kanak-kanak, menilai hubungan antara jumlah DEHP dengan ciri-ciri mainan dan mengukur potensi risiko kesihatan. **Metodologi:** Tiga puluh mainan kanak-kanak yang diperoleh dari pasar malam telah dianalisis bagi menentukan jumlah DEHP menggunakan 'high-performance liquid chromatography' (HPLC). Permukaan sampel dikikis dan ditimbang sebanyak 0.5 g. Sampel kemudian diekstrak dengan mengguncang bersama metanol selama 1 jam, sampel diletakkan dalam ultrasonik selama 30 minit, ditapis dan seterusnya dipindahkan ke botol untuk dianalisis. Risiko kesihatan yang berpotensi yang berkaitan dengan pendedahan DEHP dalam mainan kanak-kanak kanak telah dikira dan Statistik SPSS IBM digunakan untuk menganalisis data. **Keputusan dan Perbincangan:** Hasil kajian menunjukkan bahawa jumlah DEHP dalam mainan adalah dalam lingkungan 0.3 mg/g - 2556.76 mg/g. Jumlah DEHP berhubungkait dengan negara di mana mainan dibuat. Keputusan HQ lebih daripada 1 dalam sampel mainan 03, 05 dan 11 menunjukkan bahawa terdapat potensi risiko kesihatan akibat pendedahan DEHP dalam mainan kanak-kanak. **Kesimpulan:** Kesimpulannya, jumlah DEHP dalam mainan kanak-kanak haruslah di bawah pengawasan kerana ia mungkin menyebabkan potensi risiko kesihatan dalam sesetengah mainan yang dikaji mengandungi DEHP. Kajian yang lebih luas menyumbang kepada sampel mainan yang lebih besar dan pelbagai jenis 'phthalate' disarankan dalam kajian pada masa depan.

Kata kunci: Di-ethylhexyl phthalate, mainan kanak-kanak, potensi risiko kesihatan

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LIST OF ABBREVATIONS

PE	Phthalate ester
DEHP	Di-ethylhexyl phthalate
DINP	Diisononyl phthalate
DIDP	Diisodecyl phthalate
DMP	Dimethyl phthalate
DIBP	Dibutyl phthalate
DEP	Diethyl phthalate
BBP	Butylbenzyl phthalate
DIBP	Diisobutyl phthalate
MEP	Mono-ethyl phthalate
MEHP	Mono-ethyl-hexyl phthalate
MEHHP	Mono-ethyl-hexyl-hydroxy phthalate
MEOHP	Mono-ethyl-oxo-hydroxy phthalate
MMP	Mono-methyl phthalate
MBzP	Mono-benzyl phthalate
MWP	Molecular weight phthalate
EDC	Endocrine disrupting chemical
EU	European Union
MDTCC	Ministry of Domestic Trade, Cooperatives and Consumerism
ISO	International Standards Organization
US CPSC	United States Consumer Product Safety Commission
HPLC	High performance liquid chromatography
ADHD	Attention deficit hyperactive disorder

ADS	ADHD diagnostic system
ASD	Autism spectrum disorder
NHANES	National Health and Nutrition Examination Survey
BMI	Body mass index
CDGP	Constitutional Delay of Growth and
CASA	Computer-aided sperm analysis
AGD	Anogenital distance
RT	Retention time
US EPA	United State Environmental Protection Agency
ADD	Average daily dose
HRA	Health Risk Assessment
HQ	Hazard Quotient
C	Concentration
IR	Ingestion rate
EF	Exposure frequency
ED	Exposure duration
BW	Body weight
AT	Average time
CF	Conversion factor
RfD	Reference dose
IRIS	Intergrated Risk Information System
R^2	Regression coefficient
S/N	Signal to-noise-ratio

CHAPTER 1

INTRODUCTION

1.1 Background

Phthalate esters (PEs) or commonly known as phthalates are man-made chemicals that are usually used as plasticizers or softeners in most consumer products to increase the products' flexibility, stability and durability. High production volume of phthalates is driven by its high demand usage in manufacturing plastic-based products (Park et al., 2015; Sedtasiriphokin et al., 2017). Phthalates are often characterised into high and low, which is based on their molecular weight. Molecular weight of the phthalates usually determines which phthalate compound is added to a product. High-molecular weight phthalates comprise of di-2-ethylhexyl phthalate (DEHP), diisononyl phthalate (DINP) and diisodecyl phthalate (DIDP). On the other hand, low-molecular weight phthalates consist of dimethyl phthalate (DMP), dibutyl phthalate (DBP), diethyl phthalate (DEP), butylbenzyl phthalate (BBP) and diisobutyl phthalate (DIBP) (Wormuth et al., 2006; Meeker et al., 2009). DEHP is the most

important plasticizer in manufacturing polyvinyl chloride (PVC), which, in turn, used in most consumer products due to its low cost (Afshari et al., 2004).

Lipophilic properties of phthalates cause them to easily detach throughout their production, use and disposal as they are not permanently bound to the products. Therefore, phthalates are continuously dispersed into the environment and reaching humans via inhalation, ingestion and dermal contact (Afshari et al., 2004; Clark et al., 2003). The omnipresent of phthalates have alerted the concerns of public regarding multiple harmful effects of phthalates towards human health. Phthalates have also been classified as an endocrine disrupting chemical due to their capability to cause numerous toxicities towards human health. Several studies done have reported causing toxicities on neurological, respiratory, liver, reproductive, kidneys as well as cancer due to phthalates exposure (Hauser & Calafat, 2005; Meeker et al., 2009; Braun et al., 2014).

Children are more likely to be exposed to phthalates as apart from continuously inhaling phthalates present in the environment, they are also closely in contact with phthalate-containing child products as well as orally exposed to it. Children love to spend most of their times playing with anything that can spark their interest such as toys. Children's toys are one of the phthalate-containing products that are considered vital during the developmental stages of children. Apart from other modes of education, children's toys play an important role in contributing to their development of language, imagination and social skills (Korfali et al., 2013).

Seemingly, in Malaysia, many small-scale stores sell various types of children's toys that are reasonably priced, in which, most of the toys are made from the Asian countries especially China. It is claimed that China produced 80% of the world's toys. However, the safety of the toys remain questionable due to several reports on excessive toxic chemical contents in toys such as lead, claims on unsafe working conditions as well as corruptions of the standards body due to bribery (Asia News, 2010; Moore & Hall, 2011).

Apparently, children are highly vulnerable to toxic chemical exposure as compared to adults. According to Becker et al. (2010), toxic exposures in children are of particular concern because of their high metabolism, larger surface area to weight ratio, organ systems that are not fully developed, as well as rapid growth and development of organs and tissues. Hence, continuous exposure to any toxic chemical such as phthalates will eventually affect their health as their body tend to be more defenceless against any substances that may influence their growth and development. Toys in which children may come into close contact with should be given extra attention as they also pose a higher risk of coincidental ingestion. Obesity development, incidence of allergies and asthma, development of neurodevelopmental disorders and delay of pubertal development were reported from previous studies, concerning phthalates and children (Desvergne et al., 2009; Bornehag et al., 2004; Braun et al., 2013). While many countries have implemented prohibition associated with phthalate content in toys, there is still no particular regulation administered in Malaysia.

1.2 Problem Statement

As stated by Wormuth et al. (2006), several million tonnes of phthalates are used annually all over the world in PVC production as well as in other plastic-based products. Unlike other countries, Malaysia has no particular restriction concerning the allowable concentration of phthalates in consumer products as well as in children's toys. It is claimed that 10% to 40% of the total weight of children's toys are made up of phthalates (Stringer et al., 2000). There have been also limited previous studies were done in Malaysia that assessed the presence of phthalates presented in children's toys. It appears that there is still inadequate data associating with the concentration of phthalates in children's toys especially those sold at reasonably priced.

Furthermore, several developed countries have regulated the banning of phthalates in children's toys to encounter the issue as well as to secure the safety of the children. For an instance, European Union (EU) has prohibited the use of six phthalates for amount exceeding 0.1% by mass percent of toys in which DEHP, BBP and DBP were banned for all toys; DINP, DIDP and DNOP were limited only for children's toys that tend to be taken into the mouth. Meanwhile, in Malaysia, all toys must be tested to comply with the Malaysian safety standards and approved by the Ministry of Domestic Trade, Cooperatives and Consumerism (MDTCC) before being sold. Just recently, Malaysia has adopted ISO 8124-6:2018 standard which acquires all toys to be tested for certain phthalate esters in toys and children's products starting

January 2018. Even so, there is no specific regulation has been implemented yet in Malaysia concerning the permissible level of phthalates as done by other countries.

Lastly, Malaysian Standard Users (2015) has reported that majority of children's toys that needed a weekly product recall throughout 2014 were due to toxic chemical content especially phthalates, based on observations made from EU Rapex website and the United States Consumer Product Safety Commission (US CPSC). This presumes that children are highly exposed to phthalates as they tend to spend most of their times playing with toys. As children are more vulnerable to be adversely affected, several studies have reported some health effects among children associated with exposure to phthalates (Hauser & Calafat, 2005; Meeker et al., 2009; Braun et al., 2013). Even so, there remains a paucity of studies investigating if there is potential health risk associated with phthalates exposure in children's toys, especially DEHP as the most common used phthalate in consumer products including child products.

1.3 Research Justification

Majority of parents usually opt for affordable and inexpensive toys to buy for their children without considering the safety of the toys' content. The presence of toxic chemical in children's toys such as phthalates should be under scrutiny as it may be risky for their health when they are constantly exposed to it. Since there have been fewer studies conducted in Malaysia assessing the presence of phthalates in children's

toys, this study reports the concentration of the main phthalate used in all plastic products which is DEHP, detected in the children's toys marketed in Malaysia.

Next, this study assesses the associations between DEHP concentrations observed in the children's toys with the children's toys properties. Toys' properties consist of the country where the toys were made, the age suited for children to play with the toys and the type of toys.

Lastly, this study also justifies if there is a potential health risk associated with exposure to DEHP in children's toys concerning the route of exposure by ingestion. This study could be the baseline for other future studies that will investigate more on the health effects related to exposure of phthalates in children's toys.

1.4 Conceptual Framework

Figure 1.1 illustrates the conceptual framework of this study. This study focuses on two variables; the independent variable which is the presence of DEHP in children's toys and the dependent variable which is the potential health risks in children associated with DEHP exposure to phthalates in children's toys.

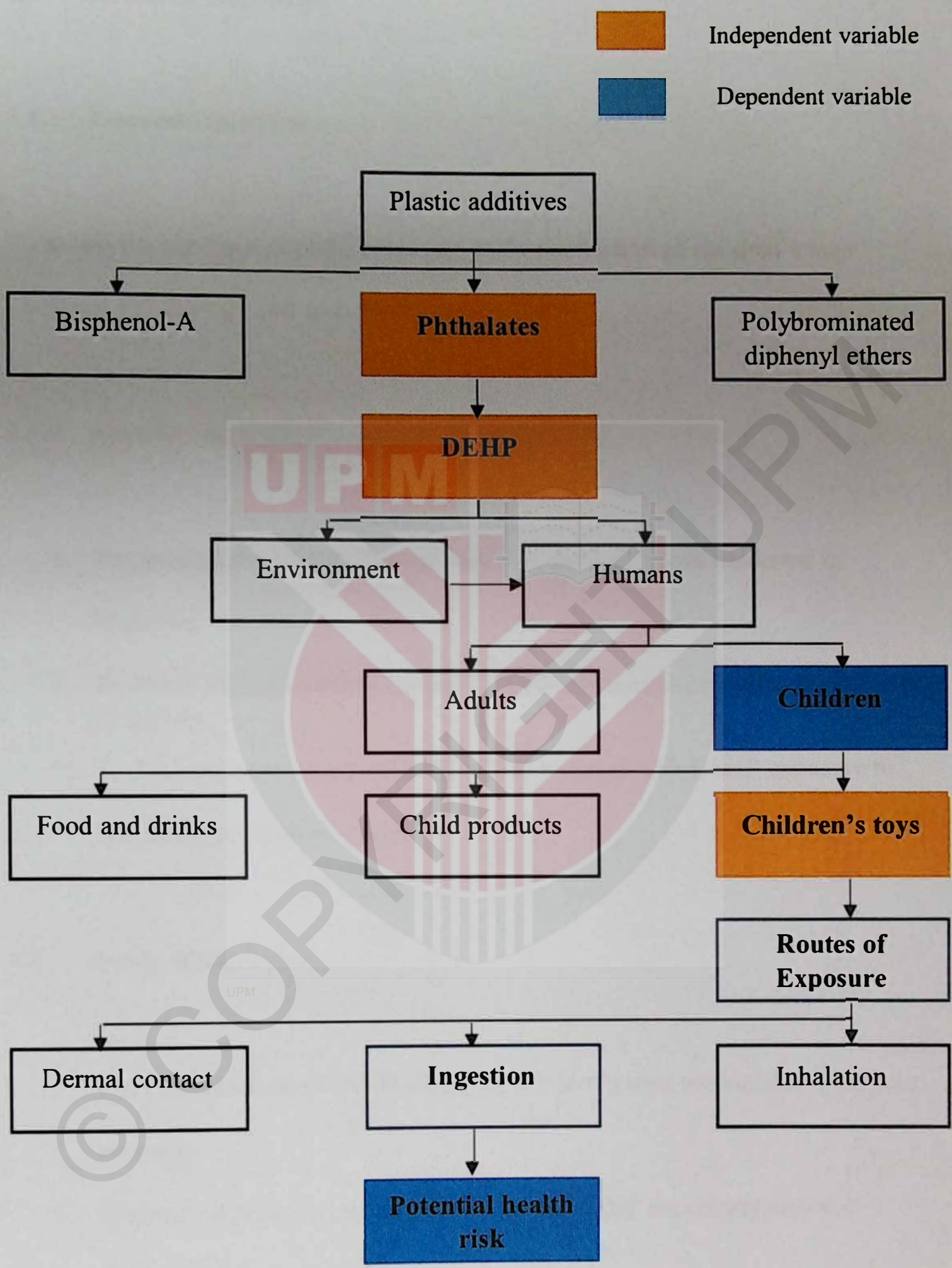

Figure 1.1: Conceptual Framework

1.5 Research Objectives

1.5.1 General Objective

To assess the presence of phthalates used in the production of children's toys marketed in Malaysia and its potential health risks.

1.5.2 Specific Objectives

1. To quantify the DEHP concentrations in children's toys marketed in Malaysia.
2. To assess the associations between DEHP concentrations with children's toys properties.
3. To determine the potential health risk associated with DEHP exposure to phthalates in children's toys.

1.6 Study Hypotheses

1. The concentration of DEHP found in children's toys marketed in Malaysia are high.
2. There are significant associations between DEHP concentrations and children's toys properties.
3. There is a potential health risk associated with DEHP exposure to phthalates in children's toys.

1.7 Definition of Terms

1.7.1 Conceptual Definition

1.7.1.1 Phthalates

Phthalates are esters of phthalic acid, primarily used as plasticizers or softeners in most consumer products, specifically plastic products, to enhance the products' flexibility, elasticity and durability (Sedtasiriphokin et al., 2017; Kim et al., 2009; Park et al., 2015).

1.7.1.2 Children's toys

Any items used by children to play with are described as toys, despite their educational character, and as such, are not linked to games or activities designed for adults (Klemenovic, 2014). Children's toys are important in the developmental process of the children as aside from giving them fun, the toys also serve as means of education (Korfali et al., 2013).

1.7.2 Operational Definition

1.7.2.1 Phthalates

Phthalates in children's toys are extracted using methanol before analysed using a high-performance liquid chromatography (HPLC). HPLC is one of the commonly used technique to determine the phthalate compound (Jing et al., 2016).

1.7.2.2 Children's toys

Children's toys need to undergo several methods to extract the phthalates before analysing it. The children's toys were scraped off or grated into pieces and weighed for 0.5 g before shaken with a known volume of methanol. The samples were then sonicated for 30 minutes before filtering and transferring the samples into Schott bottles.

1.7.2.3 Health risk assessment

An assessment involving Hazard Quotient (HQ) by calculating average daily dose (ADD) of DEHP and acquiring reference dose (RfD) of DEHP. The health risk assessment calculation was adapted from Praveena et al. (2015). HQ with more than 1 indicate an appreciable risk to human health.

CHAPTER 2

LITERATURE REVIEW

2.1 Sources of phthalates

Plastic is a widely utilized material in manufacturing a wide range of consumer products. Throughout the industrial production of plastic and its products, many additives are used in order to alter the physical properties of the polymer. This includes phthalates (Korfali et al., 2013). Phthalates are assumed to make the products more stable, flexible and durable. Nowadays, phthalates can be found nearly everywhere due to its widespread use in almost every product we used. According to Wormuth et al. (2006), a few million tonnes of phthalates are used every year across the world in manufacturing PVC and other plastic products.

Apparently, the molecular weights of phthalates determine their uses in the products. Low molecular weight phthalates which derived from alcohols of 1 to 4 carbon atoms in their chemical backbone are commonly used as solvents, adhesives as well as used in personal care products, cosmetic products and pharmaceutical

products (Meeker et al., 2009). Apart from that, several low molecular weight phthalates have also been detected in children's toys. Low molecular weight phthalates such as DBP, DIBP and BBP were among the most detected phthalates in children's toys reported in Christchurch, New Zealand. DBP and DIBP have also exceeded the limit prohibition of > 0.1% phthalates according to the EU regulation (Ashworth et al., 2018). A larger study piloted by Stringer et al. (2000) involving children's toys purchased from retail outlets in 17 countries has also assessed the presence of low molecular weight phthalates such as DEP, DIBP and BBP.

High molecular weight phthalates which produced from alcohols of 5 or more carbon backbones are the main subcategories used as plasticizers in everyday products. They are used in manufacturing soft PVC, which is then used in producing other products including construction materials, medical devices, automotive components and coatings. DEHP is the main phthalates that have been widely used in manufacturing various products such as vinyl flooring, medical tubing, toys, blood bags and paints. Other important high molecular phthalates are DINP which found profusely in children's toys while DIDP in cement, gloves, pipes, cables and toys (Meeker et al., 2009; Kamrin, 2009). DEHP was the most identified phthalates in the majority of children's toys marketed in Indian market but most of the children's toys detected with DINP and DIDP were not within the limit of EU regulation (Johnson et al., 2011).

2.2 Exposure pathways

Generally, the main route of phthalates exposure in the general population is through ingestion especially for children as compared to other routes like dermal contact and inhalation, while inhalation serves as the minimal path of phthalates exposure (Praveena et al., 2018). Younger children tend to have higher phthalates exposure due to the increased needs of food and water per unit body mass, inhale more air per kilogram of body weight and frequent hand-to-mouth activity (Becker et al., 2010; Braun et al., 2013). It was also proven by a study made by Serrano et al. (2014) that the estimated dietary DEHP exposure showed highest among young infants and adolescents compared to adults.

Children are inclined to spend most of their time with toys. Majority of the toys marketed are made up wholly or partially of plastics to enhance their softness, appearances and prolong the lifetime. Concern exists when there is a tendency of high exposure of phthalates to the children if they are mouthing the toys. As it is claimed, phthalates are not permanently bound to the products and thus may leach out from time to time or in response to the sucking and chewing of the toys (Korfali et al., 2013). Frequent sucking and mouthing of the toys cause them to be especially vulnerable in ingesting these phthalates and later enter their body. Besides, repeated dermal contact with surfaces of contaminated toys also may lead to exposure.

2.3 Metabolism of phthalates

Humans are constantly exposed to phthalates due to their ubiquitous presence in the environment as well as in most consumer products. Phthalates that have entered the humans' body will undergo biotransformation process before being excreted. Phthalates' metabolism process depends on the molecular weight of phthalates. Normally, parent diester phthalates hydrolyzed by esterase and lipase into its monoester forms in the intestine and parenchyma cells to transform into active monoesters and oxidative metabolites. Phthalates are then excreted quickly from the body through urine or feces due to their short half-lives, usually between hours to several days (Hauser & Calafat, 2005). It is claimed that the hydrolytic monoesters and the oxidative metabolites are mainly excreted in urine and in a lower degree, through feces.

Short-chain low molecular weight phthalates undergo phase I biotransformation, in which, the phthalates will hydrolyzed and converted into their respective hydrolytic monoesters in the intestine and later excreted. Contrary to the high molecular weight phthalates, the biotransformation process is more complex. The hydrolytic monoesters are further metabolized either through enzymatic oxidation or hydroxylation and produce oxidative metabolites. These oxidative metabolites may then be eliminated from the body or engage in the next phase of biotransformation. Phase II biotransformation involves the oxidative metabolites to conjugate and form

glucuronide conjugates which are also excreted in the form of urine. (Park et al., 2015; Erkekoglu & Kocer-Gumusel, 2016; Sedtasiriphokin et al., 2017).

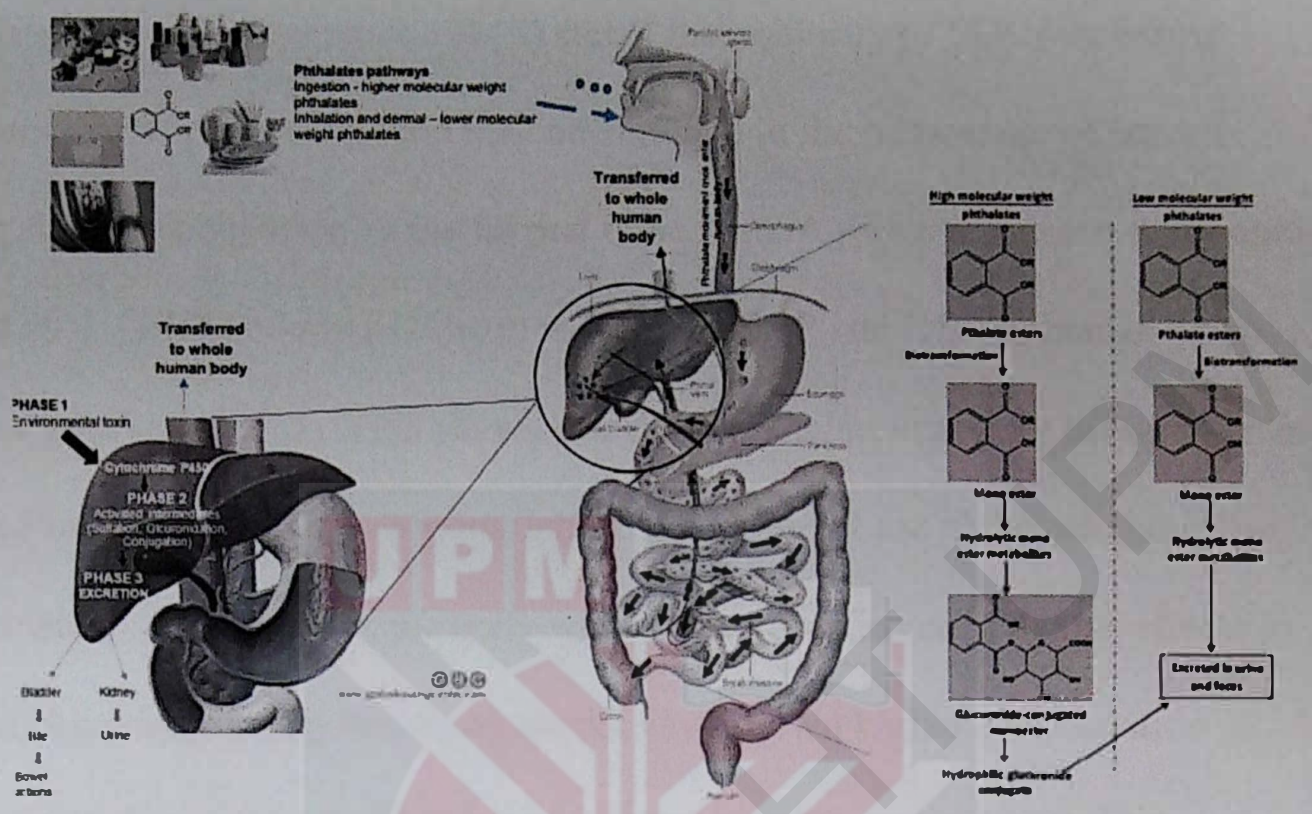

Figure 2.1: Phthalates metabolism after entering the human body (adapted from Praveena et al., 2018)

2.4 Reaction of phthalates in the body

Phthalates are a well-known synthetic endocrine disrupting chemicals (EDCs). When EDCs enter the human's body, they tend to adversely affect the endocrine system by mimicking or restraining naturally occurring hormones produced in the body and thus disrupt the levels of chemicals as well as its functions (National Institute Environmental Health Sciences, 2018). Developing fetuses, infants and

young children exposure to these EDCs were reported to have an unfortunate effect on their endocrine and reproductive development.

Furthermore, Regnier et al. (2013) stated that majority of EDCs including phthalates are a lipid soluble and may accumulate in the adiposome of fat cells causing their concentration in the fat pad to be high. A previous human study made by Mes et al. (1974) confirmed the presence of DEHP and DBP in human adipose tissue of accident victims. This shows that these phthalates may not break down and removed instantly due to the accumulation. Over time, these phthalates may also disseminate to other parts of the body which may cause several adverse effects to humans. An animal study involving chicks have shown higher accumulation of phthalates in the adipose tissue, skin and liver (Jarosova et al., 2009). The accumulation may also occur when people are constantly exposed to phthalates. Frequent exposure to chemicals will lead to inefficient detoxification system where only some of the phthalates fully break down while majority of them are not. This phenomenon may as well prolong the phthalates' elimination and making it stay longer in the body which later will lead to the long-term exposure effects of phthalates.

2.5 Associated health risks

Concerns on phthalates have been growing for these past years due to their potentiality in causing adverse effects towards human health which include the

potential of being an endocrine disruptor. Extensive studies involving *in vivo* and *in vitro* as well as epidemiological studies in humans and other scientific studies have been done assessing these toxicities. Health effects on infants and young children are in special scrutiny as they are especially vulnerable and their exposure to phthalates are thought to disrupt their growth and development. Some studies have outlined various adverse health outcomes due to phthalates exposure associated with infants and children (Hauser & Calafat, 2005; Braun et al., 2014).

One of the health effects that has been observed affecting the children were respiratory health problem. Phthalates exist in most products used at home and they tend to leak over time due to their properties. This chemical will persists in the indoor environment causing us to be constantly exposed and thus adversely affect the respiratory system. Jaakkola & Knight (2008) have outlined some studies investigating the possibility of phthalates exposure in increasing cases of respiratory inflammation and children are of particular concern. A nested case-control study made by Bornehag et al. (2004) involving children has reported those who exposed to certain phthalates found in the bedroom dust concentrations showed to develop allergies and asthmatic symptoms. From the study, there was a significant association of exposure to BBP with rhinitis and eczema while exposure to DEHP with asthmatic symptoms. A study on Bulgarian children also showed the exposure from PVC-related phthalates emission in the dust concentrations with inflammation of airways where the concentration of DEHP in indoor dust was significantly associated with wheezing among the children (Kolarik et al., 2008). Jaakkola & Knight (2008)

presumed that the modulation of the immune response to a co-allergen happened if a person was exposed to a high level of phthalates from the PVC products. Despite the fact that the main route of exposure for phthalates in the general population is through diet (Clark et al. 2003; Wormuth et al. 2006), emission of phthalates in the environment poses an important source for inhalational exposure.

Next, as is well known, phthalates are one of the widespread environmental agents and an endocrine disruptor. Hence, phthalates are capable of interfering or lowering the level of hormones in the brain which then may disrupt the neurological development. Attention deficit hyperactive disorder (ADHD) and autism spectrum disorder (ASD) are examples of a neurodevelopmental problem that may have caused by phthalates. ADHD is a neurodevelopmental disorder that is related to behavioral and attention problems in children. A previous study by Kim et al. (2009) have assessed the postnatal exposure to phthalates in the development of ADHD; involving 261 Korean children, from 8 to 11 years old. From this study, it is shown that the DEHP metabolites in the urine were significantly associated with the inattention and hyperactivity scores. Besides, a significant association was also found between the urine concentration of DBP metabolites and the scores of computerized ADHD Diagnostic System (ADS) measuring the inattention and impulsivity in children. Moreover, a study by Park et al. (2014) has reported the amount of urinary phthalate concentration in ADHD boys were higher as compared to those without ADHD. DBP metabolites were found to be significantly higher in the combined or hyperactive-impulsive subtypes as well as positively correlated with the severity of

externalizing symptoms. Imaging data also suggested a negative impact of phthalates on regional cortical maturation in ADHD children. ASD or usually known as autism is a neurodevelopmental condition characterizes by a range of symptoms including impairments of language development, social, behavior and communication skills. The causes of this disorder are poorly understood but concerns over exposure to phthalates as a risk factor in the development of autism have been unfolded recently. For an instance, a study made in Italy involving 48 children with ASD has observed that DEHP metabolites which were 5-OH-MEHP and 5-oxo-MEHP were significantly increased in children with autism (Testa et al., 2012). Similar observations have also been made by Stein et al. (2013) and Kardas et al. (2015) reporting the significant increase of phthalate metabolites among autistic children.

Other effects that have been reported among children due to phthalates exposure was the development of obesity. The growing production of industrial chemical over the years has upraised concerns over increased obesity as EDCs like phthalates are believed to disrupt the lipid metabolism pathways and thus promoting the gaining of weight and lead to obesity. For example, a prospective cohort study in New York has demonstrated the relationship between urinary phthalate concentrations and anthropometric measures among children. It was found that for both monoethyl (MEP) and low molecular weight phthalate (MWP) metabolites, there was an increase of 2 unit in BMI and 5cm in waist circumference of the children, in which, the measures were taken approximately one year after the collection of urine samples. The study showed a positive association between phthalate concentrations

and the increasing body size measures among children (Teitelbaum et al., 2012). A similar result has been shown in female adolescents in a cross-sectional study of National Health and Nutrition Examination Survey (NHANES) data, where BMI and waist circumference increased with MEP concentrations in their urine (Hatch et al., 2008). Other study using NHANES data also reported low MWP was significantly associated with higher odds for obesity in male children and adolescents (Buser et al., 2014). Emerging evidence outlined the association between these two variables has suggested the increasing risk of obesity may be associated with exposure to phthalates, as well as other environmental endocrine disruptor agents.

Puberty problem among children has also been reported in several studies involving exposure to phthalates (Braun et al., 2014). In general, factors of pubertal timing have been related to race, obesity and genetics but environmental agents like phthalates may also have a role. A prospective cohort study conducted among girls aged 6 to 8 years old reported an association between urinary phthalates exposure and pubertal development in girls, in which, observed one year later. The results of this study showed a positive association of both breast and hair development in girls with the highest exposure to LMW phthalates such as DEP and DBP as compared to those with lowest exposure. However, an inverse relationship has been observed between girls with highest exposure of high MWP such as DEHP and BBP with the development of breast and pubic hair (Wolff et al., 2010). A similar result has also been portrayed by a cross-sectional study among Danish girls, where the study showed a significant delay in the development of girls' pubic hair with higher urinary

phthalate metabolite concentration of DBP and BBP (Frederiksen et al., 2012). Besides, a case-control study in China involving 167 boys has reported that the higher concentration of urinary phthalate metabolites were detected in children with Constitutional Delay of Growth and Puberty (CDGP) and that the phthalates concentration was associated with CGDP which seemed to be mediated by circulating testosterone level (Changming et al., 2015). Another study by Yunhui et al. (2015) has suggested the effects of phthalates on pubertal onset and progression. The results reported that MBP was associated with delayed development of pubic hair in boys, while MBP, mono-methyl phthalate (MMP), MEP and mono-ethyl-hexyl phthalate (MEHP) were associated with the onset of breast development, and MEHP with accelerated breast development and earlier menarche in girls.

On the other hand, exposure to phthalates has also been linked to male and female reproductive development. Phthalates are considered to be one of the major EDCs that elicit anti-androgenic or estrogenic effects. For instance, a study conducted by Hauser et al. (2007) which involved 379 men with normal semen concentration has shown that several urinary phthalate metabolites (MEP, MBP and MBzP) were significantly associated with sperm damage. MEHP was associated with increased sperm damage only after considering both DEHP oxidative metabolites. A similar outcome has also been outlined by a study involving 269 men, in which, analyzed the association between phthalate metabolite levels in urine with semen parameters. The results showed that the urinary phthalate metabolite levels which were 5-OH-MEHP, MEHP, MINP were significantly associated with a decrease in

sperm motility, MBP with computer-aided sperm analysis (CASA) parameters and increase sperm DNA damage, MEHP with testosterone level and lastly MBzP, MBP, MEHP, and MEP with sperm aneuploidy (Jurewicz et al., 2013). Another cohort study on 463 male has established a significant dose-response relationship between mono-butyl phthalate (MBP) with low sperm count and motility, where the result was consistent with a claim that MBP is a testicular toxicant (Srivastava et al., 1990; Hauser et al., 2006). Meanwhile, female reproductive health problem can be seen by a case-control study published in Italy on the association between DEHP levels in plasma and peritoneal fluid and endometriosis. The results have reported women with endometriosis had higher plasma DEHP levels as compared to the women with no endometriosis while for DEHP and MEHP levels in the peritoneal fluid were the same for both cases and controls (Cobellis et al., 2003). Moreover, in a study conducted by Swan et al. (2015) has reported that the concentration of phthalates has been detected in the urine sample of pregnant women who have male babies with a short anogenital distance (AGD). Three metabolites of DEHP (MEHP, MEOHP, MEHHP) were significantly and inversely associated with both measures of male babies' AGD as compared to the female babies.

Lastly, exposure to phthalates may also be responsible for causing cancer. In a recent study conducted in Mexico by Lopez-Carrillo et al. (2010), a positive association was reported between the concentrations of DEHP in the urine sample of Mexican women and the risk of breast cancer development. The association also became more positive when adjusting for premenopausal women. Phthalates were

also believed to increase the risk of testicular cancer as observed in a case-control study among men working in plastic industry in Sweden. There was a significant increase of developing testicular cancer among workers who exposed to PVC, in which, assessed using questionnaires (Hardell et al., 1997). Besides, the risk of pancreatic cancer was also observed in a nested case-control study conducted in a plastic manufacturing industry. 28 men who died from pancreatic cancer as cases and 140 randomly selected controls were included in the study who have possible exposure to DEHP. A significantly increased risk of pancreatic cancer was found mainly for workers in the vinyl resins and polyethylene work area (Selenkas et al., 1995).

CHAPTER 3

METHODOLOGY

3.1 Children's toys selection

Thirty samples of colored plastic children's toys were purchased randomly from the local shops that sell low-priced children's toys in the month of March 2019. Children's toys were selected based on the basis of their physical properties which was mainly made up wholly or partially of plastic materials. Moreover, children's toy was also chosen only if it manufactured in any Asian countries. Possible mouthing of the toys was also taken into consideration in choosing the children's toys. All samples were coded and stored at room temperature until sample preparation.

3.2 Children's toy sample preparation

Figure 3.1 shows the sample preparation. The sample preparation consisted of two main steps. Firstly, the sample was pre-cleaned with water-impregnated tissue and was left to air dry. Next, each of the toy samples was cut, scrapped off or grated into pieces of about 0.5mm length using a stainless steel sharp blade and a grater. Some toy samples especially the hard ones required numerous times of grinding to obtain smaller pieces or fine powder of samples. This was to increase the contact surface of samples with the solvent as well as to ensure the efficiency of phthalates extraction process afterward. The sample was then weighed 0.5 g approximately using an electronic analytical balance (AND GR-200) before transferring them into glassware for the extraction process.

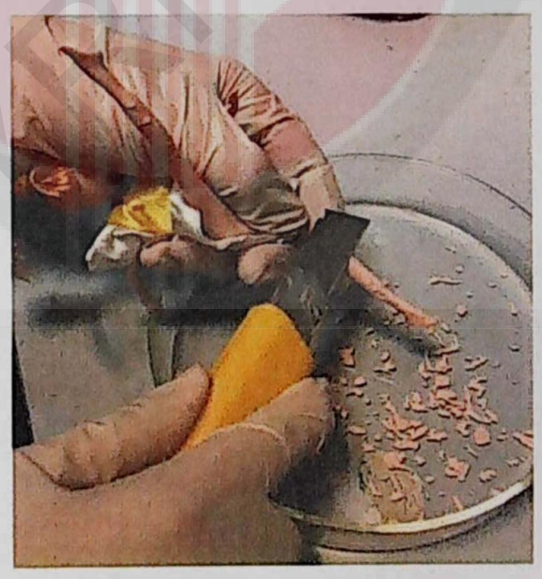

Figure 3.1: Sample preparation

3.3 Sample extraction procedures

Figure 3.2 to 3.5 show the sample extraction processes. A total of 0.5 g sample was added with 20 mL of methanol. The purpose of adding methanol into the sample was to guarantee an efficient extraction of the lipophilic compound of phthalates in the children's toys. The mixture was agitated for about 1 hour using an orbital shaker with the speed of 350 rpm to dissolve the sample. After that, to establish a complete dissolution of the sample, it was sonicated by breaking the intermolecular interactions using an ultrasonic (Elma-Hans Schmidbauer GmBH & Co. KGD – Singen, Kolpingstraße) for 30 minutes at a room temperature of 25°C in a sealed vial. Lastly, the sample was filtered using a 90 mm Whatman filter paper and transferred into a 100 mL Schott bottle. The final measurement of the sample was recorded. The Schott bottle was also labelled according to the code of the sample.

Figure 3.2: Samples added with methanol

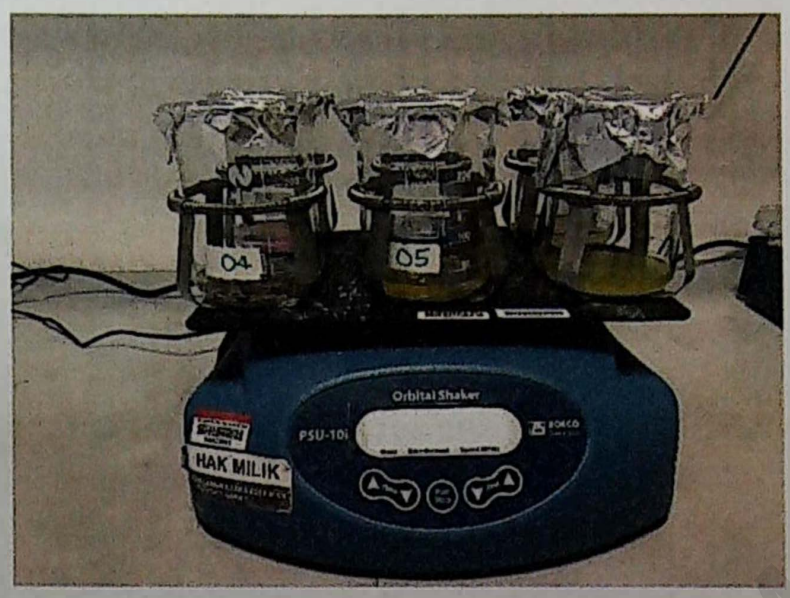

Figure 3.3: Samples agitated using an orbital shaker

Figure 3.4: Samples sonicated using ultrasonic

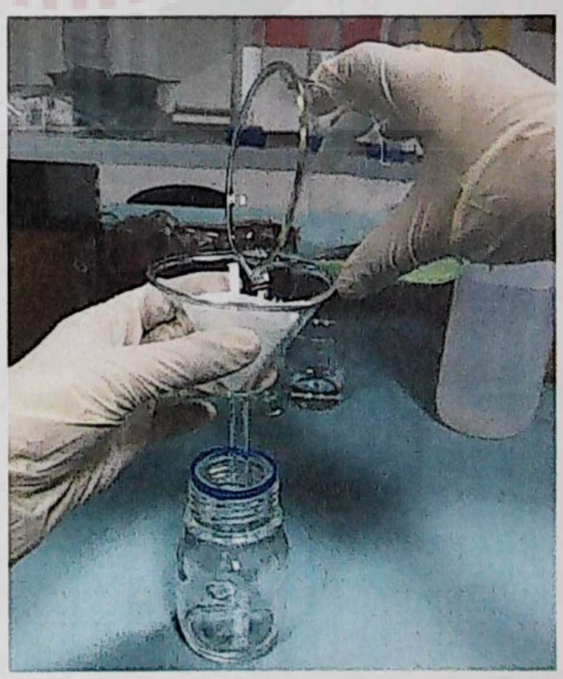

Figure 3.5: Samples filtration into the Schott bottle

3.4 High-performance liquid chromatography (HPLC) calibration and analysis

Phthalate analysis in children's toys was performed using The Shimadzu Prominence HPLC (Shimadzu Scientific Instruments, Japan) coupled with SPD-20A/20AV, UV-VIS detector. Each toy sample was analyzed for DEHP compound. DEHP and internal standard were measured using HPLC.

3.4.1 Determination of DEHP by retention time

DEHP was identified by comparing the retention time (RT) of the samples' peaks with the RT of DEHP standard. Retention time is a period of time where the compound spends on the chromatography column from the beginning of injection until the elution of the compound. The last peak of the retention time in a chromatography column is used to estimate the required interval of the chromatographic run. In this study, repeat of injection for three times was performed to confirm the retention time of DEHP compound due to interfering peak detected during the first injection. The retention time for DEHP was 3.497.

3.4.2 HPLC method

A normal phase HPLC method was applied in this study. HPLC method entailed several stages. Firstly, the solvent reservoir was equipped with solvents or also known as the mobile phase that will flow constantly into the column. The

mobile phase was an isocratic consisted of methanol. Next, the sample was stored in the sample reservoir, in which, the sample was injected automatically into the continuously moving mobile phase stream and later carried into the HPLC column Compounds were then separated on a C18 100A column (250 mm×4.6 mm, 5 µm particle size). The flow rate was 1 mL/min. After that, the UV-VIS detector produced an electronic signal proportional to the amount of sample component acquired from the column. The detection wavelength was fixed at 210 nm. Lastly, a digital microprocessor and user software displayed a peak comprised of DEHP that was detected in the sample.

3.4.3 Method validation

DEHP compound's calibration curves were prepared at the concentrations range from 0.2 to 20 µg/mL. The calibration curve was constructed by plotting concentrations ratio versus peak area ratio of the DEHP over the internal standard at different concentration levels. Linear regression analysis was used to evaluate the linearity of the calibration curves. The correlation coefficient which indicated the functional linear relationship between the concentration of the DEHP and the peak area exceeded 0.99 ($R^2 > 0.99$) which was R=0.9999.

LLOD and LLOQ of DEHP were also determined based on the signal-to-noise (S/N) ratio approach. This was performed by comparing measured signals from samples with a known low concentration of each phthalate compound with those of

blank samples. LLOD is the minimum concentration at which the compound can be reliably detected with the S/N ratio of 3:1. Whereas, LLOQ with the S/N ratio of 10:1 is the minimum concentration at which the compound can be reliably quantified.

3.5 Health Risk Assessment

Table 3.1 shows the parameters and data source as reference in this study to calculate the health risk assessment for children. Health risk assessment (HRA) due to DEHP exposure from the children's toys was determined for the main exposure route among children which was via ingestion according to the mouthing behavior of the children. Average daily dose (ADD) was derived to determine the health risk due to presence of DEHP in children's toys. ADD of DEHP was determined using equation 1, which was adapted from a study by Praveena et al. (2015).

$$ADD = \frac{C \times IR \times EF \times ED}{BW \times AT} \times \mathrm{CF} \qquad \text{(Equation 1)}$$

ADD is in mg/kg/day. C is the concentration of DEHP (mg/kg); IR is the ingestion rate (mg/day); EF is the exposure frequency (day/years); ED is the exposure duration (years); BW is the average body weight (kg); AT is the average time (days) and CF is the conversion factor (kg/mg).

The hazard quotient (HQ) was then assessed using equation 2 by dividing the ADD by reference dose (RfD) of DEHP.

$$HQ = \frac{ADD}{RfD} \qquad \text{(Equation 2)}$$

HQ ≥1 specifies that the exposure may have a potential health risk, while HQ < 1 shows that there is no potential health risk.

Table 3.1 Parameters used for estimation of average daily dose value (ADD) via ingestion route for children in health risk calculations

Parameter	Symbol	Unit	Value	Reference
DEHP concentration	C	mg/kg		
Ingestion rate	IR	mg/day	100 mg	US EPA (2002)
Exposure duration	ED	years	6 years	US EPA (2002)
Exposure frequency	EF	days/years	350	US EPA (2002)
Average body weight	BW	kg	15	US EPA (2002)
Average time	AT	days	ED × 365 years	US EPA (2002)
Conversion factor	CF	kg/mg	1×10^{-6}	USEPA (2002)
Reference dose	RfD	mg/kg/day	2×10^{-2}	IRIS (2001)

3.6 Quality Control

All glassware and Schott bottles were cleaned beforehand by using soap and water and rinsed repeatedly. All glassware was dipped with deionized water to remove traces of organics and dried in the oven at 50°C for 1 hour. Meanwhile, 100 mL Schott bottles were immersed with diluted nitric acid solution by soaking overnight. The bottles were then rinsed with tap water repeatedly and dried in the oven at 50°C for 1 hour. This was to ensure all equipment was free from any external contaminants before using it.

Moreover, all equipment used during the sample preparation and extraction process were made of glass or paper. There was no involvement of plastic-based equipment during the process to eliminate contamination of phthalates from other plastic product. A new set of methanol was also used in this study to eradicate any contaminants from the used methanol due to improper storage or mixing of other chemicals with it.

For the HPLC analysis, three method blanks were extracted and run with each batch (ten samples) to identify if there is any contamination and to check for instrumental performance. The blanks were extracted and analyzed in exactly the same way as for toy samples. Additionally, the syringe was cleaned with the sample before injecting the sample into HPLC.

CHAPTER 4

RESULTS

4.1 Toys characteristics

Table 4.1 shows the toy samples included in the study which were coded with numbers from 01 to 30 with their properties which consist of country of origin, type of the toys and range of children's age that are suited to play with the toys. The country where the toys were manufactured and range of children's age were acquired from the toys' label while the type of the toys was categorized based on the toys' physical characteristics.

Table 4.1 Children's toys and their properties

Toy No.	Country of origin	Type	Age (years)
01	China	Soft	≥ 3
02	China	Hard	≥ 3
03	Malaysia	Soft	≥ 3
04	China	Hard	0 – 3
05	Malaysia	Soft	0 – 3
06	Thailand	Hard	> 1
07	Malaysia	Soft	0 – 3
08	Malaysia	Hard	≥ 3
09	Thailand	Hard	≥ 1
10	China	Hard	> 3
11	China	Soft	0 – 3
12	China	Hard	≥ 3
13	China	Hard	≥ 3
14	China	Soft	> 0.5
15	China	Soft	≥ 3
16	China	Soft	≥ 3
17	China	Soft	≥ 3
18	China	Soft	0 – 3
19	China	Soft	0 – 3
20	China	Soft	0 – 3
21	China	Hard	0 – 3
22	China	Hard	0 – 3
23	China	Soft	≥ 1.5
24	China	Soft	≥ 1.5
25	China	Soft	≥ 1.5
26	China	Soft	≥ 1.5

27	China	Soft	≥ 1.5
28	China	Soft	≥ 1.5
29	China	Hard	≥ 3
30	China	Hard	≥ 3

4.2 DEHP concentrations in children's toys

Table 4.2 represents the DEHP concentrations in thirty samples of children's toys. All thirty samples showed the occurrence of DEHP, in which the frequency of detection of DEHP in the toys varied. The highest concentration of DEHP detected was 2556.76 mg/g in toy sample 03, while the lowest concentration of DEHP detected was 0.30 mg/g in toy sample 26. The mean concentration of DEHP was 161.34 mg/g and standard deviation was 597.50.

Table 4.2 DEHP concentrations detected in children's toys

Toy Sample No.	Concentrations of DEHP (mg/g)
01	0.58
02	0.42
03	2556.76
04	0.82
05	2140.39
06	1.19
07	3.05
08	0.53
09	0.42
10	0.85
11	126.53
12	0.61
13	0.34
14	0.46

15	0.32
16	0.48
17	0.39
18	0.44
19	0.47
20	0.58
21	0.32
22	0.48
23	0.38
24	0.62
25	0.48
26	0.30
27	0.50
28	0.57
29	0.56
30	0.50
Maximum concentration	2556.76
Minimum concentration	0.30
Mean concentration	161.34
Standard deviation	597.50

4.3 Associations between DEHP concentrations and children's toys properties

The associations between DEHP concentrations that were categorized into high and low and children's toys properties consist of the countries where the toys were made, types of toys either soft or hard and age of children suited to play with the toys were analyzed and tabulated in Table 4.3. There were no data or previous literature related to the maximum allowable concentration of phthalates in children's toys. Thus, in this study, it was assumed that the DEHP concentrations in children's toys that were equal or more than 1.0 mg/g, considered to be high while DEHP concentrations lower than 1.0 mg/g considered to be low.

The table shows there was a significant difference (P<0.05) presented between DEHP concentrations and the origin country where the toys were made. No associations were reported for DEHP concentrations and the type of the toys as well as the age suitable for the children to play with the toys.

Table 4.3 Associations between DEHP concentrations and toys' properties

Toys' properties	High[a]	Low[a]	X^2	p-value
Age				
3 years and below	3	14		
3 years and above	2	11	0.027	0.869
Country				
Malaysia	3	1		
Outside Malaysia	2	24	6.000	0.001*
Type				
Soft	4	14	1.000	0.317
Hard	1	11		

* shows a significant association observed

[a] high referred to DEHP concentrations > 1.0 mg/g and low referred to DEHP concentrations < 1.0 mg/g

4.4 Potential health impact of DEHP exposure

HRA results of reported DEHP concentrations in thirty children's toys are showed in Table 4.4. The HQ results considering the measured ADD of DEHP in children's toys and the RfD value of DEHP showed that toy sample 03, 05 and 11

showed HQs more than 1 (HQ≥1), indicated that it posed a possible human risk due to exposure. Other toy samples showed HQs less than 1 (HQ<1), indicated that there was no possible human risk due to exposure.

The highest HQ result was presented in sample 03 which was 817, followed by sample 05 with HQ result of 684 and sample 11 with the HQ result of 40.5. The lowest HQ result reported was in sample 15, 21and 26 which was 0.10.

Table 4.4 Hazard Quotient values for thirty sample toys

Toy Sample No.	Hazard Quotient
01	0.19
02	0.14
03	817.00*
04	0.26
05	684.00*
06	0.38
07	0.97
08	0.17
09	0.13
10	0.27
11	40.50*
12	0.19
13	0.11
14	0.15
15	0.10
16	0.15
17	0.12
18	0.14
19	0.15
20	0.19
21	0.10
22	0.15
23	0.12
24	0.20
25	0.15
26	0.10
27	0.16
28	0.18
29	0.18
30	0.16

*shows that HQ≥1, pose an appreciable health risk

CHAPTER 5

DISCUSSION

5.1 DEHP concentrations in children's toys

According to table 4.2, the result showed that the difference between the maximum and minimum concentration of DEHP in children's toys was too large in this study so there were several justifications outlined relating to the presence of DEHP at such concentrations. Low DEHP concentrations in the children's toys may be subjected to their role as a constituent or contaminant of another phthalate, as a component of ink or paint used on the toy or due to contamination during process through the use of phthalates as a processing aid or in another product. Meanwhile, explanations associated with high concentrations of DEHP reported was perhaps due to the contamination of DEHP from outside sources. As phthalates are ubiquitously present, DEHP contained in the dust or DEHP that are freely disperse in the environment may interfere and contaminate the samples externally or intrusion during the method procedures of samples preparation or samples extraction.

5.2 Associations between DEHP concentrations and children's toys properties

The association between concentrations of DEHP in toys and country where the toys were manufactured has shown that the countries influenced the amount of DEHP used in the toys which have been represented in table 4.3. Majority of the toy samples were manufactured outside Malaysia, which was mainly in China. China was known to be the central manufacturer of the toys in the world as they produced 4 out of 5 world's toys. However, there were several worrisome claims reported on the safety of the toys produced in China such as occurrence of toxic chemical contents such as lead, unsafe working conditions in producing the toys as well as corruptions that occur in the standards body that regulated the safety standard of the toys due to bribery. The most common safety issues in China relating to children's toys was on the chemical contents used in the toys (Asia News, 2010; Moore & Hall, 2011). This may explain the reason on how the manufactured countries affected the DEHP concentrations.

Based on the study, there was no significant association established between the DEHP concentrations and the type of the toys ($P>0.05$). The result of this study was not corresponding to a previous study by Johnson et al. (2011) which demonstrated that the soft toys comprised higher levels of phthalates as compared to hard toys. Concerning the main function of phthalates is to soften the toys, this study has shown that the type of toys affected the concentrations of phthalates used in the toys. However, this study showed no association. The age suited for the children to play

with the toys also showed no association with the DEHP concentrations in the toys. There were limited data related to discuss more on how children's toys' properties affect the concentrations of DEHP utilized in the children's toys.

5.3 Potential health impact of DEHP exposure

It is shown that there were three samples that posed a potential health risk towards the children which were sample 03, 05 and 11 as they were high in DEHP concentrations. All three samples that showed the possible health risk was shown to be soft plastic toys. The results acquired from the study was corresponding to a study by Johnson et al. (2011), in which, they claimed that the soft plastic toys contained higher level of phthalates as compared to hard plastic toys. DEHP may be used higher in the following toys to establish its function which is to soften products and so establish a possible health risk towards the children.

Continuous exposure to DEHP may cause reproductive or developmental toxicity. In a previous study involving DEHP monoester metabolite which is mono-ethylhexyl phthalate (MEHP), conducted by Hauser et al. (2006), has shown that MEHP was associated with increased sperm damage after considering for both oxidative metabolites. DEHP also reported causing endometriosis in women, where women with endometriosis tend to have higher plasma DEHP levels (Cobellis et al., 2003). A study by Swan et al. (2015) also has reported the presence of phthalates in urine of pregnant women that have male babies with a short anogenital distance

(AGD) and that three metabolites of DEHP which are MEHP, MEOHP and MEHHP showed that all metabolites were significantly and inversely associated with measures of AGD in male babies. Thus, exposure of children to DEHP in children's toys cannot be overlooked.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

In conclusion, DEHP concentrations in children's toys and its potential health risk was determined in this study. All toy samples perceived in this study showed the existence of DEHP with varied frequency of detection. The maximum concentration observed was 2556.76 mg/g, while the lowest concentration was 0.30 mg/g. The association between DEHP concentrations and the children's toys properties were also assessed. A significant association was found only between DEHP concentrations and the countries of the manufactured toys. In addition, potential human risk of DEHP exposure in children' toys was estimated. Results of HQ ratio which were more than 1 (HQ>1) for toy sample 03, 05 and 11 indicated appreciable human health while other toy samples showed an HQ ratio of less than 1 (HQ<1) indicated no appreciable human health.

This study has provided quantitative in information available on the DEHP concentrations detected in children's toys in Malaysia and potential health risk assessment as to the best our knowledge, this was the first study conducted associating with DEHP exposure in children's toys. Nevertheless, this study has some limitations. This study was targeted the children's toys sold at reasonably priced which obtained at night markets only and so does not cover the whole range of toys in Malaysian markets. A more representative study with larger samples and randomization of selecting toy samples as well as a wide range of phthalate compounds to be tested are recommended for future studies. Even so, there were some findings to be noted in the study. DEHP concentrations in children's toys should be under scrutiny as the study posed a high risk in adversely affecting the health of the children as shown by some toys containing DEHP.

To reduce the occurrence of DEHP in children's toys, there is a need for regulations in Malaysia for restriction on phthalates in toys or other children's products children as done by other countries. From that, the amount of DEHP or other phthalates used in the children's products can be controlled and so will sustain the safety of the children. The awareness among parents of the children should also be increased by developing a program about the unknown chemical contents exist in the children' toys that will affect their children's health, either in the short-term result or long-term.

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APPENDICES

Table 6.1 Children's toys

Sample No.	Picture
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02	
03	

04	
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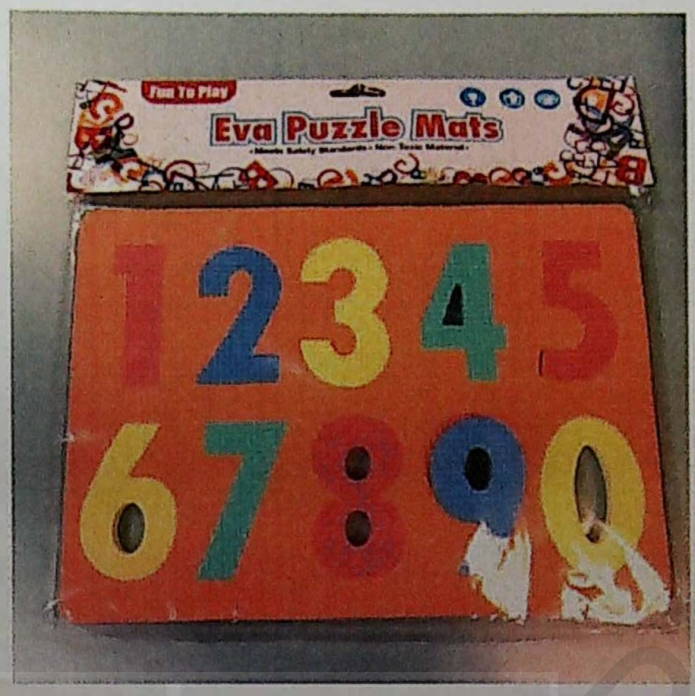
07	
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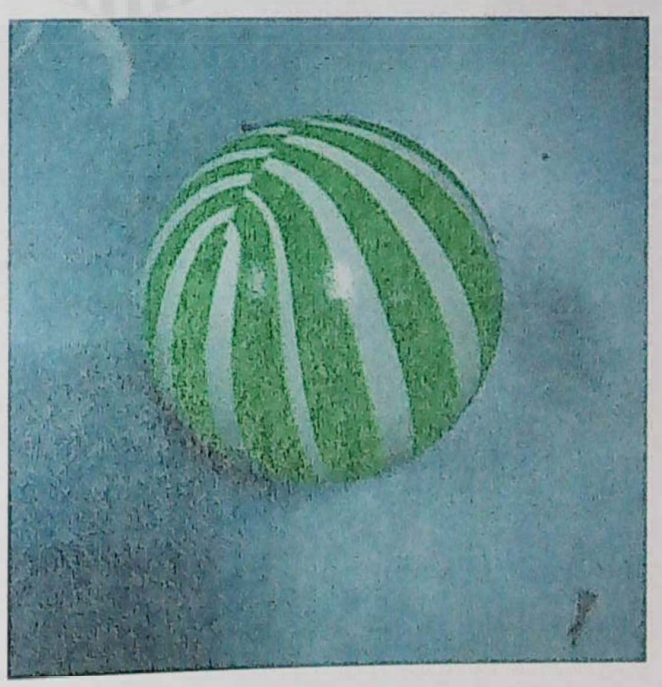
15	

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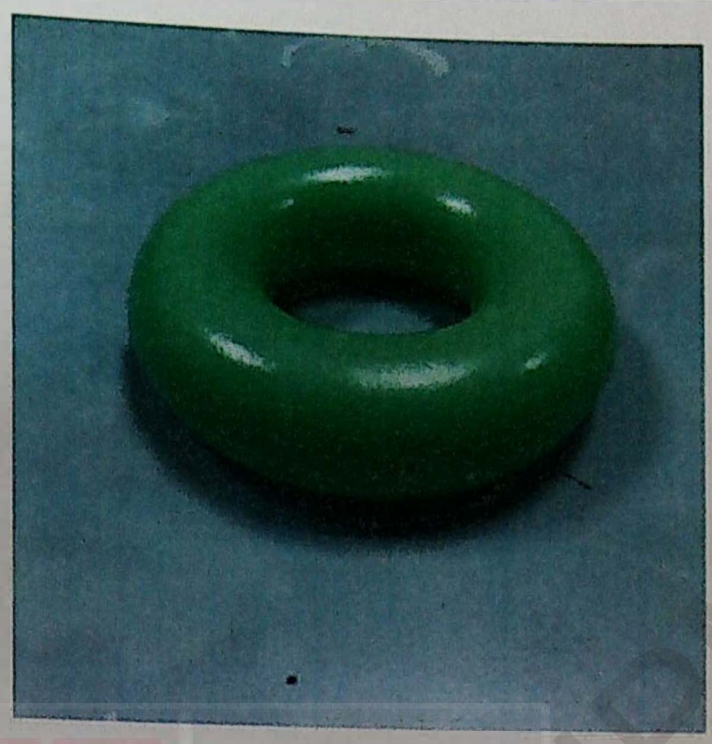
19	
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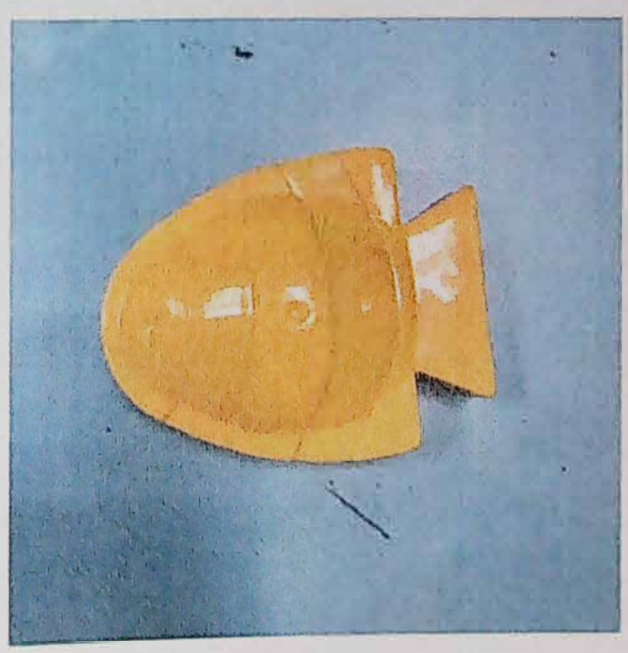
21	

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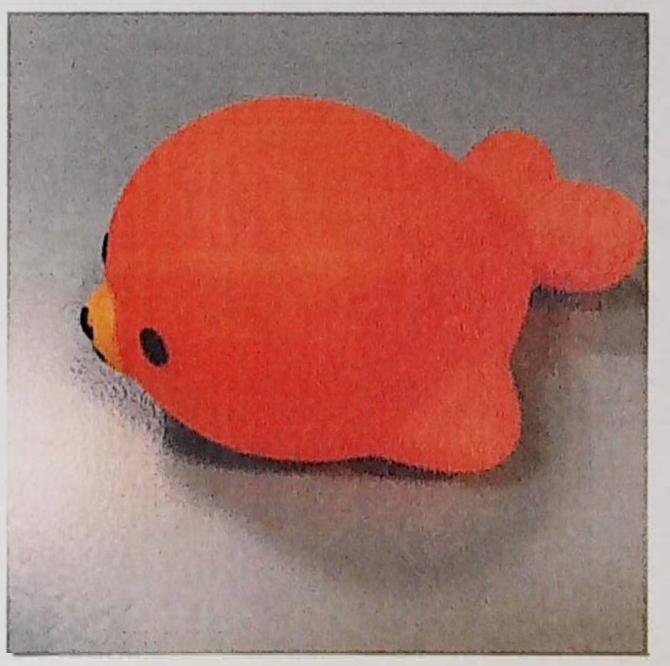
24	

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