ASSESSING THE CONCENTRATION OF HEAVY METALS (LEAD, CADMIUM AND NICKEL) IN SELECTED PERSONAL CARE PRODUCTS AND URINE AMONG THE TARGETED CONSUMERS

by

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LIST OF ABBREVIATIONS AND SYMBOL

AAS	Absorption Spectrophotometer
ACD	Allergic contact dermatitis
Ag	Silver
Al	Aluminum
ASEAN	Association of Southeast Asian Nations
USA	United States of America
Au	Gold
B	Boron
Ba	Barium
Be	Beryllium
Cd	Cadmium
Co	Cobalt
CO	Carbon monoxide
Cr	Chromium
Cu	Copper
FDA	Food and Drug Association
GDP	Gross Domestic Product
$HClO_4$	Perchloric acid
Hg	Mercury
HNO_3	Nitric acid
Li	Lithium
Mn	Manganese
Ni	Nickel
$NiCO_4$	Nickel tetra carbonyl
OSHA	Occupational Safety & Health Association
p	p value
Pb	Plumbum
PCP	Personal care product
ppm	part per million
Sn	Tin
SPSS	Statistical Package for The Social Science

µg/g	Microgram per gram
g	Gram
h	Hour
mcg/g	Micrograms per gram
mg/kg/day	Milligram per kilogram per day
mg/L	Milligram per litre
mL	Millilitre
n	Sample size
Etc.	Et cetera 'and other things'
°C	Celcius
%	Percentage
>	More than
$	Dollar

MENILAI KEPEKATAN LOGAM BERAT (PLUMBUM, KADMIUM DAN NIKEL) DALAM PRODUK PENJAGAAN DIRI YANG TERPILIH DAN AIR KENCING DALAM KALANGAN SASARAN PENGGUNA

ABSTRAK

Produk penjagaan diri diketahui secara meluas dan kerap digunakan oleh pengguna. Produk ini sering digunakan dalam kehidupan harian, dan juga dalam industri dan komersial. Di sesetengah negara, tiada peraturan yang ditetapkan berkenaan dengan keselamatan bahan-bahan. Sesetengah logam berat yang digunakan di dalam produk penjagaan diri ini adalah sebagai bahan-bahan, walaupun kebanyakannya adalah bahan yang tercemar. Jadi, kajian ini dijalankan untuk menilai kepekatan logam berat dalam pelbagai jenis produk penjagaan diri termasuk deodoran, gincu, syampu dan losyen tangan dan badan, dan sembilan belas sampel air kencing dari pengguna yang disasarkan. Lapan produk jenama yang berbeza dipilih (dalam negara dan di peringkat antarabangsa) yang terdapat di pasaran telah dianalisa. Penentuan kepekatan logam berat telah dilakukan melalui rawatan pra sampel dengan menggunakan cara *acid digestion* dengan menggunakan asid nitrik dan asid perklorik yang pekat. Sehubungan dengan itu, sampel dianalisa dengan menggunakan *Atomic Absorption Spectrophotometer* (AAS) dan ujian keputusan statistik telah dilakukan dengan menggunakan SPSS versi 22. Keputusan menunjukkan bahawa kepekatan tertinggi logam Pb, Cd dan Ni adalah seperti deodoran dengan 0.262 mg/L, gincu dengan 0.005 mg/L dan losyen tangan dan badan dengan 0.048 mg/L. Manakala, sampel air kencing menunjuk tiada kaitan antara jenis dan kekerapan penggunaan produk dan kepekatan logam berat Pb, Ni Cd di dalam air kencing pengguna dengan bacaan ($p > 0.05$). Walaupun kepekatan logam berat adalah di bawah had yang dibenarkan, ia mungkin mempunyai beberapa faktor yang berkaitan yang menyumbang kepekatan logam berat di dalam air kencing seperti status merokok, status pemakanan, air minuman, terdedah kepada asap rokok, asap kenderaan dan lain-lain. Semua logam berat berada didalam had selamat dalam ujian yang telah dijalankan keatas semua jenama.

ASSESSING THE CONCENTRATION OF HEAVY METALS (LEAD, CADMIUM AND NICKEL) IN SELECTED PERSONAL CARE PRODUCTS AND URINE AMONG THE TARGETED CONSUMER

ABSTRACT

Personal care products (PCP) are widely known and regularly used by people, often on a daily life, as well as in industry and commercial. In some countries, there are no strong regulations on safety of the ingredients. Some heavy metals are used in the personal care product as ingredients, even though most of them are contaminants. This study was conducted to assess the concentration of heavy metals in various types of PCP include deodorant, lipstick, shampoo, hand and body lotion and also in nineteen urine samples of the targeted consumer. Eight products of different brands which are locally and internationally available in the markets were analyzed. Determination of heavy metals was done by pre treatment of samples by acid digestion using concentrated Nitric Acid (HNO_3) and Perchloric Acid ($HClO_4$), then the samples were analysed using Atomic Absorption Spectrophotometer (AAS), the statistical analysis were done by SPSS version 22. Results showed that, the highest mean concentration of metal lead, cadmium and nickel was in deodorant with 0.262 mg/L, lipstick with 0.005 mg/L and hand and body lotion with 0.048 mg/L respectively. There are no an association between the types and frequency of product use and the concentration of heavy metals Pb, Cd and Ni in urine on targeted consumer with ($p > 0.05$). Even though, the trace amount of heavy metal were under the permissible limit, it might have some contributing factors that related in concentration of heavy metal in urine such as smoking status, nutritional status, drinking water, exposed to smoke vehicle and etc. All the metals are present and under the safe limits after carried out in selected brands of PCP.

CHAPTER 1:
INTRODUCTION

1.1 Study Background

Personal care products (PCPs) are regulated nationally in all regions of the world especially in terms of their impact on human health (Tolls *et al.*, 2009). In fact, the term "personal care products" is used to refer to cosmetic products in some parts of the world. In this regard, the two terms are used interchangeably referring to the same meaning (Ababneh *et al.*, 2013). Personal care products are widely known and regularly used by people, often on a daily life, as well as in industry and commercial. (Bennet *et al.*, 2010)

Humans had used the cosmetics for thousands of years. Since the dawn of civilization, cosmetics have constituted a part of routine body care not only by the upper level of society but also by middle and low class people (Chauhan *et al.*, 2010). Although beauty consciousness of people has set the demand of cosmetics in market and the issue of the presence of heavy metals as deliberate cosmetic ingredients, as these substances act as the impurities and the toxic effects has been get the attention of clinicians and researchers. (Yebpella *et al.*, 2014)

According to the Association of Southeast Asian Nations (ASEAN), PCP are defined as any substance or preparation intended to be placed in contact with the external parts of the human body or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly for cleaning them, perfuming them, changing their

appearance, and/or correcting body odours and/or protecting or keeping them in good condition. (Siti Zulaikha *et al.*, 2015)

PCP is directly applied on the outer surface of human skin. The function of human skin is as a protective barrier, however certain ingredients may penetrate. Therefore, consumers have to find for natural-based cosmetics in order to avoid allergic reactions and any side effects for their safety of health. The important criteria for the cosmetics formulation are the raw materials, which can be either synthetics or natural materials. (Kamairudin *et al.*, 2014)

Variety of PCP include skin moisturizers, perfumes, lipsticks, fingernail polishes, eye and facial makeup, shampoos, permanent waves, hair colours, toothpastes, and deodorants. (United States Food and Drug Administration, 2015)

PCP production is being use increasing by worldwide. PCP contain ingredients, such as fragrances, colours, solvents or preservatives which claims to be effective, long lasting, stable and safe to human (Adepoju-Bello *et al.*, 2012). However, many of the ingredients are hazardous mixtures or substances with different adverse effect. (Bennett *et al.*, 2010; Klaschka and Rother, 2013)

Some chemicals that are revealed in PCP such as phthalates, oxybenzone, triclosan, lead arsenic, nitrosamines, hydroquinone, and 1,4-dioxane. That chemical may be associated with health effects related with common ingredients used in PCP such as allergy, endocrine disruption, neurotoxicity, birth defects, or cancers. (Akan *et al.*, 2014; Al-Qutob *et al.*, 2013; Hepp *et al.*, 2014)

Most chemicals are added to cosmetic product in the form of preservatives and fragrances. Some of the preservatives and fragrance are toxic and restricted (Siti Zulaikha *et al.,* 2010) because it can be sensitizers or irritants. (Klaschka and Rother, 2013)

Most cosmetic ingredients are used in the formulations at 1% or greater. However, many ingredients are used in the cosmetic products which are at lower levels for different reasons because they are contaminants, and also from the by-products of the manufacturing process, residual starting materials, processing aids or others. (Bocca *et al.,* 2014)

In order to assess the exposure of chemicals in PCP, the knowledge regarding the ingredients of products, and the relevant exposure framework, including both the usage frequency and the amount of product used per time. People may be exposed to these elemental chemicals while applying personal care products through multiple exposure routes, including dermal absorption, inhalation and ingestion. (Bennet *et al.,* 2010)

Although, not all the consumers will develop obvious negative effect and advantage from the usage of PCP, but some persons can suffer considerable health problems from the hazardous ingredients. (Klaschka and Rother, 2013)

Heavy metals like lead, arsenic, mercury, aluminium, zinc, chromium and iron are found in a wide variety of personal care products including lipstick, whitening toothpaste, eyeliner and nail colour. Some metals are purposely added as ingredients, while others are contaminants. The United State regulations specify limits for elemental contaminants in colour additives used in cosmetics, but there are no limiting

specifications for these contaminants in cosmetics themselves, except for mercury. (Hepp *et al.*, 2014)

1.2 Problem statement

Many studies have reported that the concentrations of heavy metals are relatively high in the cosmetic products (Nourmoradi *et al.*, 2013). Increasing demand of cosmetics all over the world from teen to adult has increased the awareness related to safety issue and cosmetics industry itself grown by average 4.5% per year in the past 20 years. This industry was able to be one of the well-built industries despite the economic downturn is because of the demand that keep increasing all over the world. (Siti Zulaikha *et al.*, 2010)

Due to an increase in Gross Domestic Products (GDP), it was predicted that the global beauty market sales to reach $265 billion in 2017. The expansion of global beauty market is influenced greatly by the increasing demand from Europe and Asia Pacific region. In some countries for example Canada, heavy metals such as arsenic, cadmium, lead, mercury, beryllium, selenium, and thallium has been banned as intentional ingredients in cosmetics. (Siti Zulaikha *et al.*, 2010)

Many other studies have proven that cosmetic products are a possible source of heavy metal exposure to human beings. Besides, many of studies have been made to determine the level of heavy metals in cosmetics all over the world. They have tested the lead content of 20 shades of lipstick (different brands) sold in the USA. Hence, the study showed that all the lipsticks contained detectable amounts of Pb, with values ranging from 0.09 to 3.06μg/g and an average amount of 1.07μg/g. (Al-Qutob *et al.*, 2013)

A study from Yebpella *et al.*, (2014) reported that, in 2007 the Campaign for Safe Cosmetics published a report drawing attention to the lead content in lipsticks and lip glosses. While in 2009 and 2011, Food and Drug Administration (FDA) had published its own findings on lead in lipsticks. 100% of all cosmetics product tested positive for nickel and over 90% tested positive for both lead and beryllium and on the average contained at least 4 of the 8 metals of concern such as arsenic, cadmium, lead, mercury, beryllium, nickel, selenium, and thallium. (Orisakwe and Otaraku, 2013)

Environmental Working Group found that the average women use 12 products containing a total of 16 unique ingredients every day. Their study "Exposures Add Up" also showed that by using these products on a daily basis, one in 13 women are exposed to ingredients that are known or probable human carcinogens and one in 24 women are exposed to ingredients that are known or probable reproductive and developmental toxins, which can impaired fertility or developmental harm for a baby in the womb or a child. (The Centre for Science and Environment, 2014)

Heavy metals such as lead can be absorbed by children's and women's skin through cosmetic products (Nourmoradi *et al.*, 2013). In addition, recent research has reported that these metals can easily cause many types of skin problems (Alsaffar and Hussein, 2014).

Many cosmetic products contain heavy metals such as lead, arsenic, mercury, cobalt, and nickel as an ingredients or impurities. Recent research has reported that these metals can easily cause many types of skin problems. In most countries, it is legally prohibited to use lead, arsenic, and mercury in skin cosmetic products for example; lead

is prohibited as part of cosmetic compositions in Korea, the European Union, and China.

The maximum allowable level of lead is 20 mg/L in those jurisdictions. There are no specific rules on other heavy metals, such as cobalt, nickel, and copper. It is also reported that those metals can cause allergic contact dermatitis or other skin problems. (Akan *et al.*, 2014)

It is important to prevent skin contamination by toxic substance through a small injury to the skin barrier because this can significantly increase absorption through the skin. The study showed that nickel penetrated damaged skin more easily than chromium powder, which suggests that nickel has a stronger binding capacity to the proteins of the skin. (Al-Saleh and Al-Enazi, 2011)

Cosmetics often had been considered as dangerous by many dermatologists. They may contain more than 10,000 ingredients which are related to many diseases like cancer, birth defects, developmental and reproductive harms.

1.3 Research objective

1.3.1 General objective

The general objective of this study is to determine the existence of heavy metals (lead, cadmium and nickel) in selected personal care products available in the local markets.

1.3.2 Specific objectives

1. To determine the concentration of lead, cadmium and nickel in selected personal care products (deodorant, lipstick, shampoo, hand and body lotion) in the markets.

2. To determine the concentration of lead, cadmium, and nickel in urine among students in USM Health Campus as a targeted consumer.

3. To determine the association between types and frequency of products used and the concentration of heavy metal in urine among targeted consumer.

1.4 Research Hypothesis

Null hypothesis

There is no an association between the types and frequency of product use and concentration of heavy metals lead, cadmium & nickel in urine on targeted consumer.

Alternative hypothesis

There is an association between the types and frequency of product use and concentration of lead, cadmium & nickel in urine on targeted consumer.

1.5 Significance of Study

Despite much attention to the issue of heavy metals in personal care products and cosmetics, the metal continues to be detected in some products. Heavy metals can be difficult to avoid as they are not always listed on the label, so with many new products released into the market every season, it is also hard to keep track of the safety of every product and some products may carry carcinogenic contaminant. Hence, the result of this study is to provide information to the consumer about the existence and hidden of chemical inside the ingredients of PCP. Other than that, provide information to the consumer about the types and the concentration of heavy metals inside the PCP that will effect on health in order to take necessary measures and sensitize the public consumers on the hazards. Besides, reduce the exposure of hazardous substance in PCP that consumed by consumer and provide information on health effect that consumer will gain by using the PCP.

CHAPTER 2:

REVIEW OF LITERATURE

2.1 Heavy Metals

Metals are being produced naturally in the earth's crust, and their contents in the environment can vary according to different regions resulting in spatial variations of background concentrations. The distribution of metals in the environment is governed by the properties of the metal and influences by environmental factors. Of the 92 naturally occurring elements, approximately 31 metals and metalloids are potentially toxic to humans such Be, B, Li, Al, Cr, Mn, Co, Ni, Cu, As, Ag, Cd, Sn, Ba, Au, Hg, Pb and etc. (Morais *et al.,* 2012)

Their toxicity depends on several factors including the dose, route of exposure, and chemical species, as well as the age, gender, genetics, and nutritional status of the exposed individuals. Because of their high degree of toxicity, arsenic, cadmium, chromium, lead, and mercury rank among the priority metals that are of public health significance. (Tchounwou *et al.,* 2012)

Some heavy metals are used in the cosmetics as ingredients (Karimi and Ziarati, 2015). Many regulatory agencies, including the FDA, have not set limits for heavy metal contaminants in the personal care products. (Al-Saleh and Al-Enazi, 2011)

The impurities or ingredients of heavy metal commonly presence in cosmetic and personal care products. As product impurities, their presence in cosmetics is not to be listed on the label (Siti Zulaikha *et al.,* 2010). An impurity is a substance not

intentionally added to a product, but rather is either a by-product of the manufacturing process, formed by the breakdown of ingredients or an environmental contaminant of raw ingredients (The Centre for Science and Environment, 2014). In the other hand, manufacturers ought to assess raw ingredients before adding them to the final products. The ingredients should not be recycled, or contain residual by-products of other industrial processes and impurities. (Karimi and Ziarati, 2015)

Although the presences of the toxic metals were in trace amount, these metals are known to be cumulative poison. Even though slow release or present at very low concentrations, these metals has the capability to be harmful to the biological system and cause human health problems if they are allowed to accumulate over the time (Lanre-Iyanda and Adekunle, 2012). These metals could accumulate in the body organs due to their long half-life. (Siti Zulaikha *et al.*, 2010)

Lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As) are widely dispersed in the environment. These elements have no beneficial effects in humans, and there is no known homeostasis mechanism for them. They are generally considered to be the most toxic to humans and animals. Their adverse effects are associated with exposure include even at low concentrations, are diverse neurotoxic and carcinogenic actions. (Morais *et al.*, 2012)

Lead serves no useful purpose in the body and its toxicity affects virtually every organ in the human body until it reaches toxic levels. Other than that, more than 90% of lead absorbed by human is concentrated in the bones with a half-life of greater than 20 years. (Nourmoradi *et al.*, 2013)

Its ability to mimic or inhibit calcium will affects the actions of calcium-dependent or related processes. Lead also interacts with proteins such as sufhydryl, amine, phosphate and carboxyl groups. The toxic metal primarily will affect the peripheral and central nervous systems, renal functions, blood cells, as well as metabolism of vitamins D (Zirwas and Moennich, 2012). It is also associated with hypertension, reproductive toxicity, developmental effects and neurological disorders.

While, cadmium is one of the major heavy metals found in some natural colours and inorganic pigments of cosmetic products. The half-life of cadmium in human is determined to be 10-35 years (Nourmoradi *et al.,* 2013). The use of cadmium in cosmetics products are due to its colour property as it has been used as a colour pigment in many industries (Chauhan *et al.*, 2010). However, level of exposure that is well thought-out as a high limit is diverse because the cadmium effect may increase or decrease depending on other factors, example the form, type and duration of exposure. (Md *et al.,* 2016)

Besides, nickel is also an essential trace element whose role in the body is yet not fully known. It is contained in many body tissues. Ordinarily, nickel is not known to be toxic to the body as the body does not readily absorb it. However, nickel can combine with carbon monoxide (CO) to form a complex, nickel tetracarbonyl ($NiCO_4$), which is extremely poisonous, even more than CO, which is one of its precursors. Elevated dietary intake of the metal is associated with increased thyroid problems, cancer and heart disease. (Lanre-Iyanda and Adekunle, 2012)

2.2 Personal Care Products

a) Deodorant

Deodorants are the most commonly used personal care products, though with millions of consumers applying these products to their axilla everyday (Zirwas and Moennich, 2012). Antiperspirants are often confused with deodorant. Antiperspirants are astringent substances applied to the skin to reduce or prevent perspiration. While, deodorants are substances applied to the body to reduce body odour caused by bacterial growth and the smell associated with bacterial breakdown of perspiration in armpits, feet and other areas of the body. Antiperspirants primarily prevent sweating by affecting sweat gland s and can as well prevent body odour. Thus, antiperspirant can be said to be a subgroup of deodorants. (Kasim *et al.,* 2013)

According to Zirwas and Moennich, (2012), these two activities are often combined into single products. While deodorants are considered personal care products because they do not change the function of the skin, while antiperspirants are classified as drugs and are therefore subject to rules and regulations set forth by the FDA. Deodorants work by two different mechanisms which are antimicrobial agents decrease the number of bacteria that produce volatile odoriferous substances and fragrances cover any odours that are produced.

b) Lipstick

Lipstick is a lip colouring that has its earliest use dating back to the prehistoric age. In the present day, the use of this product has increased, and the choices of shades of colours, textures and luster have changed and widened. This can be seen from the fact that lipstick is now marketed with hundreds of shades of colours to satisfy the demands of women.

A good lipstick should have persuading characteristics and be multifunctional in order to be acceptable to consumers, such as having a suitable texture and antioxidant properties. Emulsifiers, emollients, binders and colorants are among the variety of components that contribute to the properties of fine lipstick. Texture, melting point and hardness of the lipstick are the influential characteristics that are altered by varying the ratio of the ingredients that are used in the formulation. (Kamairudin *et al.*, 2014)

c) Shampoo

Shampoos are good cleansing agents but not particularly good at maintaining optimum hair appearance or facilitating hair management. Even though shampoos wash and clean your hairs thoroughly and completely dry them, but they also tend to take dry out your hair. Because their purpose is to clean the hair, they end up taking away dirt, oils and moisture, thereby stripping off many nutrients that a conditioner will adds back. The purpose of a shampoo is presumably quite clear, centering on these three aspects which are to cleanse the hair and scalp, to improve appearance of the hair and to improve and facilitate manageability of the hair. (Consumer Voice, 2013)

d) Hand and body lotion

A lotion is a low-viscosity topical preparation intended for application to unbroken skin. By contrast, creams and gels have higher viscosity. While lotion may be used as a medicine delivery system, many lotions, especially hand lotions and body lotions are meant instead to simply smooth, moisturize and soften the skin. These may be used in anti-aging lotions, which can also be classified as a cosmetic in many cases, and may contain fragrances. The Food and Drug Administration voiced concern about lotions not

classified as drugs that need to advertise anti-aging or anti-wrinkle properties. (United State FDA, 2015)

2.3 Routes of Exposure

Cosmetics may have multiple exposure scenarios (Bocca *et al.*, 2014). Dermal exposure is expected to be the most significant route for cosmetic products since the majority of cosmetics are applied to the skin. Dermal absorption of heavy metals is fairly minimal, with absorption of individual elements influenced by many factors including physical-chemical properties of the mixtures. (Adepoju-Bello *et al.*, 2012)

Cosmetics are applied directly to the human body and their use provides potential routes of exposure to elemental contaminants. Products that are partially ingested such as lipsticks may provide direct oral exposure to the contaminants, and cosmetics applied to the surface of the skin such as lotions may provide indirect percutaneous exposure. (Hepp *et al.*, 2014)

In some cases, cosmetics are rinsed-off shortly after application such as shampoos and toothpaste, but in other cases the products are ''leave-on'' such as body lotion, deodorant and lipsticks. From that, it may remain in contact with the skin over several hours. Cosmetics as body emulsions may be applied over a large surface of the body with potential for much greater exposure. Some cosmetic products are applied via spray, presenting the possibility of inhalation. Lipsticks also have the higher risk of direct oral ingestion and aggravating the negative effects of their ingredients. (Bocca *et al.*, 2014)

According to the Occupational Safety and Health Administration (OSHA) of the United States Department of Labour, lead exposure both acute (a few days) and chronic

(several years) will adversely affects numerous body systems and causes many forms of health impairment and disease. (Kasim *et al.*, 2013)

Oral exposure can occur for cosmetics used in and around the mouth as well as from hand-to-mouth contact after exposure to cosmetics containing heavy metal impurities. However, inhalation exposure is typically considered to be negligible (Adepoju-Bello *et al.*, 2012). Besides, oral ingestion is highly possible when using lipsticks. (Ababneh *et al.*, 2013)

The heavy metal concentration varied depending on several factors. The causes for these differences in heavy metal concentration could be due to human activities differ from place, and change from time to time (HE *et al.*, 2011). Children are particularly sensitive to this metal because of their more rapid growth rate and metabolism, with critical effects in the developing nervous system. (Morais *et al.*, 2012)

2.4 Human Health Problems Related To Heavy Metal Accumulation

Heavy metals are toxic because they may have cumulative deleterious effects that can cause chronic degenerative changes, especially to the nervous system, liver, and kidneys, and, in some cases, they also have teratogenic and carcinogenic effects (Alissa and Ferns, 2011). Chronic symptoms are frequently associated with excessive accumulation when heavy metals are not metabolized by the body and the toxics remain in the soft tissues. (Neamtiu and Pop, 2014)

The mechanism of toxicity of some heavy metals still remains unknown, although enzymatic inhibition, impaired antioxidants metabolism, and oxidative stress may play a

role. Heavy metals generate many of their adverse health effects through the formation of free radicals, resulting in DNA damage, lipid peroxidation, and depletion of protein sulfhydryls such as glutathione. (Alissa and Ferns, 2011)

Metal are important for function of physiology of the body. It is a vital in the production of critical enzyme which cannot be replace with other elements. However, some of the metals are characterized as xenobiotics or not required for physiology function of body like mercury and lead which may give toxicity whether in low doses of exposure (Sakinah, 2011). Metals are also likely to enter to the body directly through food and drink. When the metal absorption occurs, it will spread to the organs and tissues. Though the metals will be eliminated through kidneys and digestive tract, it tends to accumulate in some organs such as the bone, liver and kidneys for a period of time and damaging the function of those organs slowly. (Sakinah, 2011)

At higher concentrations, heavy metals have been shown to have negative effects. For example, cancerous breast biopsies show higher accumulations of nickel, chromium, cadmium, mercury and lead than non-cancerous biopsies and several metals act like oestrogen in the presence of some breast cancer cells.

Furthermore, lead poisoning has been a recognized health hazard for more than 2,000 years and the concern about the physiological and behavioural effects of trace metals in human population is very well known. (Yebpella *et al.*, 2014)

Besides, lead which may be an impurity is a proven neurotoxin linked to learning, language and behavioural problems. It has also been linked to miscarriage, reduced fertility in men and women, hormonal changes, menstrual irregularities and delays in puberty onset in girls. At puberty, boys developing testes may be particularly vulnerable

to lead. Pregnant women and young children are also vulnerable because lead crosses the placenta and may enter the fetal brain. (Akan *et al.*, 2014)

The toxicity of lead at high levels of exposure is equally well known, but a major concern of today is the possibility that continual exposure to relatively low levels of lead may also entail adverse health effects. Lead and cadmium are two potentially harmful metals that have aroused considerable concern. In fact, lead has been described as the most severe environmental contaminant to arise in human civilization. (Umar and Caleb, 2013)

Characteristic features of lead toxicity, including anemia, colic, neuropathy, nephropathy, sterility and coma. Exposure to low-levels of lead has also been associated with behavioural abnormalities, learning impairment, decreased hearing, and impaired cognitive functions in humans and in experimental animals. Lead poisoning, especially among children can lead to damages of the central nervous system, causing mental impairment, affecting oxygen transport in the body and causing digestive problems, long term exposure to lead can cause coma, or death. Lead is a poisonous metal that can damage nervous connections especially in young children and cause blood and brain disorders. (Yebpella *et al.*, 2014)

Long-term exposure may result in slowly progressing physical, muscular and neurological degenerative processes that act like Alzheimer's disease, Parkinson's disease, muscular dystrophy and multiple sclerosis. Allergies are common and long-term contact with some heavy metals may even cause cancer. The possibility of skin allergy and contact dermatitis may increase due to the presence of heavy metals in cosmetics. (Karimi and Ziarati, 2015)

Lead and cadmium are two potentially harmful metals that have aroused considerable concern (Yebpella *et al.*, 2014). Cadmium as stated earlier is toxic at extremely low levels. Exposure to high cadmium levels can lead to obstructive lung disease and cadmium pneumonitis which resulting from inhaled dusts and fumes (Ekere *et al.*, 2014). Besides it also may result in a disease known as osteoporosis. Cadmium also causes bone degradation because it affects calcium metabolism. While, in long term exposure may lead to renal dysfunction, hypertension, liver damage, and suppressed immune system. (Josthna *et al.*, 2012)

Ingestion of nickel may cause hyperglyanemia, depression of the central nervous system and kidney damage. Nickel metal is frequently responsible for allergic skin reactions and has been reported to be one of the most common causes of allergic contact dermatitis (ACD) as reflected by positive dermal patch tests (Basketter *et al.*, 2003). Nickel dermatitis produces erythema, eczema and lichenification of the hands and other areas of the skin (Yebpella *et al.*, 2014). As regards Ni, this element is considered the primary source in causing ACD with a prevalence of 20% in females and 1% in males.

2.5 The Metabolic Effects of Heavy Metals

The knowledge gained about the homeostasis of heavy metals has been substantial over more than a decade. Although they have no known metabolic function, when present in the body they disrupt normal cellular processes, leading to toxicity in a number of organs. They are relatively poorly absorbed into the body, but once absorbed are slowly excreted and accumulate in the body causing organ damage. Thus, their toxicity is in large part due to their accumulation in biological tissues, including food animals such as fish and cattle as well as humans.

Distribution of heavy metals in the body relies on its binding to carrier molecules in the circulation. Metallothioneins are small proteins rich in cysteine residues, which accounts for the unique metal binding properties of metallothioneins and play a major role in the dispersal and storage of heavy metals in the body. They also accumulate in hair and toenails such as arsenic and mercury, which both can be used as indicators of long-term exposure in population studies. These heavy metals have a slow excretion rate from the body, as indicated by their long half-life time. For example, half-life of lead is 27 year in cortical bone and 16 year in cancellous bone, while half-life of cadmium is 10–30 years), compared with their uptake rate. (Alissa and Ferns, 2011)

CHAPTER 3:
METHODOLOGY

3.1 Study Design

The design for this research was an experimental study. This study was approved by the Research Ethics Committee (Human) Universiti Sains Malaysia. Human urine samples were taken from nineteen healthy subjects. Heavy metals were measured from a single urinary sample instead of a 24-hour collection which better takes into account short-term variability of metals excreted and urine dilution (Mendy *et al.*, 2012). For the sample analysis, the urine samples were analysed from the subjects according to their usage itself using all or either one of the PCP. Then, the samples were extracted using acid digestion with concentrated nitric acid and perchloric acid. The urine was analysed by Atomic Absorption Spectrometry (AAS) to determine the concentration of lead, cadmium and nickel in the urine samples.

Besides, eight PCPs include deodorant, lipstick, shampoo and hand and body lotion were purchased from the local markets. The selection criteria of each product were selected randomly includes locally available and international brands. The product was analyzed to determine the existence and the concentration of heavy metals. The flow chart of research process is shown in Figure 3.1.

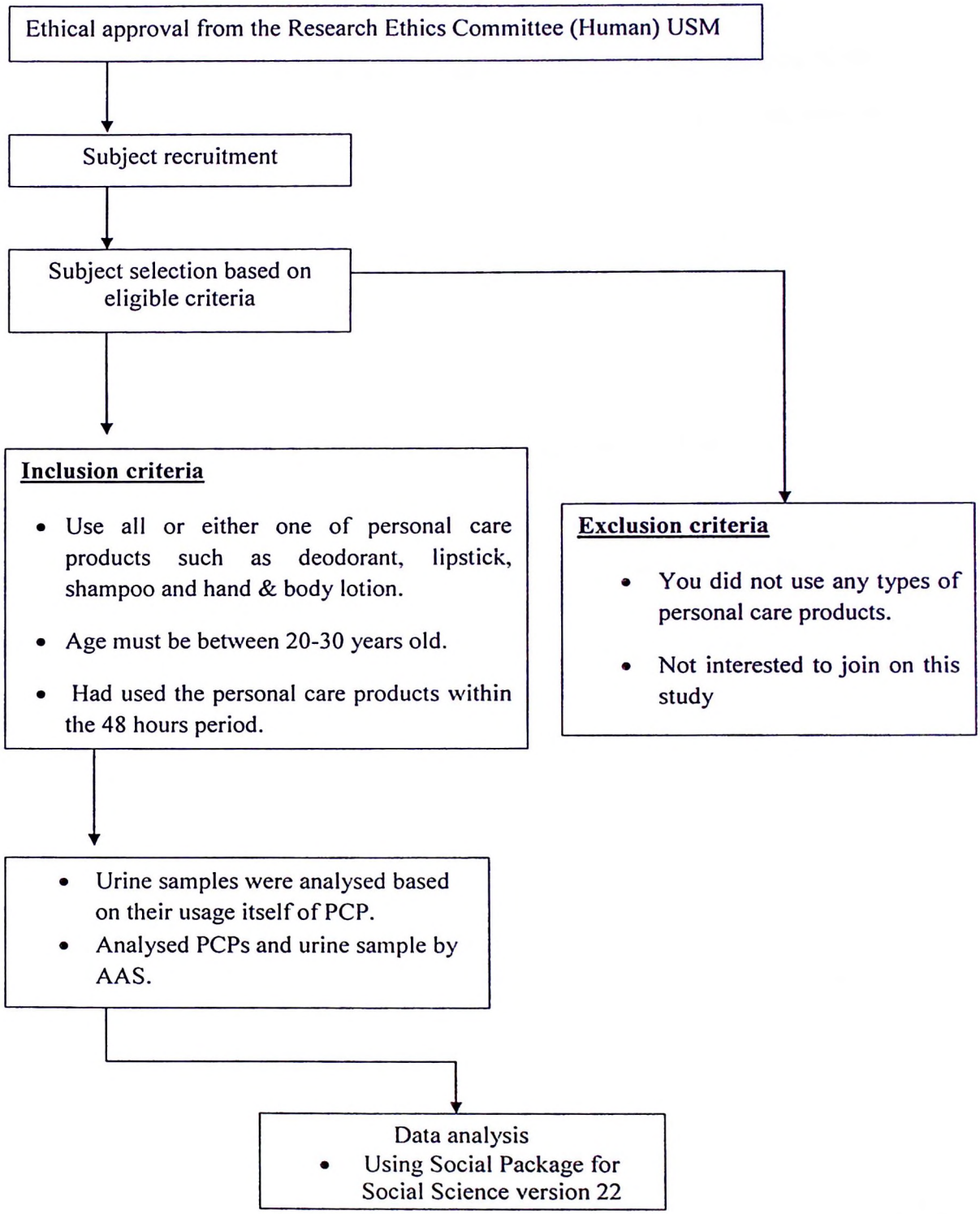

Figure 3.1: Flow chart of sampling process

3.2 Sample population

The target population of this study was a student of Universiti Sains Malaysia Health Campus. The subjects' selection was selected based on their volunteer and interested to join this study.

3.3 Study Period

This study period was from November 2015 until May 2016.

3.4 List of chemicals, biological materials, instruments and apparatus

Table 3.1: List of chemicals

No	Chemicals	Company/ sources
1	Nitric acid	Sigma Aldrich
2	Perchloric acid	Sigma Aldrich

Table 3.2: List of biological materials

No	Biological materials	Company/ sources
1	Urine	Healthy human

Table 3.3: List of instruments and apparatus

No	Instruments	Company/ sources
1	Atomic Absorption Spectrometry (AAS)	Almer Perkin A Analyst 200
2	Whatmann Filter 0.45 μm	Merck
3	Adjustable Hot Plate	Ems-Hp-700, Erla®
4	Polyethylene containers/ Urine collection containers	SAS Worldwide Sdn. Bhd.
5	Measuring Cylinder 50mL and100mL	HmbG Ev 20°C MC
6	Beaker 50mL, 100mL	Pyrex, England
7	Volumetric flask, 100mL	Iwaki Pyrex®

3.5 Determination of Heavy Metals in Selected Personal Care Products

The selected products with heavy metals used in this research were deodorant, lipstick, shampoo and hand and body lotion. The product was chosen according to the heavy metal content as reported in previous study conducted by Zulaikha *et al.*, (2015). Table 3.4 show the lists of heavy metals detected in cosmetic and personal care products. The most common heavy metals detected in cosmetic products are lead (Pb), cadmium (Cd), mercury (Hg), chromium (Cr), nickel (Ni) and copper (Cu).

Table 3.4: List of heavy metal detected in cosmetic and personal care products

Heavy metal detected						Products
Pb	**Cd**	**Hg**	**Cr**	**Ni**	**Cu**	
/		/		/	/	Cream (face, body, hand), dried powder, body lotion, skin whitening, sunscreen, lipstick
/					/	Deodorant
/	/	/	/	/	/	Shampoo

Source: Zulaikha et al., (2015)

3.5.1 Sample digestion

The sample was analyzed by using acid digestion from the study done by Sonwane and Salve, (2014). About 1.0 g of each sample was digested in 5mL mixture of concentrated acid HNO_3: $HClO_4$ (3:2) for 2-3 hours on a hot plate at 90°C. Then 5mL of acid mixture was again added and then heated for 2-3 hours to complete the digestion. The above digested samples were cooled and about 5mL ultra pure water was added and mixed

well and volume made up to 25mL in volumetric flask. The solution finally was filtered through Whatman filter paper (Number 41). The clear solution was used for metal quantification.

3.6 Determination the Heavy Metals Concentration in Urine

3.6.1 Subject recruitment and Data collection

Total of nineteen urine samples were collected from nineteen subjects. Based on variable of single mean formula, from (Blaurock-Busch *et al.*, 2011) found that Standard Deviation (SD) for Nickel is 2.09mcg/g. The assumption of different between groups, Δ is estimated 1.03mcg/g and 20% from sample size will be added to sample size for unresponsiveness subjects.

This sample size calculation was determined by using Single Mean Formula;

$$= \left(\frac{1.96\,\sigma}{\Delta}\right)^2$$

Hence, the sample size calculation = $\left(\frac{1.96\ (2.09)\ \text{mcg/g}}{1.03\text{mcg/g}}\right)^2$

$= 15.8$

20% from sample size will be added to sample size

$= 3.16 + 15.8$

$= 19$ samples needed