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THE ASSOCIATION OF MERCURY FROM DENTAL AMALGAM WITH URINARY SELENIUM

By

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Declaration

I, Rukaia Aljabo student number 3481175 declare that this Thesis THE ASSOCIATION OF MERCURY FROM DENTAL AMALGAM WITH URINARY SELENIUM is my own work, that it has not been submitted previously for any degree or examination at any other university, and that all the sources I have used or quoted have been indicated and acknowledged as complete references.

Rakaia Aljabo

Signed…… Date……

Dedication

To my family

Acknowledgements

First of all, I thank God for all the blessings He has endowed me including the completion of my thesis.

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ABSTRACT

Background: Dental amalgam has been the traditional material for filling cavities in teeth. Mercury (Hg) is a component of dental amalgam, from where it is continuously released and deposited in different tissues, mostly in the brain and the kidneys. Selenium is an important essential element in the human body. Mercury exposure from dental amalgam fillings associated with reduced the levels of selenium.

Aims and objectives: The aims of the current study were to investigate the leaching of mercury from dental amalgam fillings and also to investigate the relationship between the leached mercury from dental amalgam fillings and selenium concentrations in the bloodstream. The objective was to determine the mercury from dental amalgam fillings and urinary selenium levels.

Methods: Samples were collected from patients attending Tygerberg Oral Health Centre, Cape Town (South Africa). 107 patients who had 1-12 dental amalgam fillings provided the samples of urine, buccal swabs and did the chewing gum test. The samples were analysed by using inductively coupled plasma-mass spectrometry. The data were analysed by IBM ($p<0.05$) test with an SPSS computer software package version 24. The study involved analyses of samples of urine (n=107), chewing gum and buccal swabs (n= 102).

Results: The median urinary concentrations of mercury and selenium in female and male samples were 0.40 µg/L, 0.60 µg/L Hg and 26.29 µg/L, 29.32 µg/L Se respectively. While the median Hg concentrations in chewing gum test and buccal

swabs samples in female and male were 2.04 mg/g, 1.89 mg/g Hg and 0.16 μg/L, 0.09 μg/L respectively.

Conclusion: The excretion of urinary selenium concentration was influenced by concentration of mercury in urine and age of participants but not affected by concentrations of mercury in buccal swabs, chewing gum and gender of participants.

Keywords: Amalgam Mercury Selenium Urine Metabolic Human

List of Abbreviations

Agilent 7900 ICP-MS	Inductively coupled plasma-mass spectrometry.
CH3Se (CH2) (NH2) COOH	Selenomethionine
CH3Hg	Methyl mercury
CVD	Cardiovascular disease
CVA	Cerebrovascular disease
DNA	Deoxyribonucleic acid
FDA	Food and Drug Administration
FFQS	Food Frequency Questionnaires
GPx	Glutathione disease
GSHPx	Glutathione peroxidase
Hg	Mercury
Hg^0	Mercury vapour (elemental mercury)
Hg (II)	Inorganic mercury
HNO_3	Nitric acid
H_2O_2	Hydrogen peroxide
HCL	Hydrochloric acid
µg/L	Microgram per litter
µg/g	Microgram per gram
ROS	Reactive oxygen species
SE	Standard Deviation
Se	Selenium

$(Se)^{-2}$	Selenide
$(SeO3)^{-2}$	Selenite
$(SeO4)^{-2}$	Selenate
(Sec) NH2 COOH	Selenocysteine
US	United States
WHO	World Health Organisations

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Chapter One

1.1 Introduction and Literature review

Amalgam has been widely used in dental fillings for more than 200 years and its excellent clinical track record is well documented (Pizzichini *et al*., 2003; Clarkson *et al.*, 2003; Saber-Tehrani *et al.*, 2007; Roberts and Charlton, 2009). Generally, an amalgam is a mixture of a metallic element containing silver alloy and mercury. When the powdered silver alloy is mixed with the liquid mercury, it forms a paste which has been accepted as part of dental restorative treatment for more than 170 years (Powers and Wataha, 2014). Amalgam is composed of 50% metallic mercury (Hg) and 50% alloy, with the alloy component consisting of 35% silver (Ag), 9% tin (Sn), 6% copper (Cu) and a trace unit of zinc (Zn) (Mutter *et al*., 2004;Katchmar *et al.*, 2012 and Zwicker *et al*., 2014).

According to Chin *et al.* (2000), dental amalgam is one of the materials used in dentistry for varieties of application to restore teeth diseased by dental caries as fillings and crowns. The widespread application of mercury dental amalgam is highly connected to its physical and chemical properties as well as its ease of use and low cost compared to other dental restoration materials. The consequence of 50% metallic mercury (Hg) in dental amalgam include Hg vapour evolution, increased mercury load in urine, faeces, exhaled breath, saliva, blood and various organs and tissues such as kidney, pituitary gland, liver, and brain (Richardson *et al*., 2011).

1.2 Application of Hg in dental amalgam

Hg is a silver coloured shiny liquid natural metallic element present on earth. It is found in a variety of chemical forms, in rocks, soil, water, air, plants, and animals. Hg in its elemental state is the only metal that is liquid at room temperature. It has physical properties such as low viscosity, high density, high electrical conductance, and a reflective surface (Park and Zheng, 2012). Hg is readily vaporized at room temperature (0.0013mm at 20ºC) (Brownawell *et al.,* 2005)

Hg sources can be found from dental amalgam, air and food such as rice and freshwater fish (such as bass, swordfish tuna, shellfish, and trout) as a pollutant. Fungicides, insecticides, laxatives, paints, pesticides, tap and well water, thermometers, thermostats and vaccines are also sources of Hg (Rothenberg *et al.*, 2015).

The two major ways of human exposure to Hg are the placement of dental amalgam fillings (mercury vapour Hg°) and the consumption of fish (MeHg) (Díez *et al.*, 2008; El-Safty *et al.,* 2012). Hg can pollute the environment in three different forms;

1. Continuous elemental Hg vapour (Hg°) emission from amalgam fillings. Dental amalgams are the most common source which is a stable monoatomic gas Clarkson *et al*., 2003).

2. Inorganic Hg is sourced from the chemical industry and coal-burning power plants. They are the major source of metallic and inorganic Hg which after being emitted into the air, enters the water during precipitation and is converted to MeHg by microorganisms (Jedrychowski *et al*., 2006). People can be exposed to inorganic

Hg from silver Hg amalgam in their dental fillings, inorganic mercury present in mercury-based skin creams and infant teething powders or from the latex paints (Iqbal *et al.*, 2012).

3. Organic Hg (MeHg) exposure is usually the result of consumption of contaminated fish foods. Ethyl mercury which is another source of organic Hg (ethyl mercury is from thimerosal) is a preservative used in some paediatric vaccines. In addition, Hg is a highly reactive heavy metal that is rarely found as a free element in Nature (Guallar *et al*., 2002 and Houston, 2007).

1.3 Different ways to ingest Hg in the body

In vapour form, it is easily taken up by the body. The Hg present in an amalgam restoration exists in the metallic form and is not easily absorbed from the digestive system when swallowed. It is completely bound with the other metals present in the amalgam because the reaction is a chemical one that combines it with the metals to form an alloy. However, the metallic form of Hg can damage the immune system and can give rise to undesirable immunological responses (Bartova *et al.*, 2003; Pizzichini et al., 2003; St John, 2007).

Approximately 80% of inhaled and 0.01% of ingested elemental mercury is absorbed (Park *et al.*, 2012). For inorganic Hg, absorption of inhaled versus ingested mercury is equal (10%), whereas 2% to 3% of inorganic mercury is absorbed through the skin (Solenkova *et al.*, 2014). Organic Hg (most commonly found in fish), if ingested or inhaled, is almost completely (95%-100%) absorbed

and is the most toxic form of Hg that is distributed to all organs and tissues including the brain and the placenta (Rodrigues *et al*., 2014).

Hg vapour has high bioavailability in the mammalian respiratory tract, but in most animal species there is the negligible absorption of the liquid Hg° from the digestive tract. By contrast, there is almost 100% absorption of ingested MeHg, limited only by the efficiency of food breakdown in the stomach. Some Hg species are absorbed by organisms and transferred rapidly to the blood, while other species are excreted through the urine such as elemental Hg (Caussy *et al*., 2003; Daniel Martín-Yerga, 2013).

1.4 Impact of Hg amalgam on human health

The release of elemental Hg vapour is stimulated by chewing, tooth brushing, bruxism and the ingestion of hot foods and liquids. Most inorganic Hg released from dental amalgam fillings is excreted through the urine (Mackert *et al.*, 1997 Nicolae *et al.*, 2013).

However, number of teeth, number of surfaces, baseline Hg release, magnification factors, such as eating and tooth brushing, oral breathing habits, nose-mouth breathing ratio, inspiration-expiration ratio, swallowing, inhalation absorption, ingestion absorption and body weight are known variables that can affect the release of Hg from amalgam fillings or restorations (Varkey *et al.*, 2014).

Released Hg enters the body by inhalation and absorption of vapour, by swallowing abraded amalgam fragments, by direct absorption across the oral mucosa and by migration through teeth into tissues (Henderson *et al.*, 2001). It can lead to gum; gastrointestinal tract, lung, liver and kidney diseases; auto-immunity; foetal

abnormalities; and neurological diseases, including multiple sclerosis, motor neurone disease and other neuromuscular disorders (Reichl *et al.,* 2001). Furthermore, studies have suggested that inorganic Hg may be absorbed through the skin, and this involves the transport of mercury across the epidermis and via the sweat glands, sebaceous glands, and hair follicles (Park and Zheng, 2012).

Elemental Hg can be oxidized by the hydrogen peroxide–catalase pathway in the body to its inorganic divalent form, after exposure to elemental Hg or inorganic mercury compounds, the main route of excretion is via the urine (Risher, 2003). Gaseous elemental Hg (Hg^0) can be rapidly oxidized to inorganic Hg (II) in the blood and become a nephrotoxin; the remaining vapour form can also diffuse through the blood-brain barrier and become a neurotoxin following oxidation in the brain (Khan and Wang, 2009).

Hg vapour is oxidized to inorganic mercury, and its elimination is through the exhaled air or as inorganic Hg in the urine from the kidneys or through sweat, and saliva (Spencer, 2000). In contrast to this claim, studies have shown that Hg from dental amalgam is transformed into organic Hg compounds by microorganisms in the human gastrointestinal tract which is eventually eliminated through the faeces (Mutter, 2011; Patrick, 2002).

Cohen and Penugonda (2001) as well as Yip and Cutress (2003) reported that Hg vapour enters the body mainly through the lungs and is dangerous because approximately 80% of the inhaled Hg is absorbed into the blood and then distributed to various body tissues. The potential health effects of mercury in dental amalgams remains a subject of public debate. The possible accumulation of Hg deposits in the brain, kidneys, liver and other body tissues over an extended period remains an

issue because of the imminent danger. The US Public Health Service and the Food and Drug Administration (FDA) suggest that based on available evidence, there is no proof of Hg toxicity from dental amalgam to the patient, other than in cases of allergy Clarkson *et al*., 2003 and Bates, 2006).

MeHg easily penetrates the blood-brain barrier and causes damage to the central nervous system, particularly in fetuses (Díez, 2008). It accumulates and biomagnifies in the aquatic food chain; consequently, fish and seafood consumption is the major pathway by which humans are exposed to MeHg (de Souza *et al.*, 2013).

According to the conclusions of independent evaluations from different States in the United States health agencies (Luglie *et al.*, 2005; St John, 2007), the release of mercury from dental amalgam does not present any risk or damage to the general population. However, in some way Hg released from amalgam could cause an increase in bacterial resistance to antibiotics thereby increasing the susceptibility to bacterial diseases resistant to usual drug therapy (Donovan *et al.*, 2009). There is no proof that the emission of a minute quantity of Hg released from amalgam has a negative effect on kidney function, nerve tissue or the immune system (Donovan *et al.*, 2010).

Studies have also already begun to discover how Hg in amalgam and its vapour can be altered into MeHg within the human body. Bacteria in soil and water can convert mercury into MeHg, a form of the element sometimes consumed by fish and shellfish. This form is the most harmful to people and the natural world, because of its ability to take part in biochemical reactions and accumulate in the food chain.

Pregnant women and children are advised not to consume seafood that might contain methylmercury (Masih *et al.,* 2015).

Hg is a potent neurotoxin, even at extremely low levels of exposure, it can cause permanent harm to the human central nervous system (Park *et al.,* 2012). At higher levels, Hg can damage vital organs like the lungs and kidneys. Short-term exposure to high levels of metallic Hg vapours may cause effects, including lung damage, nausea, vomiting, diarrhoea, an increase in blood pressure or heart rate, skin rashes and eye irritation (Masih *et al.,* 2015).

In addition, there are reports that dental personnel are at risk of exposure to metallic mercury when handling amalgam for restorations (Fuks, 2015). Early reports of toxicological risk analysis of occupational diseases in dentists showed that work practices were associated with Hg exposure in dental personnel and that symptoms associated with renal function, reproductive processes and allergies were related to chronic Hg exposure (Nagpal *et al.,* 2017).

1.5 The recommendations as regards the intake of Hg

The World Health Organization estimates that the standard absorbed a dose of mercury from amalgams is 1–22 µg/d, with most people incurring doses of less than 5 µg/d. Considerable variation exists, with an upper range of ~100 µg/d associated with gum chewing. Exposure variables include the total amalgam surface area, the physical and chemical composition of the amalgam, the mechanical stresses of chewing and bruxism, the proximity to other metals, and such oral conditions as regards temperature, pH, and negative air pressure. The FDA assumes an exposure of 1–5 µg/d in its current amalgam role (Homme *et al.,* 2014; Rathore *et al.,* 2012).

According to Maqbool *et al.,* (2014), no level of Hg exposure can be considered harmless. Furthermore, the researcher believes that dental amalgam accounts for 84 % of the daily exposure to Hg.

A different nother study on U.S men aged 48–78, with an average of 19.9 amalgam surfaces had a mean urinary Hg concentration of 2.88 μg Hg/L. The study estimated that the urinary Hg increased by approximately 0.1 μg Hg/L for each surface of dental amalgam. For individuals with no amalgam fillings, the mean urinary Hg concentration was 0.70 μg Hg/L (Nicolae *et al.,* 2013).

Measuring exposure and toxicity of Hg can be measured by nail, hair, blood, and urine for distinguishing between MeHg exposure and inorganic Hg exposure (Caussy *et al.,* 2003). Also, it can be measured as the concentration of Hg in breast milk, hair and saliva (Khammar *et al*., 2015). Hair has been used and suggested to be a good biological marker of environmental exposure to Hg by many investigators (Harakeh *et al*., 2002).

Hg levels measured in urine best represent exposure to elemental Hg, while mercury levels measured in blood best represent exposure to MeHg; thus, without both, it is difficult to create a complete picture of Hg exposure (Yard *et al.,* 2012).

Urine Hg concentration is a good measure and comparatively simple. Moreover, it is a quick means of identifying those exposed to Hg. However, because organic mercury represents a very small portion of urine Hg is more useful for the analysis of metallic or inorganic Hg compounds (Zwicker *et al.,* 2014 andYe *et al.,* 2016).

Geier and co-workers (2012) studied the level of Hg in children with and without amalgam fillings. This study found that the extent of excretion of Hg in the urine is directly related to the exposure of Hg from dental amalgams in a dose-dependent

fashion (Geier *et al.*, 2012). Also, the findings from the Geier study are consistent with previous studies examining Hg exposure from dental amalgams in the Northeast US and New England. Similarly, other investigators found that the number of amalgam surfaces was directly related to the emission rate of Hg into the oral cavity and to the excretion rate of Hg in the urine (Dunn *et al.*, 2008). Furthermore, studies were carried out on 5418 Canadians between 6 and79 years old, to determine the overall urinary Hg level in the Canadian general population in relation to the number of dental amalgam surfaces.

Studies such as the one by have examined buccal cells to determine the exposure to Hg (van Wieren-de Wijer *et al.*, (2009) have been examining buccal cells to determine the exposure to Hg. For instance, buccal cell (inside cheek scrape) samples were collected from dentists and dental assistants, using by means of a cotton swab and frozen for subsequent analysis. Findings may represent a genetic predisposition to an altered biological response to Hg that could be reflected in an altered disposition to Hg-associated health risks in human subjects individuals (Woods *et al.*, 2005). The buccal swab of mercury-containing DNA was also used in the study and was stored in a laboratory freezer (Wang, 2011). The measurement of Hg in the urine, hair, and saliva shows that Hg levels in urine are related to the number of amalgam fillings, and the number of amalgam surfaces. Hg levels in the urine of patients with and without amalgam fillings are displayed, showing higher mercury levels in individuals with amalgam fillings. Levels of concentrations of Hg in the hair depend on the meals containing fish consumed per month and, Hg in saliva is not a suitable material for biological monitoring to assess Hg exposure in children, at least not in cases of low Hg exposure (Pesch *et al.*, 2002). MeHg level

can also be measured in the body and is usually determined by analysis of Hg in blood and scalp hair which has several characteristics for being an ideal tissue for epidemiological study as it can be painlessly removed, normally discarded, and easily collected (Lee *et al.*, 2000).

In a Canadian study, total blood Hg levels were measured in 5319 participants between the ages of 6 and 79 years. Results obtained showed that fish and shellfish consumption significantly influenced blood Hg levels, as did alcohol consumption and the presence of dental amalgam fillings (Lye *et al.*, 2013).

Total blood Hg is a principal biomarker for MeHg exposure, but it is assumed that a small amount of blood MeHg is metabolised to IHg and excreted in the urine. In blood, the elemental mercury is oxidized to mercuric Hg partly under the influence of catalase, and this influences the brain uptake of Hg (Syversen and Kaur, 2012).

Hair samples can be used to determine Hg levels in individuals who had amalgam fillings. A study by (Cabaña-Muñoz *et al*., 2015), in Canada , was carried out on 55 hair samples including 42 females with amalgam fillings and 13 female controls, reported . It was found that Hg levels increased in the hair of women who had dental amalgam fillings for more than ten years, in comparison with women control subjects without amalgam fillings. The values obtained agree with other studies.(Mutter *et al.*, 2004) that had reported 2–12 times higher Hg values in the body tissues of patients with dental amalgam fillings (Cabaña-Muñoz *et al*., 2015) .

1.6 Interactions between Hg and selenium

Selenium (Se) is a metalloid with the atomic number 34 and belongs to group 16 in the periodic table. It was discovered in 1817 by the Swedish chemist Jöns Jakob

Berzelius (1779–1848) (Bjørklund *et al.,* 2017). Se is essential to human and other animal health in trace amounts but is harmful in excess. Therefore, it is necessary to control its intake by humans and other animals to avoid toxicity. In general, Se has the capability to delays the onset of mercury toxicity or reduces the severity of the effects of inorganic forms of Hg and MeHg (Dye *et al.,* 2005). It is known to affect the distribution of Hg and also to reduce toxicity induced by Hg in experimental animals (Rooney, 2007). There is evidence that selenium in plasma forms a complex with inorganic Hg, which then binds to selenoprotein-P and consequently prevents Hg uptake by the kidneys. Although the role of selenoprotein-P is not well understood, however, it is suggested that it may play three separate roles including antioxidant defence; transport of selenium and as a natural heavy metal chelator (Chen and Berry, 2003 and Rooney, 2007).

Animal studies have demonstrated a protective effect of Se against Hg toxicity from dental amalgam (Høl *et al.*, 2002). Hg deposition in the tissue is mostly bound to Se, which means that the Se is no longer available for the body. Therefore, amalgam may aggravate a latent deficiency of Se (Mutter, 2011; Cabañero *et al.*, 2006). Besides, Se protects from Hg and MeHg toxicity by preventing damage from free radicals or by forming inactive SeHg complexes (Cabañero *et al.*, 2007). Hence, following an increased understanding of the biological role of Se, evidence suggests that the concept of Se protection against Hg toxicity occurs by ensuring sufficient amount of bioavailable Se so that normal selenoprotein and selenoenzyme synthesis is maintained (Mulder *et al.,* 2012;Sørmo *et al.,* 2011).

However, the effects of Hg poisoning have been identified by the quantity and rate of absorption in the body as well as the chemical and physical properties. The main target sites for Hg toxicity is the kidney, liver, digestive system and the central nervous system. This may have provided a higher Se dose to produce selenium toxicity in combination with Hg due to the action on thiols to generate superoxides and as a result of increased thiol oxidation, redox cycling and superoxide generation, in a dose dependent manner. It has been well documented that Hg and Se interact in the body of mammals, and that co-administration of both reduces the toxicity of every alternative (Jureša *et al.,* 2005 ;Agarwal and Behari, 2007). If Hg replaces Se, it will affect the neuroendocrine and nervous systems. The uniqueness of Hg is its ability to inhibit Se-dependent enzyme activities in brain tissues (Kehrig *et al.*, 2013) by hypothetically inhibiting the biochemical functions involving selenoenzymes (Carvalho *et al.,* 2008).

Hg^2 is known to interact with Se (Se^{4+}) in the body, and the co-administration of both reduces the toxicity of each element. In fact, Se^{4+} is reduced to selenide in blood cells and forms HgSe with Hg^{2+}. However, the mechanism underlying the protective action of selenite against Hg toxicity remains not completely resolved. Many lines of proof indicate that selenide (produced *in vitro* from Se^{4+} within the presence of glutathione (GSH) forms a complex (metal–Se/S) which then binds to selenoprotein P (Sel P) to form a ternary complex, (metal–Se/S)–Sel P within the bloodstream (Farina *et al*., 2003; García-Sevillano *et al.,* 2015).

Similarly, Hg and Se bind together to form complexes, and Hg selectively binds to Se to form insoluble Hg selenides. It is the mercury-selenide precipitates that have an extremely low solubility, and thus they are thought to be metabolically inert.

Therefore, it can be assumed that not only does Se affect Hg bioavailability, Hg could also affect Se bioavailability (Raymond and Relaston, 2004).

Hg propensity for Se sequestration in the brain and endocrine tissues may inhibit the formation of essential Se-dependent proteins (selenoproteins) (Mulder *et al.*, 2012). According to the recent *in vitro* studies by García-Sevillano *et al.* (2015), Hg sequestration in the bloodstream and consequent accumulation might be reduced by the use of an erythrocyte-derived reduced metabolite of Se together with the plasma selenoprotein-P (SelP) (Falnoga *et al.*, 2007). Selenoproteins may have two important roles in protecting against Hg toxicity. First, they may bind more Hg through their highly reactive selenol group, and second, their antioxidative properties help compromise the reactive oxygen species induced by Hg *in vivo*. The researchers found that the serum Se concentrations associated with glutathione peroxidase GSH-Px and selenoproteins were two times higher in the Se-exposed group than in the control group. A disruption of the GSH system by Hg leads to GSH depletion and cell destruction (Dufault *et al.*, 2009; Chen *et al.*, 2006).

1.7 Major sources of Se

Rocks are the most important natural source of Se in the environment. Rocks make up the surface of the planet. Se is found in soil in the form of elemental Se, such as selenite salts and ferric selenite it's an organic form, selenite and selenate forms which are common in most soils. (Mehdi *et al.*, 2013 ; El-Ramady *et al.*, 2015) Se concentration in plants is related to Se levels in the surrounding soils Se is also found in water and air (Dumont *et al.*, 2006).

1.8 Se in food

The main route of Se intake is via the diet. The total amount of Se in the diet varies widely depending on the food type and composition. The major sources of Se are typically provided by bread and cereals, meat, fish, eggs, milk and dairy products (Dumont *et al.*, 2006).

Selenomethionine: found in plant sources (notably cereals), Se yeast, and other Se supplements, selenocysteine: found in animal foods (from their selenoproteins), selenoneine newly discovered the major Se compound in fish such as tuna and mackerel lower concentrations in squid, tilapia, pig, and chicken. *Se*-methylselenocysteine and γ-glutamyl-*Se*-methylselenocysteine: found in plant sources such as selenium-enriched yeast, garlic, onions, and broccoli, sodium selenite and selenate components of dietary supplements; selenate occasionally appears in water supplies. Some selenate is found in fish and plant sources (e.g., cabbage). (Rayman, 2008; Rayman, 2012; Roman *et al.*, 2014).

1.9 Physical and Chemical Forms of Se

1. Organic Se (Selenomethionine CH_3Se $(CH_2)_2CH$ (NH_2) COOH and selenocysteine (Sec) NH_2) COOH are the main organic forms of Se.

2. The inorganic forms are selenite ($SeO3^2$), selenide (Se^2), selenate ($SeO4^2$) and the Se element (Se) (Mehdi *et al.*, 2013). Selenite and selenate are inorganic forms of Se used as dietary supplements (Gromadzińska *et al.*, 2008).

Se toxicity is related to chemical forms in which exposure to selenious acid or Se oxide has caused serious toxicity. Oxidation states of Se including selenate (Se^6),

selenite (Se^4), selenide (Se^2) and elemental selenium (Se^0) are commonly found in the environment. Generally, Se^0 is prepared on a nanoscale by reduction of higher oxidation states to other allotropic forms (Mishra *et al.,* 2011 ;Shakibaie *et al.*, 2013).

1.10 Determinations of urinary Se

Food frequency questionnaires (FFQS) is a good procedure that has been used for the assessment of Se from food intake for a long time (Roman-Viñas *et al.,* 2010). On the contrary, some authors (Serra-Majem *et al.,* 2009) have reported that food questionnaires do not give a reliable result for Se measurement in food intake. Besides, it is also an expensive procedure. For these reasons, scientists agree that using biomonitoring data of Se is a better approach to assess average daily exposure by whole blood, urine, hair and nail (Noisel *et al.,* 2014). Using toenail and fingernail for the assessment of Se concentrations provide a valid and objective biomarker of long-term (approximately 1 year) whereas, serum and plasma provide a short-term measure of Se in the body (Park *et al.*, 2012). Se concentrations are typically measured in plasma, serum, whole blood, amniotic fluid, and urine as well as hair and toenails (reflecting longer-term Se stores) (Mistry *et al.,* 2012).

Urinary Se has been shown to be a good indicator of the total Se absorbed from all sources, and to correlate well with serum and toenail Se measurements (Christian *et al.,* 2006). However, the major metabolites of Se excreted in urine vary considerably from person to person (Jäger *et al.*, 2016). Furthermore, the relative proportion of the urinary metabolites was altered by the source of selenium ingested (Kwak *et al.*, 2016). Se was known only as a poison but is now known to be essential for normal function of many systems in the body. Se deficiency can have adverse

consequences on the liver, skeletal, muscle and the brain (Raymond and Ralston, 2004).

1.11 Importance of Se

Se is an essential micronutrient for animals and humans. To date, the major biological functions of Se are attributed to its antioxidative properties and its role in the regulation of thyroid hormone metabolism and cell growth (Benstoem *et al;* 2015). Considering extremely binding affinity between Hg-Se and the ability of Se to cross the blood-brain barrier, it is reasonable to suspect the HgSe interaction may have a role in developmental pathophysiology (Laura and Raymond, 2004).

Se is an essential component of selenoproteins which play an important role in many biological functions such as antioxidant, defence, the formation of thyroid hormones, DNA synthesis, fertility and reproduction. However, Se can be differently bound in the organism into various metabolites and can have an effect on metabolism. Se also has a role, besides vitamin E, in muscle function by improving endurance and recovery and slowing the ageing process (Suttle, 2010; Mehdi *et al.,* 2013).

Concentrations of free Se are greatest in the renal cortex and pituitary gland, followed by the thyroid gland, adrenal glands, testes, ovaries, liver, spleen, and cerebral cortex. Se concentrations are typically measured in plasma, serum, whole blood, amniotic fluid, and urine as well as hair and toenails (reflecting longer-term Se stores) (Mistry *et al.,* 2012).

Se and fish containing omega-3 fatty acids antagonize mercury toxicity. Hg diminishes the protective effect of fish and omega-3 fatty acids and binds to Se forming seleno-mercury complexes, reducing Se availability for glutathione

peroxidase (GPx). Insoluble complexes of mercury with selenium reduces Se availability, which is a necessary cofactor for glutathione peroxidase activity to break down hydrogen peroxides and various other toxic peroxidation products, which further increases the risk for cardiovascular disease (CVD) and Cerebrovascular accident (CVA) (Houston, 2011).

Many of the lately found Se-containing enzymes and proteins are obviously essential for normal growth, development, and metabolism of an organism. Se serves in the antioxidant defence system through the glutathione peroxidase protein family (GPX1, GPX2, GPX3, and GPX4), and the mammalian thioredoxin reductases (TR). Also, iodothyronine deiodinases (types 1–3) are now known to be Se-containing proteins (Kantola *et al.*, 2004).

1.12 Recommendations for intake of Se

The daily amount of Se intake, recommended by health organizations, is 70 μg for adult males and 55 μg for females. The daily Se needs increase during childhood and pregnancy (Rayman, 2012). The Se recommended daily allowance (RDA) for male or female (excluding the states of pregnancy and lactation) individuals aged over 14 years has been set at 55 μg (Alfthan *et al.*, 2015). In South Africa, the dietary intake of Se is 55μg/day (Kolahdooz *et al.*, 2013). A protective effect of Se could directly impact on potential toxicity caused by the Hg exposure from fish consumption in Se-replete versus Se-deficient populations (Mozaffarian, 2009).

The human daily intake of Se for harmful and beneficial concentration range is very narrow (50−200) mg (Huang *et al.*, 2015). In the urine of normal subjects, i.e. a non-supplemented subject, mean urinary Se concentrations usually vary between

20 and 60 µg Se /L (Klein *et al.*, 2011). But none of these studies has estimated concentrations of Hg from dental amalgam fillings and their impact on urinary Se by a collection of samples from buccal swabs, urine samples and the chewing gum test.

1.13 Aims of the study

The aims of the current study were to investigate the leaching of Hg from dental amalgam fillings and also to investigate the relationship between the leached Hg from dental amalgam fillings and Se concentrations in the bloodstream.

1.14 Objectives

The aims of this study was achieved through the following objectives;

- To determine Hg in buccal swabs samples by using inductively coupled plasma-mass spectrometry (ICP-MS, Agilent technologies).
- To determine Hg in chewing gum samples by using ICP-MS.
- To determine Hg in urine samples using ICP-MS.
- To determine Se in urine samples by using ICP-MS.
- To determine the relationship between the concentration of Hg in buccal swabs, Hg in urine and concentration of Se in urine.
- To determine the influence of number, age and size of amalgam fillings on concentrations of buccal swabs Hg, urinary Hg and urinary Se.

- To determine the influence of daily habits on concentrations of buccal swabs Hg, urinary Hg and urinary Se.
- To determine the relationship between Hg chewing gum, Hg buccal swabs, gender and age of patients with respect to urinary Se concentrations.
- To determine the relationship between age and gender of participants and urinary Hg with respect to urinary Se concentrations.

Chapter Two

Methods

2.1 Ethical Clearance

Ethical clearance was obtained from the Ethics Research Committee of the University of the Western Cape (UWC) (Reg No: SHD EXEC 2014/19). All patients were fully informed of the research protocol and had to sign a declaration of informed consent before being allowed to participate in the study. Patients were given an option to exit from the study at any time without consequences to future treatment needs.

2.2 Methods and Materials

2.2.1 Study design

In this study, concentrations of Hg from dental amalgam fillings and Se in the urine, buccal swabs and chewing gum test, were obtained using the Inductively coupled plasma-mass spectrometry 7900 (ICP-MS, Agilent technologies). Questionnaires were also used for collection of relevant personal information. The participants completed the questionnaire form which includes information on daily habits, age and gender of patients, number, size and age of amalgam fillings and were asked to sign the consent form categorisation of variables and the reference group as described in Table 2.1

Table 2.1: Questionnaire used to collect information on subjects included in the study

Variable	**Description or categorisation**
Personal data:	Name and surname – Address - Date of birth
Age	18 – 60 years
Gender	Female – male
Number of amalgam fillings	1-3 4-7 8-12
Size of amalgam fillings	Small-medium – Large- mixed size
Age of amalgam fillings	1 year or less- 10 years or less- more than 10 years – mixed age
Consumption of hot liquids	Little – a lot – not much
Smoking habit	Less than 15 cigarettes – more than 15 cigarettes - No
Chewing gum habits	Occasionally – Daily - No
Brushing teeth habit	Once a day – Twice a day
Bruxism habit	Yes / No

References (categorical variables): 1-3 fillings (number of amalgam fillings), small fillings (size of amalgam fillings), one year or less (age of amalgam fillings) not much (hot liquids habit), No smoke (smoking habit), No chew (chewing gum habit), No (bruxism habit), once a day (brushing teeth habit) males (gender of patients)

2.2.2 Study Population

Samples were collected from 107 participants, 33 males (30.84%) and 74 females (69.15%), aged 17 to 60 years. Subjects were patients with one or more dental amalgam fillings from the dental clinic in Tygerberg Hospital (South Africa). The three collected samples (urine, chewing gum and a buccal swab) were kept in the freezer (-20 ^{o}C) until ready for analysis.

2.2.3 Trace element analysis procedure

The trace metals are analysed with the ICP-MS using the standard configuration of quartz spray chamber and torch Ni-plated sampling and skimmer cones. A 0.4ml/min micro mesh nebulizer was used to aspirate the sample. The instrument was optimised for sensitivity and oxide formation before calibration. Instrument parameters were set as described in Table 2.2

Table 2. 2 : Instrument parameters were set as follows

Instrument: Agilent 7900 ICP-MS	Value	Column1
RF Power (W)	1600	
Plasma Mode	HMI	
Sample depth (mm)	10	
Carrier gas (L/min)	0.68	
Dilution gas (L/min)	0.27	
Make-up gas (L/min)	0	
Robustness (% Ce O/Ce)	< 1	
Cell gas parameters	He flow (ml/min) for Hg	4.8
	He flow (ml/min) for Se	6
Acquisition parameters	Peak mode	1 point
	Replicates	3
	Integration time (sec)	0.3 – 1

USEPA Methods 6020A and 200.8 guidelines were followed for instrument calibration and data verification protocols; according to Cloete, (2017). The instrument was calibrated using NIST traceable standards purchased from Inorganic Ventures Inc (Christiansburg Virginia), and the accuracy of the calibration validated by a separate standard from Merck Millipore (Merck KGaA, Darmstadt, Germany). Adrift monitor standard was analysed after every 12 samples, with

internal standard elements added online to correct for drift and matrix differences between samples and standards. The instrument was housed at the Central Analytical Facility CAF at Stellenbosch University (SU).

2.2.4 The materials

1. Wrigley's double mint chewing gum

2. Swabs, sterile, cotton tip on a wooden shaft to collect buccal swabs.

3. 40 ml plastic containers from LASEC Company were used to collect urine samples.

2.3 Inclusion criteria

a. Included were patients with a number of amalgam fillings. These were recorded because it is speculated that there is an increase in urine Hg concentration with each corresponding incremental increase in the number of amalgams filled tooth surfaces (Richardson *et al.,* 2011).

b. Medical history: a questionnaire about health-related issues and behaviours and an oral health examination was done according to (Seo *et al.*, 2014).

2.4 Exclusion criteria

1: Patients having no natural teeth.

2: Patients who have chronic kidney disease as they would naturally have a high mercury level in the urine (Wang, 2011)

2.5 Determination of Hg in urine samples

The Hg determination in urine was done at the Central Analytical Facilities (CAF) in Stellenbosch University. Spot urine samples were collected in a 40 ml plastic mercury free container from each participant and stored at -20 ^{0}C until analysis. Samples were 10 times diluted in 1% HNO3. The typical parameters of analysis were: RF power, 1600 W, plasma mode HMI, Sample depth (mm) 10, Carrier gas (L/min) 0.68, Dilution gas (L/min) 0.27, Make-up gas (L/min) 0, Robustness (% Ce O/Ce) < 1, Cell gas parameters He flow (4.8 ml/min). Analysis parameters for Hg were set to measure 1 point per peak, 3 replicates before loading in ICP_MS. Urinary mercury levels were calculated as µg/ L from the data obtained on ICP_MS.

2.6 Determination of Se in urine samples

Urinary Se was determined from the same urine samples used for Hg determination. The samples were 10 times diluted in 1% HNO_3. The typical parameters of analysis were :RF power, 1600 W, plasma mode HMI, Sample depth (mm) 10, Carrier gas (L/min) 0.68, Dilution gas (L/min) 0.27, Make-up gas (L/min) 0, Robustness (% Ce O/Ce) < 1, Cell gas parameters He flow (6 ml/min). Analysis parameters for Se were set to measure 1 point per peak, 3 replicate. Determination did by using ICP-MS in CAF laboratory. The results are given as µg/L.

2.7 Determination of Hg in chewing gum samples

Each patient was given 300 mg of chewing gum (Wrigley's double mint chewing gum) to chew for 30 minutes after they rinse their mouth with distilled water. The

chewing gums were collected and stored directly in the frozen box. The chewing gums were later stored in the freezer at –20 ^{0}C until analysing in CAF laboratory. The procedure for ICP_MS digestion involved weighing the chewing gum sample before microwave digestion with 6ml HNO_3+ 2ml H_2O_2 and corrected for dilution during digestion analysed as described for urine analysis, the results given as mg/g.

2.8 Determination of Hg in buccal swabs samples

Buccal cell samples were collected by means of a cotton swab. The patients were asked to rub the swab along the inside of the cheek and against their gums for 1 minute after chewing gum directly; Cotton swabs were immediately stored in a frozen box then sent to CAF laboratory for analysis. Sample preparation involved the addition of 1ml 2% HNO_3 and 1 ml 2% HCl to the swab containers and submerged the tip. Swabs were removed, and analysis was done in the swab containers. The results were taken as the amount of Hg solubilized in 1ml solution, an absolute value cannot be determined.

Hg buccal swabs concentrations calculated as µg/L.

2.9 Statistical analysis

Statistical tests were done using IBM SPSS version 24. Normality for data was analysed using the Shapiro-Wilk test, and visual inspection of Q-Q plots and histograms. If data was found not to be normally distributed, non-parametric analysis was applied. Due to the fact that the data was not normally distributed, therefore, ANOVA could not be applied in this study. The association between variables were analysed using Spearman or Pearson correlation and multiple linear

regression for comparing the means between two different groups (males and females). Mann-Whitney U-test or Student's t-test was applied to evaluate statistical differences. Association between categorical variables were performed using the Chi-Square test, while Box and Whisker plots were used to show the distribution of data .*P values* less than 0.05 were considered statistically significant.

Chapter Three

Results

The total number of participants for this study was 107. Urine samples collected from all participants were tested for Hg and Se while only 102 participants provided chewing gum and buccal swabs samples. The participants who had between 1-12 dental amalgam fillings were included in this study and the age range of participants was between 17 and 60 years. There were 74 females (70%) with a mean age of 41 ± 9.93 years and 33 males (30%) with a mean age of 40 ± 10.08 years. The participants were patients of the dental clinic at Tygerberg Hospital (Cape Town, South Africa). The results of the statistical analysis were presented according to the highlighted aims (Section 1.14).

3.1 Relationship between gender of participants, number, size and age of amalgam filling

Pearson Chi-square test was used to investigate the relationship between the number, age, size of amalgam fillings and the gender of participants (Table 3.1).

Table 3.1: Test of association between gender and the variables, number, age and size of amalgam fillings.

Variables		Gender group		Chi-square value	P value
		Females	Males		
Number of amalgam Fillings	1-3 fillings	28	18	2.76	0.25
	4-7 fillings	39	12		
	8-12 fillings	7	3		
Age of amalgam fillings	≤ 1 year	2	1	0.88	0.82
	≥ 1 years and ≤ 10	26	13		
	≤ 10 years	48	16		
	New and old fillings	4	3		
Size of amalgam fillings	Small fillings	4	1	5.75	0.12
	Medium fillings	8	7		
	Large fillings	7	7		
	Mixed fillings	55	18		

The findings show that there is no statistical difference between the gender of participants for the number of amalgam fillings ($p = 0.25$), the age of fillings ($p = 0.82$) and size of amalgam fillings ($p = 0.12$).

3.2 The relationship between gender of participants and the daily habits (hot liquid` consumption, smoking, bruxism, brushing teeth and chewing gum)

The investigation of the relationship between the gender of participants and their daily habits was done using the Pearson Chi-square test (Table 3.2).

Table 3.2 Association between gender and the variables, hot liquid consumption, smoking, bruxism, brushing teeth and chewing gum

Variables		Gender		Chi-square value	P value
		Females	Males		
Hot liquids habit	A lot	37	14	6.39	0.04*
	Little	20	4		
	Not much	17	15		
Smoking habit	>15 cigars a day	22	8	2.88	0.23
	No smoking	48	20		
	<15 cigars a day	4	5		
Bruxism habit	Yes	28	6	4.06	0.04*
	No	46	27		
Brushing teeth habit	Once a day	16	15	6.30	0.01*
	Twice a day	58	18		
Chewing gum habit	Occasionally	52	18	3.21	0.20
	Daily	7	3		
	Did not chew	15	12		

*Statistically different at the p-value of <0.05

It was observed that there was a significant difference between the gender of participants and the consumption of hot liquids, little and not much hot liquid (p = 0.04). It was also interesting to note that there was a significant difference between the gender of participants and bruxism (p = 0.04), and asignificantly higher number of women had the habit of bruxismwhen compared to men. In addition, the results showed that females had a significantly higher percentage of participants who brushed their teeth twice a day (p =0.01). However, there was no significant gender difference for both cigarette smoking and chewing gum.

3.3 Determinations of mercury from buccal swabs and chewing gum

Hg leaching during the dental amalgam fillings can be investigated by measuring the amount of Hg in chewing gum and buccal swabs (Hansen *et al.*, 2004 and Woods *et al*., 2005) During this investigation, the amount of Hg in chewing gum and buccal swabs of both males and females were detected by using the ICP-MS as described in (Section 2.7 and .2.8). Descriptive statistics (Mann-Whitney U test) was used to evaluate statistical differences in the concentration of Hg in buccal swabs and chewing gum of both male and female participants (Table 3.3).

Table 3.3: Descriptive statistics of Hg concentration in chewing gum and buccal swab samples in the participants

Variables	**Median (Min-Max)**		**p value***
	Females	**Males**	
Buccal swabs Hg (µg/L)	0.16 (0.026-1639.90)	0.09 (0.012-25.43)	0.387
Chewing gum Hg (µg/g)	2.04 (0.05-16.40)	1.89 (0.05-12964)	0.735

Although the median concentration of Hg in the buccal swabs of females was higher than that of males, there was no significant difference between genders (p = 0.387). In addition, the median Hg concentration in chewing gum for females was higher than that of males, but was not significant.

3.2 Determinations of Hg and Se in urine

Hg leaching in dental amalgam can be investigated by determining the amount of mercury in urine and its impact on urinary Se for both males and females (Ye *et al.*, 2016 and Jäger *et al*., 2016). The detection of urinary Hg and Se was done by using the ICP-MS as described in section 2.5 and 2.6. Descriptive statistics (Mann-Whitney U test) was used to evaluate the statistical differences in the concentration of urinary Hg and Se in both male and female participants (Table 3.4).

Table 3. 4: Descriptive statistics of Hg and Se concentration in urine samples according to gender of patients.

Variables	Median (Min-Max)		p value*
	Females	Males	
Urinary Hg (µg/L)	0.40 (0.19-4.34)	0.60 (0.40-2.27)	0.387
Urinary Se (µg/L)	26.29 (2.97-89.57)	29.32 (7.96-133.41)	0.735

The Mann-Whitney U test was used to evaluate statistical differences in Hg levels between genders. Whereas, the median concentration of urinary Hg and Se in females was lower than that of males, the difference was not significant.

3.3 Correlation between concentrations of Hg, Se in urine and Hg in buccal swabs.

The Spearman correlation was used to investigate the relationship between the concentration of Hg in buccal swabs, urine and Se (Table 3.5).

Table 3.5: Correlation between Hg and Se levels (µg/L) in urine and Hg buccal swabs levels

Variables	Number of participants	Spearman's rh Correlation Coefficient	P value
Urinary Hg levels / Swabs Hg levels	102	0.133	0.183
Urinary Hg levels / Urinary Se levels	107	0.348	0.001*
Urinary Se levels / Swabs Hg levels	102	-0.170	0.088

*Statistically different representing at the p-value of <0.05.

It was observed that there was no significant difference between the Hg and Se in the urine and Hg in buccal swab. However, there was a significant positive correlation between the Hg and Se concentration in urine samples (r = 0.348, P = 0.001).

3.4. Influence of dental amalgam fillings on Hg concentrations in buccal swabs

The multiple linear regression analysis (r^2 = 0.155) was used to investigate the relationship between the number, size and age of amalgam fillings and Hg concentration in the buccal swabs.

3.4.1 Effect of number of amalgam fillings on buccal swabs Hg concentrations

The median buccal swab Hg concentrations of the participants who had 1 to 3 fillings, 4 to 7 fillings and 8 to 12 fillings were 0.08 µg/L, and 0.11 µg/L and 0.19 µg/L respectively. The group of participants who had 1 to 3 fillings was used as a reference. The multiple linear regression analysis was applied to predict the

relationship between the number of amalgam and the concentration of Hg in the buccal swabs.

Table 3.6: Multiple linear regression analysis for categorical variables; number, size and age of amalgam filling with respect to buccal swabs Hg concentrations (µg/L)

Variables		Median (µg/L)	β(coefficient)	P value
Number of amalgam fillings	4-7 fillings	0.11	33.15	0.40
	8-12 fillings	0.19	-10.60	0.87
Size of amalgam fillings	Medium fillings	0.10	-76.13	0.42
	Large fillings	0.07	-5.91	0.95
	Mixed fillings	0.11	-22.19	0.79
Age of amalgam fillings	Less than 10 years	0.08	-38.29	0.72
	more than 10 years	0.09	-60.90	0.57
	New and old fillings	0.28	-46.44	0.70

Dependent variable buccal swab
References (categorical variables) 1-3 fillings (number of amalgam fillings), small fillings (size of amalgam fillings), one year or less (age of amalgam fillings)

There was no significant relationship between the concentration of Hg in buccal swabs and the number of amalgam fillings (p = 0.40) 4-7 fillings and (p = 0.87) 8-12 fillings

3.4.2 Effect of age of amalgam fillings on buccal swabs Hg concentrations

The median buccal swab Hg concentrations of participants who had fillings aged 1 year or less was 0.39 µg/L. This group was used as a reference. However, it was

0.08 μg/L for those who had filling for more than 1 year and less than 10 years. For fillings that lasted for 10 years or more the median buccal swab Hg concentrations of participants was 0.09 μg/L. For the mixed age fillings, it was 0.28 μg/L. The multiple linear regression analysis was applied to predict the relationship between age of amalgam fillings and the concentration of Hg in buccal swabs. However, the number of amalgam fillings showed no significant association with Hg in buccal swabs (Table 3.6).

3.4.3 Effect Hg amalgam fillings size on buccal swabs Hg concentrations

The median buccal swab Hg concentration of participants who had small fillings was 0.05 μg/L. This group was use as reference. For the Medium, large and mixed fillings, the median buccal swab Hg concentration was 0.10 μg/L, 0.09 μg/L and 0.11 μg/L respectively as shown in Table 3.6. The multiple linear regression analysis was applied to predict the relationship between the size of amalgam fillings and the concentration of Hg in buccal swabs. There were no significant prediction between the size of amalgam fillings and the Hg in buccal swabs.

3.5 Influence of dental amalgam fillings on urinary Hg concentrations

The investigation into the influence of dental amalgam fillings on urinary Hg concentration was done and the multiple linear regression analysis ($r^2 = 0.190$) was used to analyse and predict the relationship between the number, size, age of amalgam fillings and urinary Hg concentrations.

3.5.1 Effect of number of amalgam fillings on urinary Hg concentrations

The median concentrations of urinary Hg was 0.40 µg/ L for participants who had 1-3 fillings and this group was used as a reference. The median concentrations for participants who had 4-7 fillings and 8-12 fillings were 0.60 µg/L and 0.40 µg/L, respectively (Table 3.7). The multiple linear regression analysis was applied to predict the relationship between number of amalgam and the urinary Hg concentrations. It was observed (Figure 3.1) that the relationship between urinary Hg and 4 to 7 fillings was significant (p = 0.01) while there was no significant relationship between the urinary Hg and 8-12 fillings (p = 0.42).

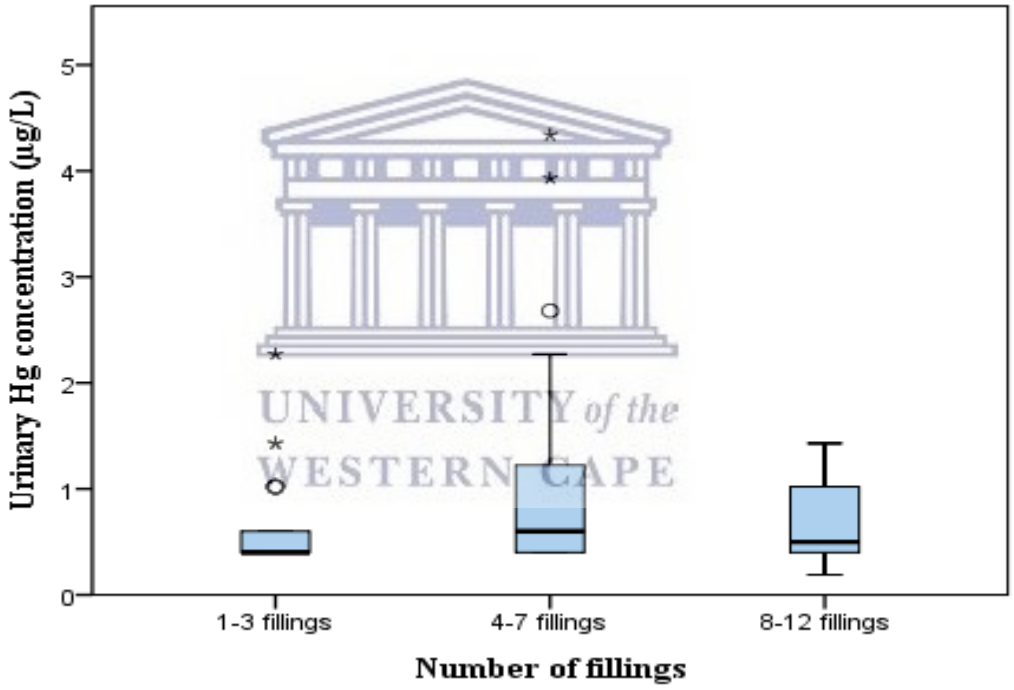

Figure 3.1: Box and Whisker plot representing the distributions of urinary Hg concentrations (µg/L) with respect to a number of amalgam fillings (In the figure above, ***** indicates outliers and ○ indicates high values)

3.5.2 Effect of age of amalgam fillings on urinary Hg concentrations

The median values of concentrations of urinary Hg for participants who had fillings aged 1 year or less was 1.02 µg/L and was used as a reference. The group of ≥1 ─

≤ 10 was 0.60 µg/L, for ≥10 years was 0.40 µg/L and for mixed age was 0.02 µg/L. The multiple linear regression analysis was applied to predict the relationship between age of amalgam fillings and the concentration of Hg in the urine (Figure 3.2).

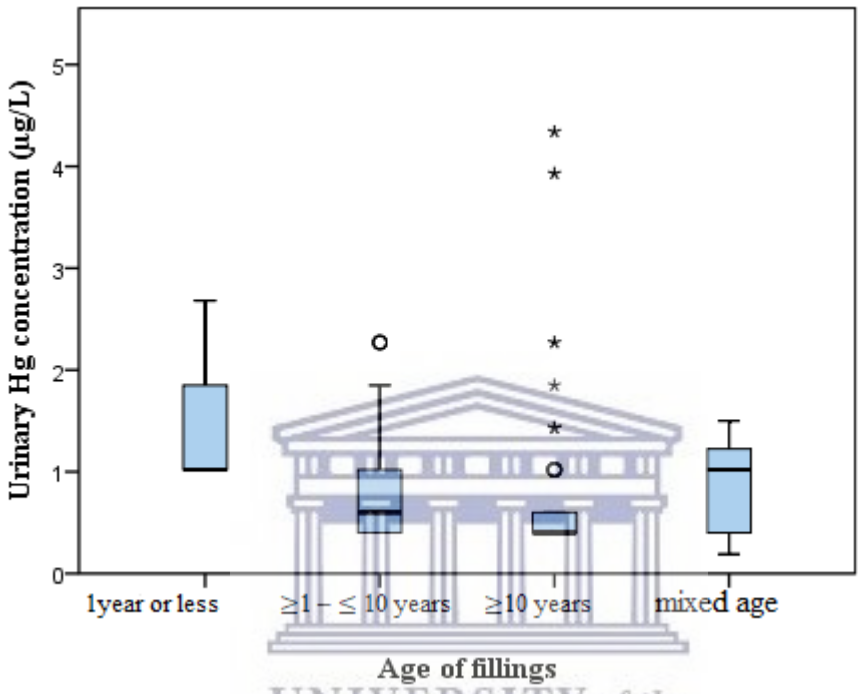

Figure 3.2: Box and whisker plot representing the distributions of urinary Hg concentrations (µg/L) with respect to age of amalgam fillings (In the figure above, * indicates outliers and ○ indicates high values)

Meanwhile, for the participants who had fillings aged ≥ 1 — ≤ 10 years (β coefficient -0.05, p = 0.02) ≥10 years (β coefficient -0.05, p = 0.02) and mixed age (β coefficient- 0.85, p = 0.04). This shows the significance of the relationship between the age of amalgam fillings and urinary Hg concentration. (Table 3.7).

3.5.3 Effect of amalgam fillings size on urinary Hg concentrations

The median values of concentrations of urinary Hg for participants according to the size of small fillings was 0.40 µg/L and this group was used as a reference. Also,

the median concentration of participants who had medium, large and mixed fillings were, 0.40 µg/L, 0.40 µg/L and 0.60 µg/L respectively (Table 3.7). The multiple linear regression analysis was applied to predict the relationship between the size of amalgam fillings and the concentration of Hg in the urine, the size of amalgam fillings did not associate with the concentrations of Hg in urine.

Table 3.7: Multiple linear regression analysis for categorical variables, number, size and age of amalgam fillings with respect to urinary Hg concentrations (µg/L)

Variables		**Median (µg/L)**	**β(coefficient)**	**p value**
Number of amalgam fillings	4-7 fillings	0.60	0.42	0.01*
	8-12 fillings	0.40	0.21	0.43
Size of amalgam fillings	Medium fillings	0.40	0.03	0.94
	Large fillings	0.40	0.17	0.65
	Mixed fillings	0.60	0.08	0.82
Age of amalgam fillings	More than 10 years	0.60	-0.05	0.02*
	Less than 10 years	0.40	-0.05	0.02*
	New and old fillings	1.02	-1.02	0.04*

Dependent Variable: Urinary Hg
References (categorical variables) 1-3 fillings (number of amalgam fillings), small fillings (size of amalgam fillings), one year or less (age of amalgam fillings)
*Statistically difference at the p-value of <0.05

3.6 Influence of dental amalgam fillings on urinary Se concentrations

The investigation into the influence of the number, size and age of amalgam fillings on urinary Se concentration was done by using multiple linear regression analysis

($r^2 = 0.330$) to analyze and predict the relationship between the number, size, age of amalgam fillings and urinary Se concentrations.

3.6.1 Effect of number of amalgam fillings on urinary Se concentrations

The median values for urinary Se in participants who had 1 to 3 filling was 30.77 µg/ L and this group was used as a reference. Also, the median concentrations of Se in urine for participants who had 4 to 7 fillings was 26.60 µg/L and 20.18 µg/L for 8 to 12 fillings (Table 3.8). The multiple linear regression analysis was applied to predict the relationship between the number of amalgam fillings and the concentration of Se in the urine. It was observed that there was no significant difference between urinary Se concentration and the number of amalgam fillings.

3.6.2 Effect of age of amalgam fillings on urinary Se concentrations

The median values of concentrations of urinary Se for participants who had amalgam fillings aged 1 year or less was 31.70 µg /L and this group was used as a reference. The group of ≥1 — ≤ 10 was 36.40 µg/L, for 10 years or more was 23.71 µg/L and for mixed age was 14.73 µg/L as shows in Table 3.8. The multiple linear regression analysis was applied to predict the relationship between age of amalgam fillings and the concentration of Se in the urine. It was observed that there was no significant relationship between urinary Se concentration and age of amalgam fillings.

3.6.3 Effect of size of amalgam fillings on urinary Se concentrations

The median concentrations of urinary Se for participants according to the size of fillings the small fillings was 45.23 µg/ L and was used as a reference. The medium, large and mixed fillings were 20.40 µg/L, 35.58 µg/ L and 26.00 µg/ L, respectively,

as presented in Table 3.8. The multiple linear regression analysis was applied to predict the relationship between the size of amalgam fillings and the concentration of Se in the urine shows no significant difference.

Table 3.8: Multiple linear regression analysis for categorical variables (number, size and age of amalgam fillings) with respect to urinary Se concentrations (µg/L)

Variables		**Median (µg/L)**	**β(coefficient)**	**P value**
Number of amalgam fillings	4-7 fillings	26.60	2.52	0.58
	8-12 fillings	20.18	-4.73	0.51
Size of amalgam fillings	Medium fillings	20.40	-15.13	0.14
	Large fillings	35.58	-9.68	0.43
	Mixed fillings	26.00	-15.21	0.10
Age of amalgam fillings	More than 10 years	36.40	-0.67	0.98
	Less than 10 years	23.71	4.03	0.74
	New and old fillings	14.73	-7.07	0.61

Dependent variable urinary selenium
References (categorical variables) 1-3 fillings (number of amalgam fillings), small fillings (size of amalgam fillings), one year or less (age of amalgam fillings)

3.7 Effect of daily habits on urinary mercury concentrations

The daily habits such as drinking of hot liquids, smoking, chewing gum, bruxism and brushing teeth were investigated for their effect on Hg leaching from dental amalgam. Multiple linear regression analysis ($r^2 = 0.190$) was used to investigate the relationship between the daily habits and concentrations of Hg in urine.

3.7.1 Effect of Hot liquids on urinary Hg concentrations

The median urinary concentration of Hg in participants who had consumed not much of hot liquids was 0.60 µg/L and this group was used as a reference. The participants who had consumed a little of hot liquids and who had consumed a lot of hot liquids were 0.40 µg/L and 0.40 µg/L respectively (Table 3.9). The multiple linear regression analysis was applied to predict the relationship between the hot liquids and urinary Hg concentrations. It was observed that there was no significant relationship between consuming of hot liquids and Hg concentrations in urine.

3.7.2 Effect of smoking on urinary Hg concentrations

The median urinary concertation of Hg in participants who did not smoke at all was 0.40 µg/L and this group was used as a reference. Participants who smoked less than 15 cigarettes per day and participants who smoked more than 15 cigarettes per day was 0.40 µg/L and 0.60 µg/L respectively (Table 3.9). The multiple linear regression analysis was applied to predict the relationship between smoking and urinary Hg and it was observed that there was no significant difference between smoking and Hg concentrations in urine.

3.7.3 Effect of chewing gum on urinary Hg concentrations

The median urinary concentration of Hg in participants who did not consume chewing gum at all was 0.60 µg/L and this group was used as reference. Participants who had consumed chewing gum daily and occasionally was 0.40 µg/L and 0.50 µg/L respectively (Table 3.9). The multiple linear regression analysis was applied

to predict the relationship between the chewing gum and urinary Hg and results show no significant relationship between chewing gum habit and urinary Hg.

3.7.4 Effect of bruxism on urinary Hg concentrations

The median values of Hg for participants who had no bruxism habit was 0.40 µg/L and served as reference. Participants with bruxism habit was 0.40 µg/L (Table 3.9) and the multiple linear regression analysis was applied to predict the relationship between the bruxism and urinary Hg. There was no significant relationship between the bruxism habit and urinary Hg concentration (Table 3.9).

3.7.5 Effect of brushing teeth on urinary Hg concentrations

The median urinary concentration of Hg in participants who brushed their teeth once a day was 0.60 µg/L and this group was used as reference. Participants who brushed their teeth twice a day was 0.40 µg/L (Table 3.9) and the multiple linear regression analysis was applied to predict the relationship between the brushing teeth and urinary Hg. It was observed that there was a negative correlation between concentrations of urinary Hg and teeth brushing habit (β coefficient -0.37, p = 0.02) (Figure 3.3)

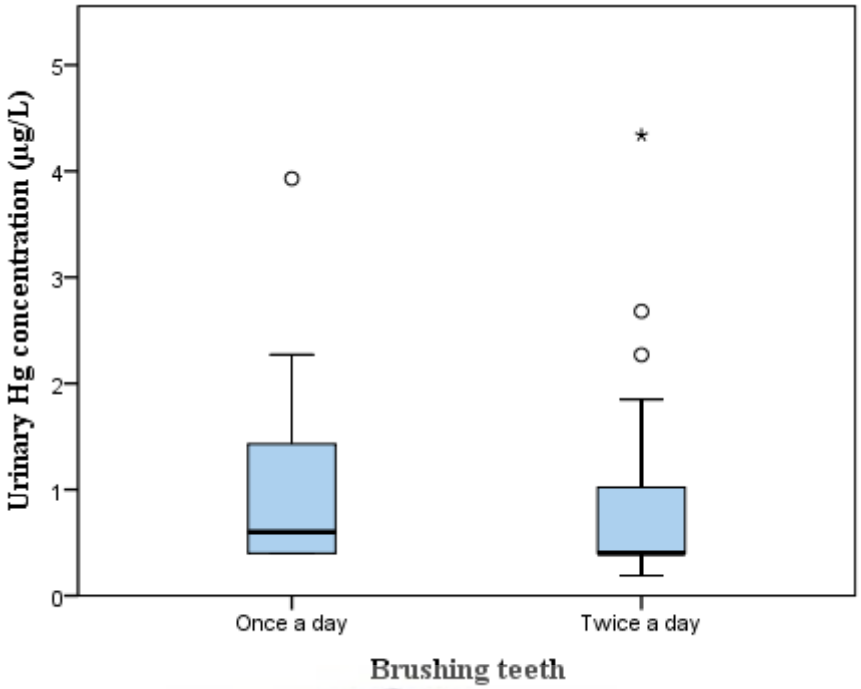

Figure 3.3: Box and whisker plot representing the distributions of urinary Hg concentrations µg/L with respect to brushing teeth habit (In the figure above, * indicates outliers and ○ indicates high values)

Table 3.9: Multiple linear regression analysis for categorical variables (daily habits hot liquids, smoking, chewing gum, bruxism and brushing teeth) with respect to urinary Hg concentrations (µg/L)

Variables		Median (µg/L)	B(coefficient)	P value
Hot liquids habit	A lot of hot liquid	0.40	0.03	0.95
	little hot liquid	0.40	-0.11	0.63
Smoking habit a day	Greater than 15 cigars	0.60	-0.23	0.31
	Less than 15 cigars	0.40	-0.16	0.35
Chewing gum habit	Daily	0.50	-0.03	0.90
	Occasionally	0.40	0.04	0.83
Bruxism	Bruxism Yes	0.40	0.02	0.89
Brushing	Brushing Twice	0.40	-0.37	0.02*

Dependent Variable: Urinary Hg
References (categorical variables) not much (hot liquids habit), No smoke (smoking habit), No chew (chewing gum habit), No (bruxism habit), once a day (brushing teeth habit)
*Statistically different representing at the p-value of <0.05

3.8. Effect of daily habits on buccal swabs Hg concentrations

The daily habits such as drinking of hot liquids, smoking, chewing gum, bruxism and brushing teeth were investigated for their effect on Hg leaching in buccal swabs from dental amalgam. Multiple linear regression analysis was used ($r^2 = 0.155$), to investigate the relationship between the daily habits and concentrations of Hg in buccal swabs.

3.8.1 Effect of Hot liquids on buccal swabs Hg concentrations

The median buccal swabs concentration of Hg in participants who had consumed not much of hot liquids was 0.10 µg/L and this group was used as a reference. The participants who had consumed a little of hot liquids and a lot of hot liquids were 0.09 µg/L and 0.10 µg/L respectively (Table 3.10). The multiple linear regression analysis was applied to predict the relationship between the hot liquids and Hg buccal swabs concentrations. It was observed that there was no significant relationship between consuming of hot liquids and Hg concentrations in urine.

3.8.2 Effect of smoking on Hg in buccal swabs concentrations

The median buccal swabs concentration of Hg in participants who did not smoke at all was 0.01 µg/L and this group was used as a reference. Participants who smoked less than 15 cigarettes per day and more than 15 cigarettes per day was 0.10 µg/L, and 0.09 µg/L respectively as shown in (Table 3.10). The multiple linear regression analysis was applied to predict the relationship between smoking and buccal swabs Hg. The result showed there was a significant difference between Hg in buccal swabs levels and in participants who smoked more than 15 cigarettes a day (β coefficient 210.473, p = 0.002).

3.8.3 Effect of chewing gum on buccal swabs Hg concentrations

The median buccal swabs concentration of Hg in participants who did not consume chewing gum at all was 0.10 µg/L and this group was used as reference. Participants who had consumed chewing gum daily and occasionally were 0.10 µg/L and 0.06

µg/L respectively (Table 3.10). The multiple linear regression analysis was applied to predict the relationship between the chewing gum and buccal swabs Hg. Also, it was observed that there is no significant difference between chewing gum habit and buccal swabs.

3.8.4 Effect of bruxism on buccal swabs Hg concentrations

The median buccal swabs concentration of Hg in participants who had no bruxism habit was 0.10 µg/L and served as a reference. In addition, participants with bruxism habit was 0.12 µg/L (Table 3.10). The multiple linear regression analysis was applied to predict the relationship between bruxism and buccal swabs Hg. It was observed that there was no significant relationship between bruxism and buccal swabs Hg concentration.

3.8.5 Effect of brushing teeth on buccal swabs Hg concentrations

The median buccal swabs concentration of Hg in participants who brushed teeth once a day was 0.08 µg/L and this group was used as a reference, while participants who brush teeth twice a day was 0.11 µg/L (Table 3.10). The multiple linear regression analysis was applied to predict the relationship between the brushing teeth and buccal swabs Hg. However, there was no significant relationship between brushing teeth habit and buccal swabs concentrations.

Table 3.10: Multiple linear regression analysis for categorical variables (daily habits hot liquids, smoking, chewing gum, bruxism and brushing teeth) with respect to buccal swabs Hg concentrations (µg/L)

Variables		Median (µg/L)	B(coefficient)	P value
Hot liquids habit	A lot of Hot liquid	0.09	48.72	0.32
	little hot liquid	0.10	32.68	0.57
Smoking habit a day	Greater than 15 cigars	0.09	210.47	0.002*
	Less than 15 cigars	0.10	-11.72	0.77
Chewing gum habit	Daily	0.06	-50.52	0.47
	Occasionally	0.10	-3.26	0.94
Bruxism	Bruxism Yes	0.12	34.45	0.35
Brushing	Brushing Twice	0.11	10.62	0.78

Dependent variable buccal swabs
References (categorical variables) not much (hot liquids habit), No smoke (smoking habit), No chew (chewing gum habit), No (bruxism habit), once a day (brushing teeth habit)
*Statistically different representing at the p-value of <0.05

3.9 Effect of daily habits on urinary Se concentrations

Multiple linear regression analysis ($r^2 = 0.330$) was to investigate the relationship between the daily habits and concentrations of Se in urine.

3.9.1 Effect of Hot liquids on urinary Se concentrations

The median urinary concentration of Se in participants who had consumed not much hot liquids was 32.31 µg/L and this group was used as reference. The participants who had consumed a lot hot liquids and a little of hot liquids was 23.86

µg/L and 31.79 µg/L respectively (Table 3.11). The multiple linear regression analysis was applied to predict the relationship between the hot liquids and urinary Se concentrations. It was observed that there was no significant relationship between consumption of hot liquids and Se concentrations in urine.

3.9.2 Effect of smoking on urinary Se concentrations

The median urinary concentration of Se in participants who did not smoke at all was 33.15 µg/L and this group was used as a reference. Participants who smoked more than 15 cigarettes and less than 15 cigarettes per day was 20.18 µg/L and 26.29 µg/L respectively (Table 3.11). The multiple linear regression analysis was applied to predict the relationship between smoking and urinary Se. The result showed no significant relationship between cigarette smoking and urinary Se concentrations.

3.9.3 Effect of chewing gum on urinary Se concentrations

The median urinary concentration of Se in participants who did not consume chewing gum at all was 27.36 µg/L and this group was use as reference. Participants who consumed chewing gum daily and occasionally was 37.71 µg/L and 28.62 µg/L respectively (Table 3.11). The multiple linear regression analysis was applied to predict the relationship between chewing gum and urinary Se. Figure 3.4 shows a significant relationship between participants who had daily chewing gum and urinary Se (β coefficient 21.03, p value 0.01).

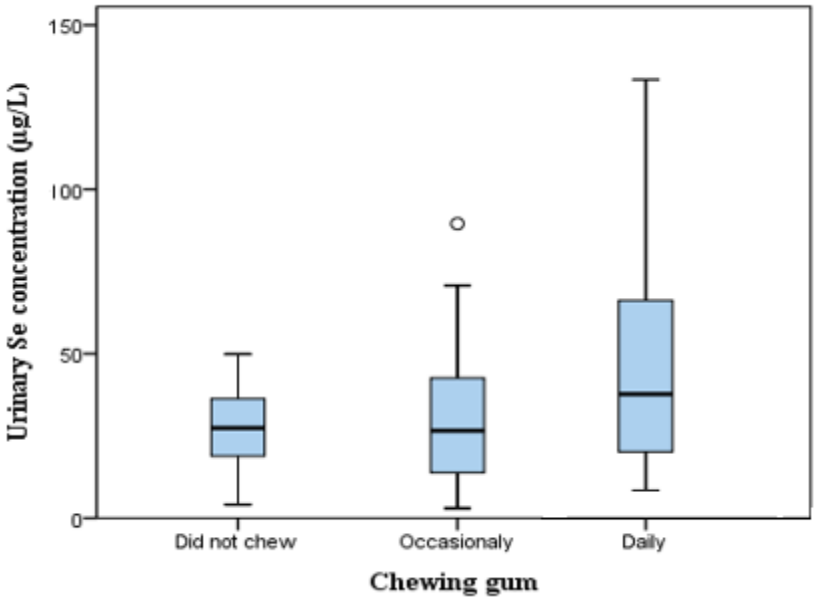

Figure 3.4: Box and whisker plot representing the distributions of Se concentrations in (µg/L) with respect to chewing gum habit (In the figure above, ○ indicates high values)

3.9.4 Effect of bruxism on urinary Se concentrations

The median urinary concentration of Se in participants who has no bruxism habit was 32.09 µg/L and served as a reference, while participants who had bruxism habit was 21.06 µg/L (Table 3.11). The multiple linear regression analysis was applied to predict the relationship between bruxism and urinary Se. There was no significant relationship between the bruxism habit and urinary Se concentration.

3.9.5 Effect of brushing teeth on urinary Se concentrations

The median urinary concentration of Se in participants who brushed once a day was 36.39 µg/L and served as a reference, while participants who brush teeth twice a day was 26.29 µg/L (Table 3.11). The multiple linear regression analysis was applied to predict the relationship between brushing and urinary Se. It was observed

that there was no significant difference between teeth brushing and urinary Se see (Table 3.11).

Table 3.11: Multiple linear regression analysis for categorical variables (daily habits hot liquids, smoking, chewing gum, bruxism and brushing teeth) with respect to urinary Se concentrations (µg/L)

Variables		Median (µg/L)	B(coefficient)	P value
Hot liquids habit	A lot of Hot liquid	23.86	-0.16	0.98
	little hot liquid	31.79	1.09	0.84
Smoking Habit	Greater than 15 cigars	20.18	-3.18	0.67
	Less than 15 cigars	26.29	-1.57	0.73
Chewing gum habit	Daily chewing gum	37.78	21.03	0.01*
	Occasionally chewing gum	28.62	1.21	0.81
Bruxism	Bruxism Yes	21.06	-4.84	0.25
Brushing	Brushing Twice	26.29	-2.35	0.59

Dependent variable urinary selenium
References (categorical variables) not much (hot liquids habit), No smoke (smoking habit), No chew (chewing gum habit), No (bruxism habit), once a day (brushing teeth habit)

3.10 The relationship between age and gender of participants and urinary Hg with respect to urinary Se

Multiple linear regression analysis (r^2 = 0.330) was use to investigate the relationship between the age, gender of participants and urinary Hg with urinary Se. Table 3.12 shows that there was a significant difference between the urinary Se and urinary Hg levels (β coefficient 8.151, p = 0.006). However, there was no

significantly difference between age, gender of the participants and urinary Se levels (Figure 3.5).

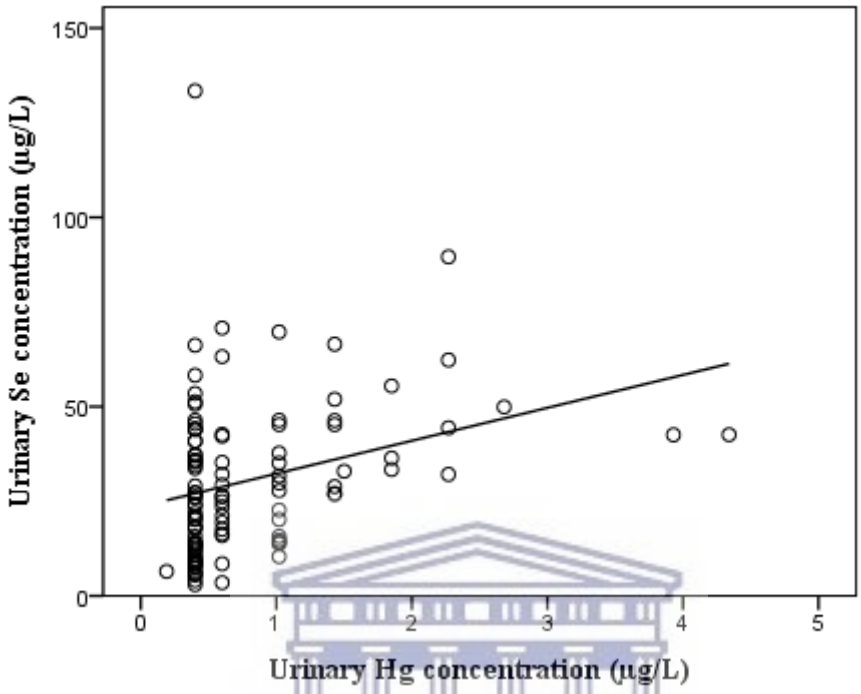

Figure 3.5: Association between urinary mercury and urinary selenium concentrations, multiple linear regression (β coefficient 8.151, p = 0.006).

Table 3.12: Multiple linear regression analysis for categorical variables (age and gender of patients and urinary Hg concentrations (µg/L) with respect to urinary Se concentrations (µg/L)

Variables	β(coefficient)	p(value)
Age of patients(years)	-0.259	0.250
Urinary Hg	8.151	0.006*
Females	-4.668	0.297

Dependent variable urinary selenium
References (categorical variables) males (gender of patients)
*Statistically different representing at the p-value of <0.05

3.11 Association between Hg concentration in chewing gum and Hg buccal swabs and gender and age of patients with respect to urinary Se levels (µg/L)

The relationship between urinary Se concentrations and Hg in chewing gum and buccal swabs, gender and age of patients was investigated by using multiple Linear regression analysis (R square = 0.096). The result revealed that the age of participants was the only variable which significantly predicted levels of urinary Se (β coefficient -0.519, p value = 0.011, Table 3.13).

Table 3.13: Multiple linear regression analysis for categorical variables (chewing gum, buccal swabs, gender and age of patients) with respect to urinary Se levels (µg/L)

Variables	β(coefficient)	p(value)
Chewing gum	-0.002	0.281
Buccal swabs	0.012	0.331
Females	-6.465	0.138
Age of patients (years)	-0.519	0.011*

Dependent Variable: Urinary Se
References (categorical variables) males (gender of patients)
*Statistically different representing at the p-value of <0.05

Notably, chewing gum, buccal swabs and the gender of participants did not associate with urinary Se levels see (Figure 3.6).

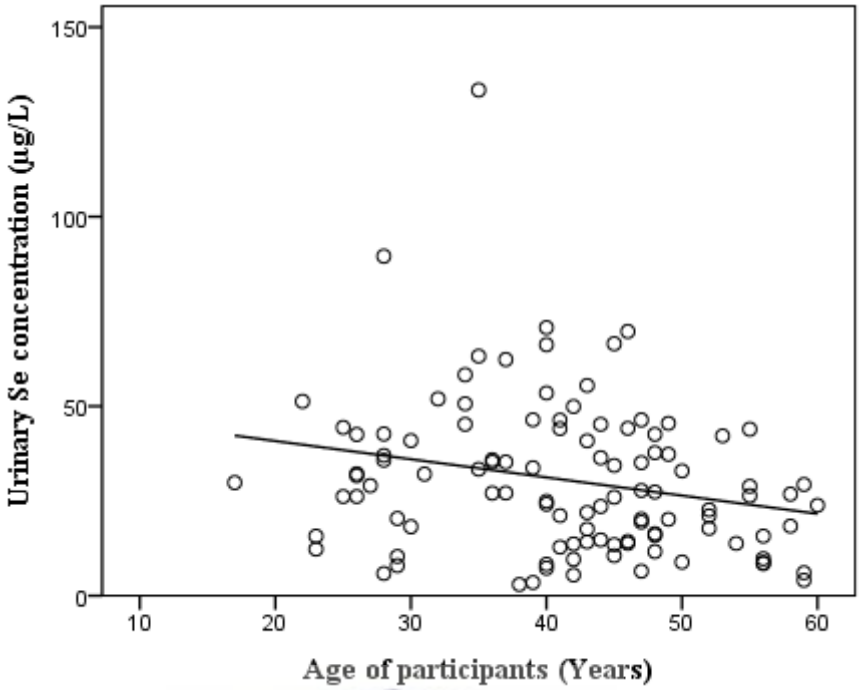

Figure 3.6: Association between age of participants and urinary Se concentrations, multiple linear regression Multiple (R-squared: 0.096, β coefficient -0.519, p =0.011)

Chapter Four

DISCUSSION

4.1 Introduction

The amount of Hg that was leached from Hg amalgam into the buccal cells and chewing gum, as well as the urinary Hg and Se, were determined in the participants. The influence of daily habits such as bubble gum chewing and smoking or factors such as gender and age on the Hg dental amalgam leaching as well as the relationship between the Hg dental amalgam and Se concentration in the body will be discussed.

4.2 Determinations of Hg from buccal swabs and chewing gum tests

It is well-known that the process of chewing gum increases the release of Hg vapour from amalgam fillings in the oral cavity and could be absorbed into the tissues. (Järup, 2003; Clarkson, 2002; Homme *et al.*, 2014 and Fuks, *et al.*, 2015). There is also evidence that gum chewing (especially nicotine gum) can increase the mercury levels in persons with Hg amalgams (Dutton *et al.*, 2013).

Findings from this study showed that the amount of Hg released in chewing gum was low (0.05 to 17.68 µg/g), except in 3 participants (12964, 1233.8 and 33.40 µg/g). It is believed that these results are possibly due to the collection of samples on the same day or after few days of filling the tooth. Our findings are in line with that of Hansen *et al.*, (2004) who investigated the values of chewing gum Hg levels

and measured values between 0 – 393 µg/g with median 27 µg/g in 2223 participants with dental amalgam fillings.

Similarly, in this study, buccal swabs samples were used to determine the levels of mercury. Previous reports indicate that Hg released directly into the mouth is absorbed into the cheeks or sublingually and both routes of absorption end up in the bloodstream (Huggins, 2007).

Our findings show that the concentration of Hg obtained from buccal swabs was low, ranging from 0.012 µg/L to 25.43 µg/L. This is in line with a study by Clarkson, (2002), who reported that low concentrations of Hg in the oral cavity could be possibly due to its small size.

4.3 Determinations of Hg and Se in urine

In this study, the median concentrations of urinary Hg in female participants was 0.40 µg/L with a concentration range between 0.19-4.34 µg Hg/L while the median was 0.60 µg/L (0.40-2.27) µg/L in males. However, there was no significant difference between gender and concentration of urinary Hg. Findings from this study is in agreement with a study by Apostoli *et al.*, (2002) who reported no significant differences between urinary Hg concentration and age or genders of 383 participants in four Italian cities.

Conversely, these findings disagree with other published data in California, where 600 participants reported a higher urinary Hg concentration in males than in females and a significant difference between genders ($p < 0.0001$) (Goodrich *et al.*, 2016).

In this study, the concentration of urinary Se was reported between 2.97 to 133.41 µg/L and the male concentrations for urinary Se was higher than those of females. In addition, there was no significant difference in urinary Se and the gender of participants. These findings agree with study by Høl *et al.,* (2002) who reported no significant difference between gender and urinary Se. Most of the participants had low levels of Se in urine when compared to the reference values for urinary Se. Previous reports indicate that low concentrations of urinary Se is possibly due to the interaction between Hg and Se in the form of HgSe not excreted in the urine (Goyer., 1996; Mehdi *et al.,* 2013 and Khan *et al.,* 2009). However, few studies have specifically investigated if the chronic exposure of mammals to Hg will decrease the concentration of Se in tissues.

4.4 Correlation between concentrations of urinary Hg and Se in urine and Hg in buccal swabs.

In this study, findings revealed that there was a positive relationship between urinary Hg and urinary Se, which indicates that persons exposed to low or high levels of elemental Hg from dental amalgam excrete less or more Se in urine. This is in agreement with a study by (Høl *et al.,* 2002 and Høl *et al.,* 2003) reporting that when more Se binds with Hg to form Hg-Se, less Se is excreted through the urine since Hg-Se is retained for a longer time than Se alone in the liver and kidneys. From a toxicological standpoint, this trapping of free Hg is favourable. However, this trapping could reduce the portion of Se in tissues available for the formation of essential selenoenzymes and may cause Se deficiency which may cause reduced protection against Hg- toxicity.

4.5 Influence of size, age and number of dental amalgam fillings on urinary Hg, urinary Se and Hg buccal swabs and concentrations

The findings from this study revealed that there was no significant relationship between the size, age and number of dental amalgam fillings with buccal swab Hg and urinary Se.

However, a significant predicted relationship was found between number and age of amalgam fillings and urinary Hg concentrations. There was a significant difference between the participants who had 4-7 fillings and urinary Hg, but not with participants who had 8-12 fillings. This is possibly due to the release rate which is dependent upon many factors including area, age, eating and individual habits, composition of the amalgam, and the quantity of the surface oxide layer (Uçar *et al.*, 2011). The group who had 4-7 fillings had new fillings and it is possible that the younger age of the fillings had an impact on the release of Hg. The fillings group of 8-12 may have had old and small fillings. relationship between the age of fillings and urinary Hg (Vahter *et al.*, 2000; Ritchie *et al.*, 2004; Bates *et al.*, 2006; Kern *et al.*, 2014 and Brownawell *et al.*, 2005).

4.6 Effect of daily habits on buccal swab and urinary Hg and S concentrations

4.6.1 Effect of drinking hot liquids

In this study, there was no significant predicted relationship between consumption of hot liquids and the concentration of urinary Hg and Se as well as Hg in buccal swabs. These findings might be due to the age of fillings which were more than 10

years old, however, some studies have reported that the release of elemental mercury vapour is stimulated by chewing, tooth brushing, bruxism, and the ingestion of hot foods and liquids (Mortazavi *et al.*, 2014 and Dodes, 2001).

4.6.2 Effect of bruxism habits

In this study, there was no significant association between bruxism and urinary Hg, urinary Se and Hg in buccal swabs. However, previous studies have demonstrated that Hg can be released from dental amalgams during trituration, condensation, setting, polishing, and removal of fillings (Spencer, 2000 ; Uçar *et al.*, 2011 ;Yalcin Cakir *et al.*, 2015).

4.6.3 Effect of brushing teeth

In this study, brushing teeth had a significant relationship with urinary Hg, however, there was no significant association with urinary Se and Hg buccal swabs. Also, findings showed that participants who brushed their teeth twice a day had a significantly negative association with urinary Hg, while participants who brushed teeth once a day had higher concentrations of Hg possible because participants might have underestimated actual reporting of brushing habit. These results are in agreement with previous reports (Mutter *et al*. 2007 and Kern *et al.*, 2014)) showing that corrosion products were found to be loosely bound on the amalgam surface and could be removed by brushing similar to tooth brushing.

4.6.4 Effect of smoking

In this study, it was observed that smoking was only associated with Hg in buccal swabs and there were no association with urinary Hg and urinary Se. This is in disagreement with the study by Decharat *et al.*, (2014), who found there was no significant difference in urinary Hg levels among the participants who smoked. Also, this disagrees with a similar study by Zolfaghari *et al.*, (2007) who reported that the Hg levels in urine among Iranian dentists, who were the patients themselves, were not affected by smoking. However, in a study by Thomson *et al.*, (2004), it was reported that the Se status of smokers was lower than that of nonsmokers.

Findings from this study also revealed that there was a positive significant association between the concentration of Hg in buccal swabs and participants who smoked more than 15 cigarettes a day, thus indicating that an increase in smoking stimulates a higher release of Hg in the oral cavity, possibly due to heat-induced release of Hg.

4.6. 5 Effect of Chewing gum habits

In this study, there was no significant association between urinary Hg, buccal swab and chewing gum. However, urinary Se was associated with chewing gum daily. Our findings on Hg and chewing gum is in disagreement with previous studies (Clarkson *et al*., (2003) and Dutton *et al.*, (2013) which indicates that one of the factors that aids the release of Hg is chewing gum and that higher urinary Hg concentrations are found in people who chew a great deal. (Mutter *et al.*, 2010).

4.7 The relationship between age and gender of participants and urinary Hg and urinary Se

In this study, the multiple linear regression analysis showed that the relationship between urinary Hg and urinary Se was positive and statistically significantly. This result was supported by the Spearman correlation which showed that this association did not depend on age and gender of participants. This is in agreement with previous findings showing Introduction and methods that Se affects the distribution of Hg and also reduces toxicity induced by Hg in experimental animals (Rooney, 2007).

4.8 Association between Hg in chewing gum, Hg in buccal swabs, gender and age of patients with respect to urinary Se levels (µg/L)

In this study, the multiple linear regression showed that there were no significant association between Hg in buccal swabs, urinary Se and gender of participants. This is possibly because of the low absorption rate of Hg in buccal cells and the rest of the body. For example, previous reports show that younger participants had higher Se excretion in urine and the opposite with the older subjects. These findings are supported by a different study (Drasch *et al.*, (2000) demonstrating the influence of the age on patients with amalgam fillings and Se concentration in the tissues. In most tissues, there is a negative correlation, this means that the higher the age, the lower the Se concentration (Nylander *et al.*, 1991).

Conclusion

It was discovered that a substantial amount of Hg was leached from the dental amalgam fillings. The amount of Hg in the buccal swabs of female and male participants was in the range of 0.026-1639.90 μg/L or 0.012-25.43 μg/L, respectively. Also, the amount of Hg in the urine of female and male participants was in the range of 0.19-4.34) μg/L or (0.40-2.27) μg/L respectively. These findings indicate that the number of dental amalgam filling, the age of dental amalgam fillings, teeth brushing and smoking habit had a significant influence on the release of Hg. However, the size of filling, consumption of hot liquids, bruxism and chewing gum was not implicated as responsible for releasing of Hg. Ultimately, the study could not demonstrate a causal relationship between any of the Hg measures and urinary Se.

Limitations and recommendations

- The study was limited by measuring selenium only in the urine samples. It was not possible to get blood or other samples such as hair and toenails because of ethical and financial constraints.
- The study was limited by the small sample size. It is possible that with a bigger sample size, the correlations observed would be more robust, and less likely to be the result of chance.
- Maybe a bigger sample size will also allow a better opportunity to consider more confounders in the regression models to provide more convincing results/evidence.
- We only measured the exposure of mercury from dental amalgam fillings. It could be that other sources are responsible for the release of mercury.

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Appendixes

Appendix 1

QUESTIONNAIRE

A questionnaire will be used to collect information on subjects included in the study

Patient Name: _____

Address: _____

Age:

Sex:

Cell phone No:

Number of amalgam fillings:

2 □ 3□ 4□ 5 □ 6 □ □ more:

Period of amalgam fillings:

□ Days □ Months □Years

Size of amalgam fillings:

Large□ Medium□ Small□

Teeth brushing frequency:

Once□ twice□ three times per day□

Chew gum:

Yes□ No□

Times of chewing gum:

Daily□ occasionally □

Smoking habit:

No□ □≥15 cigarettes/day □≤ 15 cigarettes/day

Bruxism:

Yes□ No□

Previous renal disease:

Yes□ No□

Drinking hot beverages:

Little□ Not much□ A lot□ No□

Appendix 2

Information and Consent Letter

The Dean of dentistry department in

Tygerberg Hospital in Cape Town

Dear Sir

Re: Request for permission to conduct research at Tygerberg hospital TH

I am a Full-time MSc student in the Department of Medical Biosciences at the University of Western Cape. To fulfill the requirements of the degree, I am undertaking research into effect of mercury from dental amalgam on the metabolic function of selenium. This research study will take place over the period of 1 month. Therefore, I believe that your hospital would be a suitable place to conduct my study.

Data for this one-month quantitative action research project will be collected through post-samples results, examination, and interviews. Research participants will be asked for their permission for the data collection.

I undertake that my study will cause no harm to the hospital or any of the staff or patients. None of the participants will be asked to pay any money. I also ask you to allow me to use the instruments that TH using by the dentists help.

I would be very grateful if you could allow me to conduct this research at your hospital.

If you require any information about this study or any other questions regarding your rights as the place where the study will be conducted, please do not hesitate to ask.

Yours sincerely

Rukaia Aljabo

0764182855

3481175@myuwc.ac.za

I have read the above information regarding this research study on effect of mercury from dental amalgam on the metabolic function of selenium, and allow Rukaia Aljabo to conduct her study at TH and to use the instruments that TH is using by the dentists help.

_____ (Name)

_____ (Signature)

_____ (Date)

Appendix 3

Information consent

I, (Name…….) have been informed about the study entitled effect of mercury from dental amalgam on the metabolic function of selenium, by Rukaia Aljabo.

I understand the purpose and procedures of the study.

I have been given an opportunity to ask questions about the study and have had answers to my satisfaction.

I declare that my participation in this study is entirely voluntary and that I may withdraw at any time without affecting any treatment or care that I would usually be entitled to.

If I have any further questions/concerns or queries related to the study I understand that I may contact the researcher at cell phone number (0764182855) or via e-mail 3481175@myuwc.ac.za

If I have any questions or concerns about my rights as a study participant, or if I am concerned about an aspect of the study or the researchers then I may contact: The Dean of Dentistry.

Francie van Zyl Drive

Private Bag Xl

Tygerberg 7505

Cape Town, SOUTH AFRICA

_____ _____

Signature of Participant Date References:

Appendix4

Bylae 4
Ingeligte Toestemming

Hiermee bevestig ek, (Naam …….), dat ek ten volle ingelig was oor die navorsingstudie, genaamd: ‘‘DIE VERENIGING VAN MERCURIE VAN DENTALE AMALGAM MET URINÊRE SELENIUM’’ deur Rukaia Aljabo .

Ek verstaan die doel en prosedures van bogenoemde studie.

Ek is 'n geleentheid gegee om vrae oor die studie te vra en is tevrede met die antwoorde.

Ek verklaar dat my deelname aan hierdie studie heeltemal vrywillig is en dat ek te eniger tyd kan onttrek sonder dat enige behandeling of sorg, wat ek gewoonlik geregtig op sou wees, beinvloed sou wees.

Ek verstaan dat ek die navorser enige tyd kan kontak as ek enige verdere vrae / knelpunte of navrae het, wat verband hou met die studie. Selfoonnommer: **076 418 2855** of per e-pos: **3481175@myuwc.ac.za**

As ek enige vrae of kommentaar oor my regte as 'n studie deelnemer het, of as ek bekommerd is oor 'n aspek van die studie of die navorsers kan ek kontak:

Dekaan: Fakulteit Tandheelkunde
Tygerbergkampus
Francie van Zyl Rylaan
Privaatsak X1
Tygerberg 7505
Kaapstad, Suid-Afrika

A master's degree practical map for Rukaia Aljabo

03/05/2016

The Structural work to collect the samples at the dentistry in Tygerberg Hospital (TBH)

Time

Work time will be from 8:30 am to 2:00 pm every day

Steps of the procedures

1- Study explanation and dental examination will be done for every person according to the study criteria as following:

a- How many amalgam fillings and surfaces.
b- How old are the amalgam fillings.
c- How old is the patient.
d- Does the patient have a chronic disease or taking any medicine or not.

2- After the examination, patient will be informed about the study then sent to the next clinic to sign the consent letter, fill in the questionnaire and to do the interview to collect more information about the participants.

3- Start taking samples

a- A piece of chewing gum will be given to the participants to chew for 30 minutes.

b- Participants will be sent out to get urine samples in special tubes.

c- After finishing chew the gum, buccal samples will be taken from each parson by special sterile cotton swabs.

4- All samples will be kept in an Ice container after the collecting directly.

5- At the end of the working day, samples will be transferred to the main campus by our own transport then kept in one of the medical bioscience department's fridges in the right way and hermetically sealed until analysis.

6- Samples will be sent to the laboratory for analysis when all complete and ready by the university or our own transport.

7- Results will be received personally then finished up for the thesis.

The end

Appendix 6

Information's of participants

N.	Sex	Age	N of fillings	Size	Period of fillings	Had	Brushing	Chewing Gum	Bruxism	Smoking	Hot Liquids
1	M	25	4	4M	10Y		1	No	No	No	Little
2	F	47	12	4L 3M 5S	2W to 10Y	3from 10Y	2	Occ	Yes	No	A lot
3	F	44	9	4L 5M	+10Y		1	No	No	No	Not Much
4	M	55	6	3L 3S	1W to 40Y		2	No	No	No	A lot
5	F	43	5	4M 1S	4to25Y	7-4Y	1	No	Yes	No	A lot
6	F	46	3	3S	+10		2	No	No	No	A lot
7	F	42	6	1L 5S	2Wto1m		2	No	No	No	No
8	F	29	4	4M	6 Y		2	Occ	No	No	Little
9	F	46	6	3L 2M 1S	+_20Y	4-18M	1	Occ	Yes	No	A lot
10	F	40	3	2L 1S	+_10 Y	1 2M	1	Occ	No	No	Little
11	M	47	3	3L	+_10Y		1	Occ	No	No	Little
12	F	28	3	3M	+_20Y		2	Occ	No	No	Little
13	F	23	4	2L 2S	3to4Y	3 1-2W	2	Occ	yes	Yes	A lot
14	F	49	4	4L	30Y	1 2Y	2	No	No	No	L
15	F	46	8	7L 1M	12Y		3	Occ	No	No	L
16	M	48	3	1L 2M	3 to4Y		2	Occ	No	No	A lot

17	F	42	4	3L 1S	+_15	2 6Y	2	Occ	No	Yes	A lot
18	F	44	5	3L 1M 1S	1m to20Y		1	Occ	No	No	Little
19	F	28	5	3M 2S	+10Y		1	Occ	No	No	Little
20	F	50	5	3L 1M 1S	+10Y	4 3Y	3	Occ	No	No	Little
21	F	28	7	6L 1M	7Y	2 5Y	2	Occ	No	Yes	Little
22	F	43	6	1L 5M	+_20		2	Occ	No	No	Little
23	M	17	3	3M	1W		2	Occ	No	Yes	Little
24	M	47	5	3L 1M 1S	6Y	2 1M - 2Y	3	Occ	No	No	Little
25	F	39	4	4L	10Y		2	Occ	No	No	A lot
26	M	41	5	3M 1S	5Y			Daily	No	No	Little
27	F	35	5	3M 2L	2-6Y		2	No	No	No	Not Much
28	F	40	6	2L 4M	+_18Y		2	Occ	No	No	A lot
29	F	26	7	5M 2S	+-10Y	1 3Y	2	Occ	No	Yes	A lot
30	F	38	8	3L 5M	4 to 15Y		2	Occ	No	Yes	A lot
31	M	40	2	2M	30 Y	2 10 - 12Y	1	Daily	No	Yes	A lot
32	F	32	5	1L 4M	17Y		2	Occ	Yes	Yes	Yes
33	F	25	2	1M 1S	14 Y		2	Occ	Yes	Yes	A lot
34	M	36	3	3L	18 Y	1 5Y	2	No	No	No	Not Much
35	F	37	6	5L 1S	10Y		2	No	yes	NO	A lot

36	F	26	3	2M 1S	+10Y		2	No	No	No	A lot
37	M	47	3	2M 1S	15 to 20 Y		2	Occ	No	No	Not Much
38	M	46	3	3S	5 to 20 Y		1	Occ	No	Yes	Little
39	M	23	2	2L	2		2	No	No	Yes	A lot
40	F	34	2	2S	10Y	2 1-5 Y	1	Daily	No	No	Little
41	F	42	6	3L 3M	20Y		2	Occ	Yes	No	A lot
42	F	30	4	1L 3M	16 to 20Y		1	Occ	No	Yes	A lot
43	M	42	2	1L 1M	15 Y		1	No	No	No	Not Much
44	F	45	2	1L 1M	+10Y		1	Occ	No	No	A lot
45	F	30	3	2L 1M	10 Y		1	Occ	No	No	A lot
46	F	45	2	1L 1S	+25 Y		2	Daily	No	No	A lot
47	F	28	2	2M	10 Y		2	Occ	No	yes	No
48	F	56	5	5M	25 Y	2 3D	2	No	Yes	yes	Not much
49	M	35	5	2L 2M 1S	20 Y	1 1 Y	1	Occ	No	Yes	A lot
50	M	49	2	2M	+10 Y		2	Occ	Yes	Yes	Not Much
51	F	40	5	3L 2M	20Y		2	Occ	Yes	Yes	A lot
52	F	40	2	2S	14 Y	1 2M	2	Occ	No	No	A lot
53	M	55	4	2L 1M 1S	5 Y		2	Occ	No	No	A lot
54	F	60	7	4L 1M 2S			2	Occ	Yes	Yes	Not much
55	M	47	3	3M	2 Y	3 1M	1	No	No	Yes	Not Much

56	F	54	7	6L 1S	37 Y	3 5Y	2	Occ	Yes	No	A lot
57	F	26	3	2L 1S	1 Y		2	Occ	No	Yes	Not much
58	F	40	8	5L 2M 1S	30 Y		2	Occ	Yes	NO	A lot
59	F	40	3	3M	10 Y	2 2Y	1	Occ	No	No	A lot
60	M	45	3	3M	30 Y	5 10 Y	1	Occ	No	No	A lot
61	F	47	2	1M 1S	20 Y		2	Occ	No	No	Little
62	M	37	2	1L 1S	15 Y		2	Occ	No	No	A lot
63	F	48	10	5L 4M 1	4Y		2	Occ	No	Yes	A lot
64	F	55	7	4L 2M 1S	8 to 20 Y		2	No	Yes	Yes	A lot
65	M	58	5	3L 2M	10 Y	4 4W	1	No	No	Yes	Not Much
66	M	37	7	7L	15 Y		1	Occ	No	No	Not Much
67	M	36	3	3L	6 Y		2	Occ	Yes	Yes	Little
68	M	42	3	1M 2S	10 Y		1	Occ	No	Yes	A lot
69	M	22	2	2L	8 Y		1	Occ	No	No	A lot
70	F	43	5	2L 2M 1S	10to35Y		2	Occ	No	No	A lot
71	F	59	5	4L 1M	+_40 Y		2	No	Yes	No	Not Much
72	F	45	2	2L	+_30 Y		2	Occ	No	Yes	A lot
73	M	29	5	5M	+_12 Y	3 12Y	2	Occ	Yes	Yes	A lot
74	F	47	9	5L 3M 1S	30 Y	1 10Y	2	Daily	No	Yes	A lot

75	F	56	5	3L 2M	+_20 Y	1 1Y	2	Occ	No	No	Little
76	F	48	2	1L 1M	+_10 Y		2	Occ	Yes	No	Not Much
77	M	52	4	2L 2M	+_30 Y		2	Occ	No	Yes	A lot
78	F	52	7	4L 3M	+_30 Y		2	Occ	Yes	Yes	A lot
79	M	44	5	2L 3M	+_10 Y		1	Occ	Yes	No	A lot
80	F	56	4	1L 2M 1S	25 Y		2	Daily	No	No	A lot
81	F	43	3	1M 2S	+_30 Y		2	Occ	Yes	Yes	A lot
82	F	59	3	1L 2M		3 20Y		Occ	No	No	Little
83	F	28	3	2L 1M	1m to +_6Y		2	Occ	Yes	No	Not Much
84	M	50	8	4L 1M 2S	+_35 Y		3	Occ	Yes	No	Little
85	F	31	2	2L	3 Y		1	Occ	No	No	A lot
86	F	48	8	7L 1M	+_10 Y		1	No	Yes	No	Not Much
87	F	56	3	1L 2M	4 Y		1	Occ	Yes	Yes	A lot
88	F	58	4	3L 1M	+_30 Y	1 2m	2	No	Yes	No	A lot
89	F	28	2	2L			2	Occ	No	Yes	Little
90	F	52	5	2L 3M	+_40 Y		1	No	Yes	Yes	Little
91	F	34	1	1L	6 Y		1	Occ	No	No	Little
92	M	41	1	1L	10 Y	3 3Y	2	Occ	No	No	Not Much

Appendix 7

Results of levels of mercury and urinary selenium

	urine results of Hg	Se in urine	Gums test	Swab test
Sample 1	1.02	37.74		
Sample 2	2.27	44.40	4.963	0.079
Sample 3	0.19	6.46	8.605	
Sample 4	1.43	45.21	6.242	0.230
Sample 5	1.43	28.88	0.636	0.285
Sample 6	1.02	14.21	7.492	0.049
Sample 7	1.5	32.92		0.371
Sample 8	3.93	42.54	1.646	0.101
Sample 9	0.4	44.14	2.037	0.051
Sample 10	2.68	49.91	3.279	0.396
Sample 11	1.02	10.38	6.487	0.113
Sample 12	0.4	14.33	6.537	0.247
Sample 13	1.43	46.34	12.804	0.071
Sample 14	1.02	45.23	3.155	0.045
Sample 15	1.02	46.38	3.973	0.039
Sample 16	0.4	5.85	6.590	0.054
Sample 17	1.02	15.72	0.360	0.037
Sample 18	0.4	37.32	5.757	0.045
Sample 19	0.4	46.40	6.223	0.111
Sample 20	1.43	27.07	3.489	0.169
Sample 21	0.6	16.38	3.933	0.089
Sample 22	0.4	12.83	2.237	0.088
Sample 23	1.02	14.73	0.186	0.140
Sample 24	0.4	37.03	12.018	0.103
Sample 25	0.4	45.50	2.111	0.146
Sample 26	2.27	89.57	11.153	0.187
Sample 27	0.6	17.59	0.995	0.221
Sample 28	1.02	29.83	3.085	0.389
Sample 29	0.4	13.83	17.684	0.111
Sample 30	0.4	40.86		
Sample 31	0.4	33.74	2.610	0.047
Sample 32	0.6	21.13	2.295	0.131
Sample 33	1.85	33.37	4.265	0.154
Sample 34	0.6	3.49	1.650	0.431
Sample 35	0.6	32.16	0.321	0.058
Sample 36	0.4	2.97	12.159	0.108
Sample 37	0.4	20.40		

Sample 38		0.4		66.22		0.046		0.020
Sample 39		1.43		51.94		0.380		0.060
Sample 40		0.6		26.19		7.332		0.067
Sample 41		0.4		35.30		0.485		0.037
Sample 42		0.4		27.09		2.309		0.096
Sample 43		0.4		26.16		0.122		0.026
Sample 44		1.02		27.77		0.558		0.043
Sample 45		4.34		42.58		13.958		0.733
Sample 46		0.6		23.56				
Sample 47		1.02		69.72		0.334		0.038
Sample 48		0.4		12.30		0.079		0.012
Sample 49		0.4		133.41		2.031		0.061
Sample 50		0.4		58.29		0.239		0.035
Sample 51		0.4		5.49		3.875		0.059
Sample 52		0.4		18.19		1.511		0.051
Sample 53		0.4		9.61		0.325		0.026
Sample 54		0.4		26.00		0.443		0.101
Sample 55		0.4		40.91		1.480		0.028
Sample 56		1.43		66.48		0.100		0.064
Sample 57		0.6		42.68		0.144		0.043
Sample 58		0.6		42.17		8.876		
Sample 59		0.4		9.78		0.195		
Sample 60		0.6		63.20		0.628		0.093
Sample 61		0.4		20.17		0.246		0.094
Sample 62		0.6		24.90		0.940		0.114
Sample 63		0.4		24.15		0.074		0.077
Sample 64		0.4		43.92		0.711		0.099
Sample 65		0.4		23.86		5.577		0.160
Sample 66		0.6		19.41		3.405		0.090
Sample 67		0.4		10.57				
Sample 68		1.02		13.79		8.000		
Sample 69		0.4		53.47				
Sample 70		1.02		31.70		2.794		0.106
Sample 71		0.4		8.32		12.663		0.149
Sample 72		0.6		70.75		1.324		0.085
Sample 73		0.4		13.49		0.958		0.127
Sample 74		1.02		35.12		1.504		0.096
Sample 75		0.6		35.26		1.941		0.090
Sample 76		0.4		11.66		16.401		0.211
Sample 77		0.6		26.40		5.292		0.130
Sample 78		1.43		26.79		2.053		0.130
Sample 79		2.27		62.31		11.854		0.121
Sample 80		0.4		35.86		0.731		0.132

Sample 81		0.4		13.68		0.181		0.070
Sample 82		0.4		51.28		0.802		0.080
Sample 83		0.6		29.33				
Sample 84		1.85		55.47		0.631		0.981
Sample 85		0.4		4.09		2.240		0.343
Sample 86		0.4		34.39		0.408		0.094
Sample 87		0.4		7.96		3.618		0.139
Sample 88		1.02		20.18		5.442		0.205
Sample 89		0.4		15.73		0.362		0.080
Sample 90		0.6		15.95		1.292		0.082
Sample 91		0.4		21.08		0.062		0.097
Sample 92		0.4		17.76		0.667		0.143
Sample 93		0.4		7.28		10.749		0.132
Sample 94		1.85		36.40		3.259		0.077
Sample 95		0.6		8.47		0.295		0.057
Sample 96		0.4		21.96		0.441		0.065
Sample 97		0.4		6.05		0.050		0.080
Sample 98		0.4		35.75		1.874		0.249
Sample 99		0.4		8.87		8.162		0.140
Sample 100		2.27		32.09		0.649		0.065
Sample 101		0.4		27.37		5.395		0.068
Sample 102		0.4		8.74		0.066		0.122
Sample 103		0.4		18.35		2.056		0.100
Sample 104		0.4		29.04		0.097		0.147
Sample 105		1.02		22.67		0.903		0.075
Sample 106		0.4		50.65		0.964		0.131
Sample 107		0.4		44.08		1.341		0.081