The influence of pH on the *in vitro* skin permeation of rhodium

S.J. Jansen van Rensburg

21619719

B.Sc., B.Sc. Hons.

Mini-dissertation submitted in partial fulfilment of the requirements for the degree *Magister Scientiae* in Occupational Hygiene at the Potchefstroom Campus of the North-West University

Supervisor: Ms. A. Franken

Co-supervisor: Prof. J.L. du Plessis

Assistant-supervisor: Prof. J. du Plessis

May 2014

Acknowledgements

The author would like to thank everyone who contributed to this study, especially the following persons:

- Ms. A. Franken, for being an exceptional supervisor and guiding me throughout this study, as well as for her patience and tremendous help regarding all the aspects of this study.
- Prof J.L. du Plessis, for his expert opinion, direction and advice during the completion of this study.
- My parents, for their support and motivation throughout the duration of my studies.
- Mrs V. Anderson, for completing the analyses for this study, as well as providing the necessary training to complete the laboratory studies.
- Mr I. van der Sandt, for his support and encouragement, as well as his assistance in the laboratory.
- My classmates, as well as the honours students in the subject group Physiology for their enormous help with regard to collection of skin and assistance in the laboratory.
- Prof. L.A. Greyvenstein for the language and technical editing of this mini-dissertation.
- All the doctors, nurses and administrative staff at the hospitals and the patients for their willingness to contribute towards this study and for donating skin.
- This work is based on the research supported in part by the National Research Foundation of South Africa for the grant No. 80635.

Preface

In this mini-dissertation the article format is used. The reference style in the mini-dissertation follows the guidelines of *Annals of Occupational Hygiene*, the journal chosen for potential publication. This journal requires that references in text should be inserted in Harvard style and the list of references should be set out in the Vancouver style of abbreviation and punctuation. The list of references should be in alphabetical order by name of first author. Details on the requirements of this reference style can be found in Chapter 3. For the sake of uniformity, this style of referencing was used throughout the mini-dissertation.

The outline of this mini-dissertation is as follows:

Chapter 1 is an introductory chapter and provides background with regard to the study. It includes the problem statement, aims and hypothesis of the study.

Chapter 2 presents a basic summary of the relevant literature regarding the platinum group metals and specifically rhodium, and the possible health effects thereof. It also gives an account of the structure of the skin, its barrier function and skin surface pH. The influence of pH on ionisation of metals and permeation through the skin is critically discussed.

Chapter 3 is the manuscript (article) to be submitted for publication. It includes background information, the materials and methods used, results obtained, as well as a discussion and conclusion.

Chapter 4 is the concluding chapter with conclusions, recommendations for occupational settings and future studies, and the limitations to which this study was subjected.

Chapter 5 is the appendix and includes the report from the language editor.

Disclaimer: Any opinion, finding and conclusion or recommendation expressed in this material is that of the author(s) and the NRF does not accept any liability in this regard.

Author contributions

This study was planned and executed by a team of researchers. The contribution of each researcher is described as follows:

Ms. S.J. Jansen van Rensburg (Author): Responsible for planning, design and writing of the mini-dissertation under the supervision of Miss. A. Franken and Prof. J.L. du Plessis, as well as researching and reviewing of the relevant literature, collection of data and interpretation of the results.

Ms. A. Franken (Supervisor): Involved in all aspects of this study, specifically supervising the design and planning of the experimental method, critically reviewing the mini-dissertation and guiding the interpretation of results and the writing of the mini-dissertation.

Prof. J.L. du Plessis (Co-supervisor): Contributed towards the design and planning of the sampling method; responsible for critically reviewing the mini-dissertation and guiding the interpretation of results and the writing of the mini-dissertation.

Prof. J. du Plessis (Assistant-supervisor): Responsible for critically reviewing and supervising the writing of the mini-dissertation.

The following is a statement from the researchers involved, confirming each individual's role in the study:

I declare that I have approved the above mentioned study and that my role in the completion thereof as indicated above is representative of my actual contribution. I hereby give my consent that it may be published as part of S.J. Jansen van Rensburg's mini-dissertation.

_____

Ms. S.J. Jansen van Rensburg (Author)

_____

Ms. A. Franken (Supervisor)

_____

Prof. J.L. du Plessis (Co-supervisor)

_____

Prof. J. du Plessis (Assistant-supervisor)

Table of contents

Abstract

In occupational settings where rhodium is produced or used, such as the mining industry, refineries and catalytic industries, workers are at risk of being dermally exposed to this metal in either the metallic form or its salt compounds. A considerable amount of contradictory literature has been published with regard to the sensitising abilities of rhodium and no published information is available on the occupational dermal exposure of rhodium as well as its ability to permeate through the skin. Previous studies conducted on the *in vitro* permeation of metals, such as nickel, cobalt and chromium, have indicated that certain metals undergo oxidation in the presence of sweat and form ions which are able to permeate through skin. For some metals, this ionisation takes place more rapidly in an acidic environment and a decrease in the environmental pH would cause an increase in the release of ions from those metals. *Aim*: The aim of this study was to determine whether rhodium in the form of rhodium trichloride ($RhCl_3$) would be able to permeate through the skin *in vitro*, as well as to determine whether any differences exist between the *in vitro* permeation of rhodium at a pH of 4.5 and a pH of 6.5. *Methods*: Full thickness abdominal skin was obtained as biological waste after surgery from Caucasian females ranging between 39 and 42 years of age. The Franz diffusion cell method was used in which the experimental cells contained synthetic sweat with $RhCl_3$ and the blanks did not contain any $RhCl_3$ in the donor compartment. All of the cells contained a physiological receptor solution in the receptor compartment. At intervals of 8, 12 and 24 hours, 2 ml of the receptor solution were removed for analysis. The receptor compartment was rinsed with 2 ml receptor solution which was also removed for analysis and 2 ml of fresh receptor solution was added to the compartment. After 24 hours, the receptor and donor solution was removed respectively for analysis and the skin was removed for digestion, prior to analysis. The mass of rhodium in the receptor solutions were determined using Inductively Coupled Plasma Mass Spectrometry. The donor solutions and digested skin solutions were analysed using Inductively Coupled Plasma Optical Emission Spectrometry. *Results*: At both pH values of 4.5 and 6.5, rhodium was able to permeate through the skin with a cumulative increase in permeation over prolonged exposure time. After 8, 12 and 24 hours, the amount of rhodium that permeated through the skin was higher at pH 4.5 than for pH 6.5. After 12 hours, the permeation of rhodium was statistically significantly higher for pH 4.5 than for pH 6.5 ($p = 0.02$). At both pH values, the percentage of rhodium that accumulated in the skin was higher than the percentage of rhodium that diffused through the skin and the lag time was less than six hours.
Conclusion: At both pH values of 4.5 and 6.5, rhodium was able to permeate through the skin. A decrease in the pH of synthetic sweat led to an increase in the permeation of rhodium and it is recommended that future *in vitro* permeation studies be conducted at a pH of 4.5, as the skin surface pH of workers are generally considered to be below 5. A higher percentage of rhodium

was retained in the skin than the percentage that diffused through, indicating the ability of rhodium to accumulate in the skin, from where it may exert health effects, such as sensitisation.

Key words: Skin surface pH, Franz diffusion cells, platinum group metals, rhodium, ionisation, oxidation.

Opsomming

In die werkomgewing waar rodium geproduseer of gebruik word, soos die mynindustrie, raffinaderye en katalitiese industrieë, bestaan die risiko dat werkers dermaal blootgestel kan word aan hierdie metaal, in die metaal vorm of die sout verbindings daarvan. ‘n Groot hoeveelheid teenstrydige literatuur is gepubliseer met betrekking tot die sensitiserings eienskappe van rodium en geen gepubliseerde inligting is beskikbaar oor die dermale beroepsblootstelling van rodium en die vermoë daarvan om deur die vel te beweeg. Vorige studies wat uitgevoer is op die *in vitro* diffusie van metale, soos nickel, kobalt en chroom, het bewys dat sekere metale oksidasie ondergaan in die teenwoordigheid van sweet en ione vorm wat die vermoë het om deur die vel te beweeg. Vir sommige metale vind hierdie ionisering vinniger plaas in 'n suur omgewing en 'n afname in die pH van die omgewing sal lei tot 'n toename in die vrystelling van ione vanaf sulke metale. *Doelstellings*: Die doel van hierdie studie was om te bepaal of rodium, in die vorm van rodium trichloried ($RhCl_3$) deur die vel kan beweeg *in vitro*, asook om te bepaal of enige verskille bestaan tussen die *in vitro* diffusie van rodium by 'n pH van 4.5 en 'n pH van 6.5. *Metode*: Vol dikte abdominale vel is verkry as biologiese afval vanaf blanke vroue tussen 39 en 42 jaar oud. Die Franz diffusie sel metode is gebruik waarin die eksperimentele selle sintetiese sweet met $RhCl_3$ bevat het en die blanko selle geen $RhCl_3$ in die skenker kompartement. Al die selle het `n fisiologiese reseptor oplossing in die reseptor kompartement bevat. By intervalle van 8, 12 en 24 uur is 2 ml van die reseptor oplossing onttrek vir analise. Die reseptor kompartement is gespoel met 2 ml reseptor oplossing wat ook onttrek is vir analise en 2 ml vars reseptor oplossing is teruggeplaas in die kompartement. Na 24 uur is die reseptor en skenker oplossings, onderskeidelik onttrek vir analise en die vel is verwyder vir vertering, waarna dit ook geanaliseer is. Die konsentrasie van rodium in die reseptor oplossings is bepaal deur middel van Induktief Gekoppelde Plasma Optiese Emissie Spektrometrie. Die skenker oplossings en verteerde vel oplossings is geanaliseer met Induktief Gekoppelde Plasma Massa Spektrometrie. *Resultate*: By beide pH waardes van 4.5 en 6.5 het rodium deur die vel beweeg en die kumulatiewe massa rodium wat deurbeweeg het, het verhoog met tyd. Na 8, 12 en 24 ure was die massa rodium wat deurbeweeg het, hoër by pH 4.5 as 6.5. Na 12 ure was die diffusie van rodium statisties betekenisvol hoër by pH 4.5 as by 6.5 ($p = 0.02$). By beide pH waardes was die persentasie van rodium wat in die vel geakkumuleer het hoër as die persentasie wat deur die vel beweeg het en die vertragingstyd was minder as ses ure. *Gevolgtrekking*: By beide pH waardes van 4.5 en 6.5 het rodium deur die vel beweeg en die kumulatiewe hoeveelheid van rodium wat deurbeweeg het, het verhoog met tyd. 'n Afname in die pH van sintetiese sweet het gelei tot 'n verhoging in die diffusie van rodium. Dit word aanbeveel dat toekomstige *in vitro* diffusie studies uitgevoer word met `n skenker oplossing met ‘n pH van 4.5, omdat die vel oppervlak pH oor die algemeen laer is as 5. Meer rodium het in die vel agtergebly as wat deurbeweeg het en

dui aan dat rodium die vermoë het om in die vel te akkumuleer, vanwaar dit gesondheidseffekte kan ontlok, soos sensitisering.

Sleutelwoorde: Vel oppervlak pH, Franz diffusie selle, platinum groep metale, rodium, ionisasie, oksidasie.

Chapter 1: Introduction

Chapter 1: Introduction

1.1 General Introduction

Rhodium belongs to the platinum group metals (PGMs) and is a rare, durable metal with unique catalytic properties (Cawthorn, 1999; Foti *et al*., 2002). The PGMs are six metals which are closely related and chemically very similar, with platinum being the most well-known and widely used. The remaining four metals include iridium, osmium, ruthenium and palladium (Cawthorn, 1999). Despite platinum being the most frequently used and investigated of the PGMs, there has been a growing interest during the last few years in some of the other PGMs and in particular rhodium. Previously rhodium, palladium and platinum were used in vehicle exhaust catalysts (VEC's) where platinum was usually the main element. An increased amount of converters are, however, being fitted with palladium or rhodium only (Gómez *et al*., 2000; Merget and Rosner, 2001). Several manufacturers of automobiles are increasing the amount of rhodium used in VEC's in order to sustain the durability thereof and to enhance their performance (Matthey, 2012). In addition, it is also used in various other industries, such as chemical, electronic and petroleum industries, as well as in production of jewellery and glass. It is also used in dentistry and in medicine for its anti-carcinogenic abilities (Wiseman and Zereini, 2009; Zereini *et al*., 2012). The occupational exposure of workers to hazardous substances and chemicals in occupational settings is a general occurrence (Chang *et al*., 2012). Several routes exist by which workers can be exposed to hazardous substances, such as ingestion, inhalation and the skin. Skin as a route of exposure has, however, been considered to be a less apparent route of exposure than the other two (Kissel, 2010). Despite dermal exposure becoming a popular topic of research, a lack of adequate information still exists in the literature with regard to dermal exposure to rhodium. Studies conducted on the presence of PGMs in the working and general environment focused primarily on platinum as airborne particulate matter (Gómez *et al*., 2000; Gómez *et al*., 2001; Wiseman and Zereini, 2009; Zereini *et al*., 2012). In all of these occupational settings, and especially in PGM refineries, workers are at risk of being dermally exposed to rhodium, either in the metallic state or the salt form (Boscolo *et al*., 2004; Goossens *et al*., 2011).

Although very little is known about the ability of rhodium to cause adverse health effects, some researchers have reported rhodium to have sensitising abilities, especially when in salt form (Bocca and Forte, 2009; Goossens *et al*., 2011; Sartorelli, 2012). Much controversy still surrounds this statement, with other researchers finding inconclusive or contradictory results. Yajun and Xiaozheng (2012) indicated that particles containing PGMs and soluble rhodium species emitted from catalysts may be a possible health concern, but did not find rhodium to have any sensitising ability. Bedello *et al*. (1987) confirmed the sensitising ability of rhodium, reporting a jeweller who developed contact dermatitis after coming into contact with a rhodium

salt. De La Cuadra and Grau-Massanés (1991) also found sensitisation to occur in a worker after being dermally exposed to a highly concentrated rhodium solution. Other toxic effects of rhodium may include its ability to induce oxidative, as well as cytogenetic damage, but no evidence has been found to suggest that rhodium may act as a carcinogen (Migliore *et al*., 2002; Boscolo *et al*., 2004).

Although the sensitising abilities of rhodium have been investigated, no research has been conducted on the ability of rhodium to permeate through the skin following dermal exposure. This is an important topic of research, as the ability of hazardous substances to permeate the skin is crucial when investigating the health risks associated with such substances, especially due to possible dermal exposure in the workplace (Byford, 2009).

During the investigation of *in vitro* permeation of other metals, such as nickel, cobalt, chromium, silver and gold, synthetic sweat was used in order to simulate normal skin conditions (Tanojo *et al*., 2001; Larese Filon *et al*., 2004; Larese Filon *et al*., 2007; Larese Filon *et al*., 2009; Larese Filon *et al*., 2011; Larese Filon *et al*., 2012). The ability of a metal to permeate through the skin is dependent, amongst other factors, on its ability to form ions through the process of oxidation. Hostýnek *et al*. (2006) stated that the formation of soluble compounds will influence the permeation of metals *in vitro*. They explained sweat to act as an electrolyte, causing electrochemical oxidation, which leads to the formation of metal ions and other compounds, such as chloride ions that permeate the skin more easily. Metals forming ions will permeate the skin more readily than non-ionised metal molecules, due to the smaller size of the ions (Tanojo *et al*., 2001; Larese Filon *et al*., 2007)

1.2 Problem Statement

The possible health risk, such as sensitisation, associated with dermal exposure to rhodium in refineries and the mining industry should raise concerns regarding the safety of workers in these workplaces. Very little published information is available with regard to the dermal exposure of rhodium. Although the ability of some metals, such as nickel, cobalt and chromium to permeate through the skin have been confirmed, the permeation ability of rhodium remains unknown. The influence that the skin surface pH will have on permeation has also not yet been investigated. In order to simulate the normal physiological conditions of the skin, the pH of synthetic sweat used in *in vitro* studies were representative of the normal skin surface pH, which is considered to be between 4.5 and 6 (Hanson *et al*., 2002; Larese Filon *et al*., 2007). The presence of acidic elements and substances in occupational settings, as well as the skin of workers in the working environment being metabolically active may cause the skin surface pH of workers to be lower than normally considered (Larese Filon *et al*., 2007; Larese Filon *et al*., 2008). For some metals, ionisation takes place more rapidly in an acidic environment, and a

decrease in pH would cause an increase in the release of ions from that metal, which is beneficial for permeation (Tanojo *et al*., 2001; Hostýnek *et al*., 2006; Larese Filon *et al*., 2007). Contradictory to this, an environment that is generally acidic has been found to have a beneficial influence on the functioning of the skin as a barrier (Gunathilake *et al*., 2009). The barrier function of the skin is effective in preventing the permeation of xenobiotics through the skin (Byford, 2009). To determine the ability of rhodium to permeate through the skin, as well as inconsistencies as mentioned above were some of the motivations for conducting this study. It has been found that the solubility of rhodium increases with a decrease in the environmental pH (Ek *et al*., 2004). However, the influence of the pH of synthetic sweat on the permeation of metals, has not been fully investigated. It has not been determined whether a pH of 4.5 or a pH of 6.5, as used in previous studies, should be used for future *in vitro* permeation studies. With regard to rhodium, the processes of oxidation and ionisation in synthetic sweat, as described for other metals, remain yet to be proven by investigation. The *in vitro* permeation of rhodium through the skin and the clinical effects thereof has not yet been demonstrated.

1.3 Research Aims and Objectives

The general aim of this study was to determine whether rhodium is able to permeate through the skin at a pH of 4.5 and 6.5.

The specific objectives of this study were:

- to apply the Franz diffusion cell method to determine the *in vitro* permeation of rhodium, in the form of $RhCl_3$, through intact human skin over a period of 24 hours;
- to determine whether statistically significant differences exist between the permeation of rhodium dissolved in synthetic sweat at different pH values through intact human skin;

1.4 Hypothesis

Metals such as nickel, chromium and cobalt may be more readily ionised at a lower pH and would consequently permeate the skin more easily in this ionised form. A decrease of one unit in pH would lead to a 10 to 100 fold increase in permeation through the skin (Larese Filon *et al*., 2009). Therefore, it is hypothesised that the *in vitro* permeation of rhodium at a pH of 4.5 will be significantly higher than that at a pH of 6.5.

1.5 References

Bedello PG, Goitre M, Roncarolo S. (1987) Contact dermatitis to rhodium. Contact Dermatitis; 17:111 – 112.

Bocca B, Forte G. (2009) The epidemiology of contact allergy to metals in the general population: prevalence and new evidences. Open Chem Biomed Meth J; 2: 26 – 34.

Boscolo P, Di Giampaolo L, Reale M, *et al*. (2004) Different effects of platinum, palladium and rhodium salts on lymphocyte proliferation and cytokine release. Ann Clin Lab Sci; 34: 299 – 306.

Byford T. (2009) Environmental Health Criteria 235: Dermal Absorption. Int J Environ Stud; 66: 662 – 788.

Cawthorn RG. (1999) The platinum and palladium resources of the Bushveld complex. S Afr J Sci; 95: 481- 489.

Chang YC, Chen CP, Chen CC. (2012) Predicting the skin permeability of chemical substances using a quantitative structure-activity relationship. Procedia Eng; 45: 875 – 879.

De La Cuadra J, Grau-Massanés M. (1991) Occupational contact dermatitis from rhodium and cobalt. Contact Dermatitis; 25: 182 – 184.

Ek KH, Morrison GM, Rauch S. (2004) Environmental routes for platinum group elements to biological materials – a review. Sci Total Environ; 334 – 335: 21 – 38.

Foti C, Amoruso A, Cassano N, Vena GA. (2002) Contact sensitisation to nickel from rhodium-plated 'nickel-free' earrings. Contact Dermatitis; 46: 309.

Gómez MB, Gómez MM, Palacios MA. (2000) Control of interferences in the determination of Pt, Pd and Rh in airborne particulate matter by inductively coupled plasma mass spectrometry. Anal Chim Acta; 404: 285 – 294.

Gómez B, Gómez M, Sanchez JL, *et al*. (2001) Platinum and rhodium distribution in airborne particulate matter and road dust. Sci Total Environ; 269: 131 – 144.

Goossens A, Cattaert N, Nemery B, *et al*. (2011) Occupational allergic contact dermatitis caused by rhodium solutions. Contact Dermatitis; 64: 158 – 184.

Gunathilake R, Schurer NY, Shoo BA, *et al*. (2009) pH-Regulated mechanisms account for pigment-type differences in epidermal barrier function. J Invest Dermatol; 129: 1719 – 1729.

Hanson KM, Behne MJ, Barry NP, *et al*. (2002) Two-photon fluorescence lifetime imaging of the stratum corneum pH gradient. Biophys J; 83: 1682 – 1690.

Hostýnek JJ, Dreher F, Maibach HI. (2006) Human stratum corneum penetration by copper: *In vivo* study after occlusive and semi-occlusive application of the metal as powder. Food Chem Toxicol; 44: 1539 – 1543.

Kissel J. (2010) The mismeasure of dermal absorption. J Expo Sci Environ Epidemiol; 21: 302 - 309.

Larese Filon F, Maina G, Adami G, *et al*. (2004) *In vitro* percutaneous absorption of cobalt. Int Arch Environ Health; 7: 85 – 89.

Larese Filon F, Gianpiero A, Venier M, *et al*. (2007) *In vitro* percutaneous absorption of metal compounds. Toxicol Lett; 170: 49 – 56.

Larese Filon F, D'Agostin F, Crosera M, *et al*. (2008) *In vitro* percutaneous absorption of chromium powder and the effect of skin cleanser. Toxicol In Vitro; 22: 1562 – 1567.

Larese Filon F, D'Agostin F, Crosera M, *et al*. (2009) Human skin penetration of silver nanoparticles through intact and damaged skin. Toxicology; 225: 33 -37.

Larese Filon F, Crosera M, Adami G, *et al*. (2011) Human skin penetration of gold nanoparticles through intact and damaged skin. Nanotoxicology; 5: 493 - 501.

Larese Filon F, Crosera M, Timeus E, *et al*. (2012) Human skin penetration of cobalt nanoparticles through intact and damaged skin. Toxicol In Vitro; 27: 121 – 127.

Matthey J. (2012) Platinum interim review: Other platinum group metals. Available at: http://www.platinum.matthey.com/media/1393522/platinum_2012_interim_review.pdf; Accessed: 5 July 2012.

Merget R, Rosner G. (2001) Evaluation of the health risk of platinum group metals emitted from automotive catalytic converters. Sci Total Environ; 270: 165 – 173.

Migliore L, Frenzilli G, Nesti C, *et al*. (2002) Cytogenetic and oxidative damage induced in human lymphocytes by platinum, rhodium and palladium compounds. Mutagenesis; 5: 411 – 417.

Sartorelli P, Montomoli L, Sisinni AG. (2012) Percutaneous penetration of metals and their effects on skin. Prevent Res; 2: 158 – 164.

Tanojo H, Hostýnek JJ, Mountford HS, Maibach HI. (2001) *In vitro* permeation of nickel salts through human stratum corneum. Acta Derm Venereol; 21: 19 – 23.

Wiseman CLS, Zereini F. (2009) Airborne particulate matter, platinum group elements and human health: a review of recent evidence. Sci Total Environ; 407: 2493 – 2500.

Yajun W, Xiaozheng LE. (2012) Health risk of platinum group elements from automobile catalysts. Procedia Eng; 45: 1004 – 1009.

Zereini F, Alsenz H. Wiseman CLS, *et al*. (2012) Platinum group elements (Pt, Pd, and Rh) in airborne particulate matter in rural vs. urban areas of Germany: concentrations and spatial patterns of distribution. Sci Total Environ; 416: 261 – 268.

Chapter 2: Literature Study

Chapter 2: Literature Study

This literature study will investigate the potential ability of rhodium to permeate the skin and the influence that skin surface pH will have on this permeation. Existing information on the PGMs will be addressed with special emphasis placed on the physical and chemical characteristics of rhodium, the occupational exposure thereof and the consequential health effects this metal may have. The barrier function of the skin will be briefly discussed in order to provide a better understanding of the mechanisms by which permeation may occur through the skin. Previous studies on *in vitro* permeation will be critically reviewed to investigate the ionisation of metals in sweat, the influence of pH on such ionisation and the effect that pH will consequentially have on permeation. The *in vitro* Franz diffusion cell method will be discussed as a method to investigate skin permeation of rhodium.

2.1 Platinum group metals

Platinum group metals (PGMs) are a group of six closely related metals with unique chemical characteristics. These metals include platinum, palladium, iridium, osmium, ruthenium and rhodium (Cawthorn, 1999). Although the PGMs are of the least abundant elements, they occur in close association in the earth crust in concentrations ranging from 0.4 to 5 µg/kg (Yajun and Xiaozheng, 2012; Zereini *et al*., 2012). Since its discovery in 1803 by William Hyde Wollaston, the demand for and use of rhodium has grown tremendously due to its various applications (Cawthorn, 1999; Goossens *et al*., 2011). By 1998 the worldwide demand of platinum alone had increased to 5 million ounces (oz.) per year. Approximately 75% of this demand was supplied by South Africa (Cawthorn, 1999). In 2010, approximately 632 000 oz. of rhodium was supplied by South Africa and 641 000 oz. in 2011. In 2012, the rhodium supply decreased to 580 000 oz. (Matthey, 2012).

PGMs have exceptional chemical features and the majority of these metals are utilised for their catalytic properties. PGMs are mostly used as vehicle exhaust catalysts (VEC's), which was introduced in the United States of America and Europe in the 1970's and 1980's, respectively (Yajun and Xiaozheng, 2012). VEC's are fitted to vehicles to control the emission of hazardous substances such as nitrous oxide (NOx), carbon monoxide (CO) and hydrocarbon (HC) and reduce them to less harmful substances such as water (H_2O), carbon dioxide (CO_2) and nitrogen (N_2). The lesser-known of the PGMs, iridium, osmium, ruthenium and rhodium are usually produced as by-products of the more widely used palladium and platinum. There has, however, been a general increase in the use of rhodium, with approximately 78% of the rhodium demand being used for VEC's, while only 38% of the platinum demand was used for the same application in 2011 (Yajun and Xiaozheng, 2012). PGMs are utilised in various other industries as well, including chemistry, electronics and petroleum and dentistry. Other applications include

the production of jewellery and glass, as well as in medical applications for its anti-carcinogenic abilities (Wiseman and Zereini, 2009; Yajun and Xiaozheng, 2012; Zereini *et al*., 2012).

2.2 Physicochemical characteristics of rhodium

Rhodium is naturally found as a rare, durable metal with a silvery colour. It has approximately the same density as silver at 12.41 g/cm^3 and an extremely high melting point of 1960 ^{o}C (Foti *et al*., 2002; Matthey, 2012). It belongs to the same elemental group as platinum, group VIII of the periodic table, and possesses the same physicochemical qualities, such as size and reactivity (Murdoch *et al*., 1986). The electrical resistance of rhodium at 0 ^{o}C is 4.33 microhm/cm and it is highly resistant against corrosion. It has good mechanical strength and malleability (Matthey, 2012). Rhodium can be present in either the metallic state or as a salt compound, such as $RhCl_3$ (Goossens *et al*., 2011).

2.3 Occupational exposure to rhodium

Due to the various applications of rhodium, several occupational settings exist where employees (workers) may be exposed to this metal or its salt compounds. These include the mining industry, where metals are extracted from platinum ore, foundries and in refineries. Exposure may also occur in electronic and chemical industries where VEC's, ornaments, decorations, jewellery, radio equipment and camera fittings are manufactured. Workers working at electroplating baths may also be exposed, as well as those in laboratories where research is conducted (Boscolo *et al*., 2004; Goossens *et al*., 2011).

Several routes exist by which a person can be occupationally or environmentally exposed to metals, such as ingestion, inhalation and the skin. In the field of occupational hygiene, exposure via inhalation has been previously considered as the most apparent exposure route to hazardous substances. A study conducted by Schierl *et al*. (1998) indicated airborne platinum concentrations between 1.7 – 6.0 µg/m^3 in PGM refineries where exposure to platinum salts occurred. They reported that some workers stopped working due to hypersensitive reactions to PGMs. Venables *et al*. (1989) reported workers to develop respiratory symptoms following occupational exposure to platinum salts in PGM refineries. Very little information is available on dermal exposure to PGMs and rhodium in particular. The majority of occupational exposure to PGMs, and specifically platinum, that has been conducted, investigated the inhalation of airborne particulate matter (Gómez *et al*., 2000; Gómez *et al*., 2001; Wiseman and Zereini, 2009; Zereini *et al*., 2012).

2.4 Adverse health effects caused by rhodium

Several uncertainties exist with regard to the possible negative effects associated with exposure to rhodium. While some researchers reported PGM-containing particles and soluble rhodium species emitted from catalysts to be a health concern, others found no evidence to support this statement (Merget and Rosner, 2001; Yajun and Xiaozheng, 2012). Rhodium may be a significant health risk due to occupational exposure to this metal in the working environment. One of the health concerns of rhodium which has been investigated, although not thoroughly, is the ability of the metal to cause sensitisation. This and other health risks associated with rhodium will be briefly discussed in the following sections.

2.4.1 Sensitisation and allergic contact dermatitis

Rhodium metal is generally regarded as allergen safe and especially in the metallic form is considered to be inert and unable to elicit any physiological reactions in the body (Ravindra *et al*., 2004; Goossens *et al.,* 2011). It is, therefore, often used to plate other metals with sensitising potential, such as nickel and cobalt in order to prevent allergic contact dermatitis and other diseases (Foti *et al*., 2002; Goossens *et al*., 2011). This may cause less nickel to be released from the object (such as jewellery), but it has not yet been proven that contact allergy will be completely avoided (Goossens *et al*., 2011).

With regard to allergenic metals, the formation of ions in sweat may have an influence on the onset of contact allergy (Flint, 1998). As rhodium is highly resistant to corrosion, it is unlikely to react with sweat in the metallic form and will, therefore, act as a sensitiser only in the salt form, as confirmed by Goossens *et al*. (2011).

Flint (1998) considered soluble rhodium compounds to act as potent sensitising agents. This is supported by Bocca and Forte (2009) and Sartorelli *et al.* (2012) who stated rhodium to be a sensitiser in the salt form, but not as a metal. Bedello *et al*. (1987) confirmed the sensitising ability of rhodium, reporting a goldsmith who developed contact dermatitis after coming in contact with a rhodium salt. Allergic contact dermatitis is an inflammatory occupational disease that affects millions of workers worldwide when xenobiotics come in contact with the skin (Nosbaum *et al*., 2009; Merget *et al*., 2010). It may result in various immunological reactions and if severe, may even prevent workers from continuing their work and have a negative impact on the overall health of the worker (Merget *et al*., 2010). De La Cuadra and Grau-Massanés (1991) also found contact dermatitis to occur in a worker after being dermally exposed to a highly concentrated rhodium solution. In yet another study, Goossens *et al.* (2011) investigated the reaction of two patients who had previously been sensitised to rhodium compounds. The first patient had a positive reaction to a rhodium chloride compound and developed contact dermatitis. The other developed airborne-induced skin lesions due to exposure to rhodium

compounds. Wiseman and Zereini (2009) reported soluble rhodium salt to cause dermatitis and urticaria among workers in refineries and catalyst manufacturers through airborne exposure, but found a general shortage of information available with regard to the effects thereof on the skin. Boscolo *et al*. (2004) found no evidence of occupational allergies caused by rhodium alone. They suggested that rhodium may induce allergic reactions by cross-reactions with other PGMs, leading to ACD. Yajun and Xiaozheng (2012) did not find enough evidence to confirm the sensitising ability of rhodium on humans, as opposed to the sensitisation it caused in mammalian cells. Several studies were found that confirms rhodium salt as a sensitiser and although the results from some studies were inconclusive, no studies were found that absolutely denied it. Therefore, in the opinion of the author, it is more likely for rhodium salts to act as a sensitiser than for it to be non-allergenic.

2.4.1.1 Mechanism of action of sensitisation and elicitation of allergic reaction

Some metals are only able to permeate through the skin and cause sensitisation, after forming a complete antigen, or a hapten (Adams, 2006). The potential of platinum for instance, to act as an allergen is dependent on its ability to form a complex with circulating proteins or tissue proteins in the epidermis. These proteins include amino acids or albumin (Bordignon *et al*., 2008). These proteins act as a carrier for the metal and enable the immune system to detect the hapten (Adams, 2006).

After a substance has permeated through the skin, it causes several epidermal cells, such as keratinocytes to release cytokines and chemokines. This is the initial signal for an allergic response to occur in the skin. In the case of prior contact to a xenobiotic, sensitisation will be induced in the body, causing specific T cells to be induced (Nosbaum *et al*., 2009). When the hapten comes into contact with the skin yet again, a cellular immune response is induced against the hapten complex. The specific T cells previously induced are activated by the process of antigen presentation, under the influence of a major histocompatibility complex (MHC) class I or II molecule (Bordignon *et al*., 2008; Nosbaum *et al*., 2009). These T cells secrete cytokines, such as interferon-gamma (IFN-γ), interleukin-2 (IL-2) and IL-17. The function of these and other cytokines is to destroy skin cells by the process of apoptosis, which leads to the occurrence of inflammation and eventually the production of new skin cells (Nosbaum *et al.*, 2009).

2.4.2 Carcinogenicity

A carcinogenicity study done on mice found that $RhCl_3$ increased tumour incidences (Schroeder and Mitchener, 1971). Merget and Rosner (2001) criticised this study for having too many methodological deficiencies. Rhodium is not listed as a carcinogen by the American Conference of Industrial Hygienists (ACGIH), International Agency for Research on Cancer

(IARC) or the National Toxicology Program (NTP) (Acros Organics, 2014). No other evidence was found to suggest that rhodium may act as a carcinogen under any circumstances.

2.4.3 Genotoxicity

The immunotoxicity of palladium is higher than that of rhodium and platinum, while the genotoxic ability of platinum and rhodium compounds are higher than that of palladium, due to their ability to induce oxidative damage (Boscolo *et al*., 2004). In accordance with this, Wiseman and Zereini (2009) found that compounds, such as platinum (II) chloride ($PtCl_2$), platinum (IV) chloride ($PtCl_4$) and $RhCl_3$ are more genotoxic than palladium salts, such as palladium (II) chloride ($PdCl_2$) and palladium (IV) chloride ($PdCl_4$), due to the ability of platinum and rhodium metals to induce oxidative damage. The ability of rhodium compounds to induce substantial cytogenetic damage has been confirmed (Migliore *et al*., 2002).

2.5 Barrier function of the skin

The skin is a dynamic, heterogeneous organ, the largest in the body and the only one continuously exposed to the environment (Byford, 2009; Ngo *et al*., 2009). It has an average weight of 5 kg (Godin and Touitou, 2007). The functions of the skin include maintenance of fluid balance, and controlling body temperature mainly via sweat production (Ngo *et al*., 2009). It also responds to mechanical forces and is responsible for defence and repair (Benson, 2005; Byford, 2009). These functions are achieved by the ability of the skin to act as an effective, although not complete barrier (Byford, 2009). The skin can act as a barrier in one of two ways. The first is by protecting against the loss of endogenous water and nutrients from inside the body to the external environment via evaporation. The second function, which relates to this study, is to prevent permeation of hazardous chemicals and pathogenic substances and xenobiotics from the external environment through the skin and into the body tissue (Byford, 2009; Rubio *et al*., 2011).

This barrier is mainly achieved by the specific organisation and physiological structure of the skin, and specifically the outermost layer (Byford, 2009; Rubio *et al*., 2011). This layer, the epidermis is one of three layers in the skin and contains no blood supply. The other two layers are the dermis, which contains the capillaries, nerve endings and skin appendages and the subcutaneous layer, which is the deepest layer of the skin (Ngo *et al*., 2009). The epidermis consists mainly of four layers, the stratum corneum, the stratum granulosum, the stratum spinosum and the stratum germinativum, as well as appendages, such as sweat glands, hair follicles and sebaceous glands (Byford, 2009). The stratum corneum is considered to be the major barrier against the permeation of substances. This is due to its brick-and-mortar-like structure created by the 10-15 layers of differentiated corneocytes, surrounded by an

extracellular lipid matrix. These lipids include primarily fatty acids, triglycerides, cholesterol and ceramides (Benson, 2005; Godin and Touitou, 2007; Lee *et al*., 2010).

2.6 Permeation through the skin

Percutaneous absorption describes the transport of substances from the external environment to the systemic circulation via unbroken skin. This is a complex process and can be considered as consisting of three distinct processes. The first is penetration, which involves the movement of a substance into a specific structure or layer, such as the stratum corneum (Byford, 2009). This is achieved mainly through passive diffusion and active transport plays no role (Byford, 2009; Jepps *et al*., 2013). The second is permeation, which describes the transport of a substance from one layer to another that is functionally and structurally different from the first. The last process is resorption, where a substance is taken into the skin and transported to the lymphatic system and blood vessels (Byford, 2009).

The permeation pathway that a substance will follow will depend primarily on its affinity for the lipid environment, its affinity for corneocytes and on the substance's ability to permeate the corneocytes' cell membranes (Jepps *et al*., 2013). Substances can permeate through the stratum corneum via one of three pathways. These include the transcellular route, the intercellular route and the shunt route (Benson, 2005). The transcellular route, also known as the intracellular route, is characterised by a series of partitioning and diffusion processes through the corneocytes. Thus, for a substance to follow this route, it should be able to diffuse through both the corneocytes and the intercellular lipid matrix (Jepps *et al*., 2013). The molecule would first undergo segmentation before diffusing through each keratinocyte that it encounters. Between each keratinocyte, approximately 4-20 lipid lamellae are located through which this molecule must partition and diffuse before moving on towards the next keratinocyte. The molecule must complete this complex process across multiple hydrophilic, as well as lipophilic layers before being completely absorbed into the skin (Benson, 2005; Jepps *et al*., 2013). In the second pathway, the intercellular route, substances follow the intercellular lipid matrix by diffusing between the corneocytes (Byford, 2009). The last pathway, the shunt routes occurs via the appendages. This pathway contributes the least to permeation. It is known as the shunt route, due to substances following this route bypassing the corneocytes and being transported via the hair follicles, the sweat glands and the sebaceous glands (Byford, 2009). This route of permeation is discussed later in this chapter.

2.6.1 Permeation through the stratum corneum

The stratum corneum provides the major contribution to the barrier function of the skin against permeation and may, therefore, be considered as the major route for permeation to take place. Permeation through the stratum corneum is mainly achieved via passive diffusion (Jepps *et al*.,

2013). While this lipid-rich structure is beneficial for the permeation of lipophilic substances, it may prevent the permeation of water-soluble compounds (Ngo *et al*., 2009). The mechanism of permeation through the stratum corneum is the same as previously described for permeation through the skin in general. Permeation through the stratum corneum may occur through either the intercellular or the transcellular route. Some controversy exists with regard to which of these routes is the predominant route for permeation through the stratum corneum. When following the intercellular route, the permeation is dependent on the affinity of the permeant for the lipid environment and when following the transcellular route, it depends on the affinity of the permeant for the internal environment of the corneocyte (Jepps *et al*., 2013). The rate by which a substance will permeate through the skin, known as the flux, will depend on the specific vehicle, as well as the concentration of the substances (Ngo *et al*., 2009).

2.6.2 Permeation through the deeper layers of the epidermis

A substance that is able to permeate the stratum corneum, will reach the epidermis and the underlying skin layers. These deeper layers also act as a barrier, provided by the presence of various proteins. Although this barrier is not as effective as the stratum corneum, it provides some obstruction to the diffusion process of xenobiotics. Due to the high water content of the epidermis, this layer of skin is more effective in protecting against lipophilic compounds (Jepps *et al*., 2013). If these viable aqueous layers fail to provide resistance against further permeation, the substance will be transported to the dermis and subcutaneous tissue, from where it may be transported to the systemic circulation (Jepps *et al*., 2013). Due to $RhCl_3$ being a soluble salt compound, it should be possible for rhodium to permeate through this layer, provided it is able to permeate through the stratum corneum first.

2.6.3 Permeation through the dermis and deeper skin layers

The deeper skin layers serve an important function in preventing permeation of substances through the skin, specifically for lipophilic substances, due to the aqueous nature of the dermis (Jepps *et al*., 2013). Permeation through the dermis differs significantly from absorption through the epidermis, especially with regard to the ability of the dermis to contribute to transport and distribution of substances in the skin. The dermis may, however, enhance the barrier function of the skin by providing opportunities for some substances to be bound and sequestrated to the skin (Jepps *et al*., 2013). Such substances include aluminium (III), which has been found to form complexes with the skin and form insoluble compounds (Hostýnek *et al*., 2003).

2.6.4 Permeation through the appendages

Although permeation via the appendages, also known as the shunt route, provides a very inviting alternative to the resistant stratum corneum, this route only contributes approximately

0.1 % of the total permeation through the skin (Benson, 2005; Jepps *et al*., 2013). In general, this route provides less resistance to delivery of substances through the skin (Benson, 2005). Lipophilic substances may easily diffuse into the hair follicles and sebaceous glands, due to lipophilic sebum, but this route is quite resistant to hydrophilic substances (Jepps *et al*., 2013). This route of permeation may provide a very important alternative for larger molecules, such as proteins to be absorbed through and stored in the skin (Ngo *et al*., 2009). The accumulation of substances within the appendages may change the physiological structure of the skin barrier, leading to effective uptake into the body (Schneider *et al*., 2009).

In studies where permeation is investigated via *in vitro* methods, the appendages play a very small role in the permeation of the substance investigated. This is due to the fact that the constant hydration caused by the solution placed on the surface of the skin, causes swelling of the skin and closes off the appendages (Benson, 2005).

A substance that is able to permeate all the above mentioned layers of the skin, may accumulate in the skin itself or be distributed to the vascular network and lymphatic system (Ngo *et al*., 2009; Jepps *et al*., 2013). The lymphatic and vascular system may have one of two roles to play in the distribution of an absorbed substance. It may either facilitate the distribution of the substance in the body or contribute towards the clearing thereof. If the lymphatic system does not completely remove the substance it may be cleared from the body via skin metabolism. This may lead to accumulation of some substances in the body, from where it may exert several local or systemic effects, depending on its nature (Jepps *et al*., 2013).

2.7 Skin surface pH

Skin surface pH is considered to be a marker of the integrity and wellbeing of the skin. It is a measure of the negative logarithm of the free hydrogen ion concentration (H^+) present in the skin (Ehlers, 2001). It can be measured on a scale between one and fourteen, where a measurement of seven is considered to be neutral, with an acidic range below seven and an alkaline range above seven (Schmid-Wendtner and Korting, 2006). In general the surface pH of human skin is thought to be acidic, ranging between 4 and 6 or even lower (Hanson *et al*., 2002; Larese Filon *et al*., 2006; Byford, 2009). This acidity may be attributed to several factors, such as the presence of water-soluble elements in the stratum corneum, diffusion of CO_2, as well as secretion of sebum and sweat (Ehlers, 2001; Parra and Paye, 2003; Schmid-Wendtner and Korting, 2006).

The pH of the skin has an important influence on the composition of the stratum corneum and various enzymes in the skin are pH dependent. The pH values in the extracellular spaces need to be maintained in the acidic range, as this is beneficial for the regulation of enzyme activities that are responsible for maintaining keratinisation and regeneration of the skin barrier (Schmid-

Wendtner and Korting, 2006). The acidic nature of the skin surface thus contributes to the various functions of the skin, such as antimicrobial functions, regulating homeostasis of the skin barrier and maintaining the integrity of the stratum corneum (Gunathilake *et al*., 2009). The pH of the internal body environment is generally closer to neutral, with a range between 7.35 and 7.46 (Schmid-Wendtner and Korting, 2006).

In occupational settings, the skin surface pH of workers may be lower than normally considered. This may be due to the presence of acidic elements and substances in the working environment, as well as the metabolically active state of the skin (Larese Filon *et al*. 2007; Larese Filon *et al*., 2008). The evaporation of eccrine sweat causes the pH of the skin surface to decrease. The barrier function, which is effective in protecting against permeation of hazardous substances, is influenced by the surface pH of the skin (Schmid-Wendtner and Korting, 2006; Byford, 2009). A change in the pH of the skin surface would, therefore, indirectly lead to a change in the permeation of a substance, due to reduced or increased barrier function of the skin, as demonstrated by Sartorelli *et al*. (2012). Gunathilake *et al*. (2009) found a decrease in the pH to increase the barrier function of the skin and Schmid-Wendtner and Korting (2006) supported this by stating that an increase in the pH has been proven to contribute to dramatic deviation of the skin barrier.

The pH of eccrine sweat varies between 5 and 6. The presence of lactic acid in the sweat is generally considered to be the cause of the acidity of the skin surface. Ammonia, a product of bacterial degradation, may cause the sweat to become more alkaline, but due to the rapid evaporation of the ammonia, the skin will return to its acidic state (Parra and Paye, 2003). The pH of the skin surface is, therefore, maintained at an acidic value between 4.5 and 6 and thus contributes to the acid mantle of the skin, which is influenced by several factors, including anatomical position and gender (Hanson *et al*., 2002; Levin and Maibach, 2008).

2.7.1 Buffering capacity of the skin

A buffer refers to the ability of a chemical system to limit changes in pH that occur due to the addition of a base or an acid. In human skin, this buffer is mainly achieved by the presence of lactic acids and amino acids in sweat. The contact of aqueous acid or alkaline solutions with the skin causes a temporary change in the skin pH. This change is, however, rapidly restored, an indication of the significant buffering capacity of the skin (Levin and Maibach, 2008).

The buffering capacity of the skin contributes towards maintaining the elasticity of the stratum corneum and the acidic nature of the skin surface. It contributes to the ionisation of several compounds in the skin, specifically in the stratum corneum, and plays a role in the regulation of the pH gradient in the stratum corneum (Parra and Paye, 2003).

2.7.2 Natural moisturising factor

One of the functions of natural moisturising factor (NMF) is to serve as a buffer against the loss of water from corneocytes. The NMF of the skin is comprised of various components, such as lactic acid, urea, carbohydrates, ammonia, peptides and amino acids. The main function of the NMF is to maintain the water retention capacity in the skin via the corneocytes. (Parra and Paye, 2003).

2.7.3 pH gradient across the stratum corneum

The stratum granulosum lies approximately 10 µm beneath the skin surface, directly below the stratum corneum. In contrast to the acidic nature of the stratum corneum, the stratum granulosum reaches neutrality, creating a significant pH gradient between the uppermost and deepest layers of the stratum corneum (Hanson *et al*., 2002; Parra and Paye, 2003). This is achieved by an increase in the pH with each deeper layer of corneocytes in the stratum corneum (Hanson *et al*., 2002). This sharp gradient serves an important function in the maintenance of cellular metabolism regulated by various enzymes in the skin. Several processes in the skin serve to sustain this gradient across the stratum corneum, such as the secretion of sebum and sweat (Parra and Paye, 2003).

2.8 Influence of ionisation on permeation

Metals such as silver and iron have been found to be ionised quite readily when in contact with human sweat, giving rise to several problems, such as staining the skin (Lidén *et al*., 1998). The action of nickel salts in synthetic sweat were also demonstrated by Lidén and Carter (2001) who found that more nickel salts were extracted from nickel-containing coins when immersed in synthetic sweat than in water. They attributed this to the corrosive effect of sweat. Hostýnek *et al*. (2003) found that copper undergoes electrochemical reactions in the presence of synthetic sweat, leading to the formation of cupric ions (Cu^{2+}) with the ability to permeate through the skin. Hostýnek *et al*. (2006) attributed the ability of certain metals to undergo ionisation in the presence of sweat, to play a significant role in the ability of those metals to be permeated through the skin, as the permeation is highly dependent on the formation of soluble compounds. In the presence of sweat, reactions may occur between the skin and the metal, causing the metal to undergo oxidation. This leads to the formation of permeable compounds with anions identical to the skin, such as chloride ions on the skin surface. Sweat functions as an electrolyte, leading to the formation of metal ions via the process of electrochemical oxidation (Hostýnek *et al*., 2006).

Some researchers considered this ion formation to be detrimental to the permeation process across the skin. Guy and Hadgraft (1989) found that chemicals in the ionised form permeate

poorly through the skin, as they are unable to partition into the stratum corneum. A molecule in the ionised form would possess a charge and due to high polarity would not permeate through the skin effectively. This is supported by Smith (1990) who stated that ionised molecules would permeate through the skin much less rapidly than non-ionised molecules.

More recently, and in direct contrast to this, Tanojo *et al*., (2001) found that free nickel ions permeate the skin faster than non-ionised nickel molecules, due to the smaller size of the ions. The more nickel ions that exist, the more permeation will take place. Larese Filon *et al*. (2007) found that certain cobalt and nickel compounds released metallic ions when stirred in synthetic sweat and permeated more easily across the skin in the ionised form. Since then the same has been confirmed for chromium, silver and gold (Larese Filon *et al*., 2008; Larese Filon *et al*., 2009a; Larese Filon *et al*., 2011).

2.8.1 Influence of pH on ionisation

Menek *et al*. (2012) investigated the release of nickel ions from stainless steel crowns placed in artificial saliva at different pH values of 2.5, 3.75, 5 and 6.25. With each increase in pH value, a decrease in nickel (II) concentration occurred due to the easier dissolution of nickel alloys in an acidic medium. Skin permeation of nickel, cobalt and chromium was investigated using synthetic sweat with a pH of 6.5. While nickel and cobalt permeated through the skin quite readily, the permeation of chromium was low. This was attributed to the inability of chromium to be oxidised at such a high pH (Larese Filon *et al*., 2007). In a following study, the solubility of chromium immersed in synthetic sweat at pH values of 5.5, 4.5 and 3.5 was investigated, as well as the permeation of chromium at a pH of 4.5. The dissolution of chromium was found to increase with a decrease in pH. Chromium was able to permeate through the skin at a pH of 4.5, due to the formation of ions through the process of oxidation (Larese Filon *et al*., 2008). In a later study, Larese Filon *et al*. (2009a) stated that certain chemical elements, such as chromium are more readily ionised in an acidic environment. A decrease in pH of one unit would lead to a 10 to 100 fold increase in the permeation across the skin. In this and other studies, a lower pH of 4.5 allowed metallic powders to be oxidised to soluble ions prior to permeation of the metals through the skin (Larese Filon *et al*., 2009a; Larese Filon *et al*., 2009b; Larese Filon *et al*., 2011; Larese Filon *et al*., 2012). During the investigation of the permeation of cobalt, nickel and chromium through human skin at a pH of 4.5 it was found that the permeation values were higher than those from previous studies in which synthetic sweat with a pH of 6.5 was used (Larese Filon *et al*., 2009b).

2.9 Franz diffusion cell method

Several *in vivo* as well as *in vitro* methods exist that are applied to investigate permeation of substances through the skin (Venter *et al*., 2001). *In vivo* studies are mostly performed on

animals such as mice and rats. It is, however, very difficult to relate these results to humans (Larese Filon *et al*., 2007).

The Franz diffusion cell method is one of the most widely accepted and used methods to investigate the *in vitro* permeation of various substances. These cells can be applied to determine the bioavailability of substances by examining their diffusion properties and abilities (Larese Filon *et al*., 2007). This method provides a very useful way to characterise the relationship between the skin, its barrier and substances that are able to permeate this barrier. Franz diffusion cells are used in various fields of study, such a pharmaceutics where new pharmaceutical products are developed and occupational toxicology when screening for hazardous toxicants in occupational settings (Larese Filon *et al*., 2007).

In studies utilising Franz diffusion cells the intact human skin is simulated by using excised human skin, mostly obtained as biological waste from surgeries. Full-thickness human skin can be utilised. This refers to skin from which the subcutaneous fat has been removed, but all the layers of the skin are kept intact (Larese Filon *et al*., 2009a). *In vitro* studies are generally simple to carry out and the experimental conditions can be accurately maintained (Larese Filon *et al*., 1999). Another benefit of this method is the fact that the researcher can sample directly from the receptor compartment beneath the skin via the sampling port. The other chamber of the Franz diffusion cell, the donor compartment usually contains a physiologically relevant solution, such as synthetic sweat for toxicology studies. The synthetic sweat is adjusted to a pH that resembles that of normal skin in order to simulate normal intact skin conditions as closely as possible (Larese Filon *et al*., 2007).

There are a few disadvantages associated with this method. Physiological changes may occur in the skin after surgery, mainly because of the removed blood supply. This method also fails to take factors such as cutaneous metabolism into account (Venter *et al*., 2001). In *in vitro* methods, such as Franz diffusion cells, the absorption rates and permeation are determined by passive diffusion through the stratum corneum (Venter *et al*., 2001). Therefore, these methods represent a realistic means of determining permeation through the skin.

2.10 Summary

From the above literature study, it is evident that a many contradictions still exist with regard to the *in vitro* permeation of metals and there is a general lack of information with regard to this. The ionisation of metals following contact with human sweat remains a topic yet to be fully understood. Although studies have proven the ability of various metals to permeate through skin at different pH values, no studies have been conducted with the aim of comparing the permeation ability of metals at different pH values. A lack of information also exists with regard

to the permeation of rhodium through the skin, as well as that of other PGMs in general. It is, therefore, essential that more studies be conducted on this topic.

2.11 References

Acros Organics. (2013) Material safety data sheet: Rhodium on alumina powder. Available at: http://www.fishersci.com/ecomm/servlet/msdsproxy. Accessed: 6 May 2014.

Adams S. (2006) Allergies in the workplace. Curr Allergy Clin Immunol; 19: 82 – 86.

Addicks WJ, Flynn GL, Weiner N. (1978) Validation of a flow-through diffusion cell for use in transdermal research. Pharmaceut Res; 4: 337 – 341.

Benson HAE. (2005) Transdermal drug delivery: penetration enhancement techniques. Curr Drug Deliv; 2: 23 – 33.

Bocca B, Forte G. (2009) The epidemiology of contact allergy to metals in the general population: prevalence and new evidences. Open Chem Biomed Meth J; 2: 26 – 34.

Bordignon V, Palamara F, Cordiali-Fei P, *et al*. (2008) Nickel, palladium and rhodium induced IFN-gamma and IL-10 production as assessed by *in vitro* ELISpot-analysis in contact dermatitis patients. BMC Immunol; 9: 19 – 29.

Boscolo P, Di Giampaolo L, Reale M, *et al*. (2004) Different effects of platinum, palladium and rhodium salts on lymphocyte proliferation and cytokine release. Ann Clin Lab Sci; 34: 299 – 306.

Byford T. (2009) Environmental Health Criteria 235: Dermal Absorption. Int J Environ Stud; 66: 662 – 788.

Cawthorn RG. (1999) The platinum and palladium resources of the Bushveld complex. S Afr J Sci; 95: 481- 489.

Ehlers C, Ivens UI, Moller ML, *et al*. (2001) Females have lower skin surface pH than men: A study on the influence of gender, forearm site variation, right / left difference and time of the day on the skin surface pH. Skin Res Technol; 7: 90 – 94.

Flint GN. (1998) A mettalurgical approach to metal contact dermatitis. Contact Dermatitis; 38: 213 – 221.

Foti C, Amoruso A, Cassano N, Vena GA. (2002) Contact sensitisation to nickel from rhodium-plated ‘nickel-free’ earrings. Contact Dermatitis; 46: 309.

Godin B, Touitou E. (2007) Transdermal skin delivery: predictions for humans from in vivo, ex vivo and animal models. Adv Drug Delivery Rev; 59: 1152 – 1161.

Gómez MB, Gómez MM, Palacios MA. (2000) Control of interferences in the determination of Pt, Pd and Rh in airborne particulate matter by inductively coupled plasma mass spectrometry. Anal Chim Acta; 404: 285 – 294.

Gómez B, Gómez M, Sanchez JL, *et al*. (2001) Platinum and rhodium distribution in airborne particulate matter and road dust. Sci Total Environ; 269: 131 – 144.

Goossens A, Cattaert N, Nemery B, *et al*. (2011) Occupational allergic contact dermatitis caused by rhodium solutions. Contact Dermatitis; 64: 158 – 184.

Gunathilake R, Schurer NY, Shoo BA, *et al*. (2009) pH-Regulated mechanisms account for pigment-type differences in epidermal barrier function. J Invest Dermatol; 129: 1719 – 1729.

Guy RH, Hadgraft J. (1989) Chapter 6: Structure-activity correlations in percutaneous absorption. In Bronaugh RL, Maibach HI, editors. Percutaneous absorption: Methods – Methodology – Drug Delivery. 2nd ed. New York: Marcel Dekker, Inc. p. 95 – 109. ISBN 0-8247-8036-1.

Hanson KM, Behne MJ, Barry NP, *et al*. (2002) Two-photon fluorescence lifetime imaging of the stratum corneum pH gradient. Biophys J; 83: 1682 – 1690.

Haudrechy P, Foussereau J, Mantout B, Baroux B. (1993) Nickel release from 304 and 316 stainless steels in synthetic sweat. Comparison with nickel and nickel – plated metals. Consequences on allergic contact dermatitis. Corr Sci; 35: 329 – 336.

Hostýnek JJ, Dreher F, Pelosi A, *et al*. (2003) Human stratum corneum penetration by nickel. Acta Derm Venereol Suppl; 212: 5 – 10.

Hostýnek JJ, Dreher F, Maibach HI. (2006) Human stratum corneum penetration by copper: In vivo study after occlusive and semi-occlusive application of the metal as powder. Food Chem Toxicol; 44: 1539 – 1543.

Jepps OG, Dancik Y, Anissimov YG, Robers MS. (2013) Modeling the human skin barrier – towards a better understanding of dermal absorption. Advanced Drug Delivery Reviews; 65: 152 – 168.

Larese Filon F, Boeniger M, Giovanni M, *et al*. (2006) Skin absorption of inorganic lead (PbO) and the effect of skin cleansers. J Occup Environ Med; 48: 692 – 699.

Larese Filon F, Gianpiero A, Venier M, *et al*. (2007) *In vitro* percutaneous absorption of metal compounds. Toxicol Lett; 170: 49 – 56.

Larese Filon F, D'Agostin F, Crosera M, Adami G. (2008) *In vitro* percutaneous absorption of chromium powders and the effect of skin cleanser. Toxicol In Vitro; 22: 1562 – 1567.

Larese Filon F, D'Agostin F, Crosera M, *et al*. (2009a) In vitro absorption of metal powders through intact and damaged human skin. Toxicol In Vitro; 23: 574 – 579.

Larese Filon F, D'Agostin F, Crosera M, *et al*. (2009b) Human skin penetration of silver nanoparticles through intact and damaged skin. Toxicol; 225; 33 – 37.

Larese Filon F, Crosera M, Adami G, *et al*. (2011) Human skin penetration of gold nanoparticles through intact and damaged skin. Nanotoxicol; 1-9.

Larese Filon F, Crosera M, Timeus E, *et al*. (2012) Human skin penetration of cobalt nanoparticles through intact and damaged skin. Toxicol In Vitro; 27: 121 – 127.

Lee PH, Conradi R, Shanmugasundaram V. (2010) Development of an in silico model for human skin permeation based on a Franz cell skin permeability assay. Bioorg Med Chem Lett: 20: 69 – 73.

Levin J, Maibach H. (2008) Human skin buffering capacity: an overview. Skin Res Technol; 14: 121 – 126.

Lidén C, Nordenadler M, Skare L. (1998) Metal release from gold-containing jewellery materials: no gold release detected. Contact Dermatitis; 39: 281 – 285.

Lidén C, Carter S. (2001) Nickel release from coins. Contact Dermatitis; 44: 160 – 165.

Matthey J. (2012) Platinum interim review: Other platinum group metals. Available at: http://www.platinum.matthey.com/media/1393522/platinum_2012_interim_review.pdf; Accessed: 5 July 2012.

Merget R, Rosner G. (2001) Evaluation of the health risk of platinum group metals emitted from automotive catalytic converters. Sci Total Environ; 270: 165 – 173.

Merget R, Sander I, Van Kampen V, *et al*., (2010) Occupational immediate-type asthma and rhinitis due to rhodium salts. Am J Ind Med; 53: 42 - 46.

Migliore L, Frenzilli G, Nesti C, *et al*. (2002) Cytogenetic and oxidative damage induced in human lymphocytes by platinum, rhodium and palladium compounds. Mutagenesis; 5: 411 – 417.

Murdoch RD, Pepys J, Hughes EG. (1986) IgE antibody responses to platinum group metals: a large scale refinery survey. Brit J Ind Med; 43: 37 – 43.

Ngo MA, O'Malley M, Maibach HI. (2009) Percutaneous absorption and exposure assessment of pesticides. J Appl Toxicol; 30: 91 – 114.

Nosbaum A, Vocanson M, Rozieres A, *et al*. (2009) Allergic and contact dermatitis. Eur J Dermatol; 19: 325 – 332.

Parra JL, Paye M. (2003) EEMCO Guidance for the in vivo assessment of skin surface pH. Skin Pharmacol Appl Skin Phsysiol; 16: 188 – 202.

Ravindra K, Bencs L, Van Grieken R. (2004) Platinum group elements in the environment and their health risk. Sci Total Environ; 318: 1 – 43.

Rubio L, Alonso C, López O, *et al*. (2011).Barrier function of intact and impaired skin: percutaneous penetration of caffeine and salicylic acid. Int J Dermatol; 50: 881 - 889.

Sartorellli P, Montomoli L, Sisinni AG. (2012).Percutaneous penetration of metals and their effects on the skin. Prevent Res; 2: 158 – 164.

Schierl R, Fries HG, Van de Weyer C, Fruhmann G. (1998). Urinary excretion of platinum from platinum industry workers. Occ Environ Med; 55: 138 – 140.

Schmid-Wetner MH, Korting HC. (2006) The pH of the skin surface and its impact on the barrier function. Skin Pharmacology and Physiology; 19: 296 – 302.

Schneider M, Stracke F, Hansen S, Schaefer UF. (2009) Nanoparticles and their interaction with the dermal barrier. Dermato Endocrinol; 4: 197 – 206.

Schroeder HA, Mitchener M. (1971) Scandium, chromium, gallium, yttrium, rhodium, palladium, indium in mice: effects on growth and life span. J Nutr; 101: 1431 – 1438.

Smith KL. (1990) Chapter 2: Penetrant characteristics influencing skin absorption. In Kemppainen BW, Reifenrath WG, editors. Methods for skin absorption. Boca Raton: CRC Press. p. 23 – 34. ISBN 0-8493-4651-7.

Tanojo H, Hostýnek JJ, Mountford HS, Maibach HI. (2001) *In vitro* permeation of nickel salts through human stratum corneum. Acta Derm Venereol; 21: 19 – 23.

Venables KM, Dally MB, Nunn AJ, *et al*. (1989) Smoking and occupational allergy in workers in a platinum refinery. Br Med J; 299: 939 – 942.

Venter JP, Müller DG, Du Plessis J, Goosen C. (2001) A comparative study of an in situ adapted diffusion cell and an *in vitro* Franz diffusion cell method for transdermal absorption of doxylamine. European Journal of Pharmaceutical Sciences; 13: 169 - 177.

Wiseman CLS, Zereini F. (2009) Airborne particulate matter, platinum group elements and human health: a review of recent evidence. Sci Total Environ; 407: 2493 – 2500.

Yajun W, Xiaozheng LE. (2012) Health risk of platinum group elements from automobile catalysts. Procedia Eng; 45: 1004 – 1009.

Zereini F, Alsenz H. Wiseman CLS, *et al*. (2012) Platinum group elements (Pt, Pd, and Rh) in airborne particulate matter in rural vs. urban areas of Germany: concentrations and spatial patterns of distribution. Sci Total Environ; 416: 261 – 268.

Chapter 3: Article

Chapter 3: Article

3.1 Instructions to Authors

Annals of Occupational Hygiene publishes original research and development material that helps reduce risk of ill-health resulting from work, and welcomes submissions in these areas.

Language: Manuscripts must be in English and authors should write in a way which is simple and clear. British or American styles and spelling may be used, but should be used consistently, and words or phrases which might be unclear in other parts of the world should be avoided or clearly explained.

Title, abstract and keywords: These are important because most readers find papers by internet search of subjects, not by browsing the journal. Titles should be constructed to succinctly describe the major issue or question examined by the paper and should not assert the research findings as a truth. Recognisable, searchable terms and keywords must be included to enable readers to more effectively find your paper. To optimise the visibility of your paper we advise you to make a list of the 10 most likely search terms (words and phrases) that your intended readers will use to find your work, and to ensure that these appear in your title, the abstract and the keywords. The 'number one' search term from your list should appear somewhere in the paper's title. This will usually not be just a single word; rather a short phrase summarising the main subject of the paper. The 'top 5' search terms (including 'number one') should each appear at least once in the abstract, with the 'top 3' appearing more than once if possible. It is important that your abstract is written in a naturalistic and engaging style that will encourage readers to follow up by reading the full paper. The 'bottom 5' search terms can then be added as keywords. It is important to include variants of the 'top 5' here if they exist, e.g. alternative names for chemicals or processes.

Authorship: Persons should only be named as authors if they have made significant identifiable intellectual contributions to the work; other contributions may be recognised by acknowledgement at the end of the submission.

Structure of paper: Papers should generally conform to the pattern: Introduction, Methods, Results, Discussion, and Conclusions, unless these are clearly inappropriate. A paper must be prefaced by an abstract of the argument and findings, which may also be arranged under the same headings. As with many other journals, we are unable to publish footnotes to the text. Please therefore incorporate this sort of material into the body of the paper, in brackets if appropriate.

Design and analysis: The quality of the data and analysis must always be good enough to

justify the inferences and conclusions drawn. Particular attention should be given to design of sampling surveys, which should be planned using modern statistical principles, and to the treatment of results below the limit of detection.

Units and symbols: SI units must be used, though their equivalent in other systems may be given as well.

Figures: These include photographs, diagrams and charts. The first submission should include good quality low resolution copies of Figures, and may be incorporated into the text or at the end of the manuscript.

Tables: Tables should be numbered consecutively and given a suitable caption. As with Figures, it is helpful to incorporate them into the text of the first submission, but in the revised version each table should be presented on a separate page. Footnotes to tables should be provided below the table and should be referred to by superscript lowercase letters.

References: References should only be included which are essential to the development of an argument or hypothesis, or which describe methods for which the original account is too long to be reproduced. References in the text should be in the form Jones (1995), or Jones and Brown (1995), or Jones et al. (1995) if there are more than two authors, and they should be incorporated naturally into the text. For example:
Jones and Brown (1995) and Hospath et al (2006) observed total breakdown of control..., or
Total breakdown of control has sometimes been observed (Jones and Brown, 1995; Hospath et al., 2006).
Papers whose references are not properly arranged may be returned for revision without review.

At the end of the paper, references should be listed in alphabetical order by name of first author, using the Vancouver Style of abbreviation and punctuation. ISBNs should be given for books and other publications where appropriate. Material unobtainable by readers should not be cited. Personal Communications, if essential, should be cited in the text (e.g., Professor O.H. Poobah, Institute for Dusty Sciences). Internet material can be referred to if it is likely to be permanently available; the date on which it was last accessed should be given. References will not be checked editorially, and their accuracy is the responsibility of authors.

Examples:
Simpson AT, Groves JA, Unwin J, Piney M. (2000) Mineral oil metal working fluids (MWFs)—Development of practical criteria for mist sampling. Ann Occup Hyg; 44: 165–72.

Vincent JH. (1989) Aerosol sampling: science and practice. Chichester, UK: John Wiley. ISBN 0 471 92175 0.

Swift DL, Cheng Y-S, Su Y-F, Yeh H-C. (1994) Ultrafine aerosol deposition in the human nasal and oral passages. In Dodgson J, McCallum RI, editors. Inhaled Particles VII. Oxford: Elsevier Science. p. 77–81. ISBN 0 08 040841 9 H.

British Standards Institution. (1986). BS 6691: 1986. Fume from welding and allied processes. Part 1. Guide to methods for the sampling and analysis of particulate matter. London: British Standards Institution.

Morse SS. (1995) Factors in the emergence of infectious diseases. Emerg Infect Dis [serial online] 1995 Jan–Mar;1(1). Available from: URL: http://www.cdc.gov/ncidod/ EID/eid.htm (accessed 25 Oct 2010).

The influence of pH on the *in vitro* skin permeation of rhodium

SANÉ J JANSEN VAN RENSBURG[1], ANJA FRANKEN[1], JOHANNES L DU PLESSIS[1], JEANETTA DU PLESSIS[2]

[1] Subject Group Physiology, Faculty of Health Sciences, North-West University, Potchefstroom Campus, South Africa.

[2] Centre of Excellence for Pharmaceutical Sciences, North-West University, Potchefstroom Campus, South Africa.

Corresponding Author

Miss SJ Jansen van Rensburg

School of Physiology, Nutrition and Consumer Sciences,

North-West University, Potchefstroom Campus

Private Bag X6001

Potchefstroom

2520

South Africa

Tel: 018 299 2433

Fax: 018 299 1053

Email: 21619719@nwu.ac.za

Word Count: 5524

3.2 Abstract

Rhodium is one of the platinum group metals (PGMs) and in occupational settings such as catalytic industries and PGM refineries, workers are at risk of being dermally exposed to this metal. This exposure can occur either in the metallic state or its salt compounds. It has been found that rhodium in the salt form is able to act as sensitiser, but not in the metallic state. Very little information is available on the dermal exposure to rhodium, as well as its ability to permeate through the skin. Previous studies conducted on the *in vitro* permeation of metals have yielded evidence that certain metals, such as nickel, cobalt and chromium, undergo oxidation under the influence of synthetic sweat. This leads to the formation of ions that are able to permeate through the skin. The pH of the solution, such as synthetic sweat, greatly influences the ionisation process. With regard to other metals, such as chromium, a decrease in the pH has led to an increase in permeation through skin due to an increase in the ionisation in the acidic environment. The ability of rhodium to permeate through the skin has not yet been investigated and the influence of pH on such permeation has not yet been described. *Aim*: The aim of this study was to determine whether rhodium applied as rhodium trichloride ($RhCl_3$) is able to permeate through the skin and if any differences exist between the *in vitro* permeation of rhodium at a pH of 4.5 and 6.5. *Method*: Synthetic sweat containing $RhCl_3$ was prepared as the donor solution and adjusted to pH values of 4.5 and 6.5 respectively. Circular pieces of full thickness abdominal skin from donors between the ages of 39 and 42 years were mounted on Franz diffusion cells and the donor solution, containing $RhCl_3$ was applied to experimental cells for 24 hours. Blank cells did not contain any $RhCl_3$. The receptor compartment contained a physiological receptor solution, which was removed at time intervals of 8, 12 and 24 hours to be analysed. The receptor solutions were analysed using Inductively Coupled Plasma Mass Spectrometry and the donor and digested skin solutions were analysed using Inductively Coupled Plasma Optical Emission Spectrometry. *Results*: At both a pH of 4.5 and 6.5, rhodium was able to permeate through the skin with a cumulative increase in permeation over prolonged exposure time. After 8, 12 and 24 hours, the mass of rhodium that permeated through the skin was higher for pH 4.5 than for pH 6.5 with the mass of rhodium that permeated after 12 hours being statistically significantly higher at a pH of 4.5 than at a pH of 6.5. At both pH values, the percentage of rhodium that accumulated in the skin was exponentially higher than the percentage of rhodium that diffused through the skin and the lag time was less than six hours. *Conclusion*: From these results, it can be concluded that a decrease in the pH of synthetic sweat led to an increase in the permeation of rhodium and it is recommended that future *in vitro* permeation studies be conducted at a pH of 4.5, as the skin surface pH of workers are generally at or below this value. A greater percentage of rhodium was retained in the skin than the percentage that diffused through, indicating the ability of rhodium to accumulate in the skin, from where it may exert health effects, such as sensitisation. Although the lag time was less

than six hours, it should not be wrongfully assumed that a worker with a work shift less than six hours is safe regarding exposure to and permeation of rhodium.

3.3 Introduction

Rhodium is a rare, durable metal, belonging to the platinum group metals (PGMs) (Cawthorn, 1999; Foti *et al*., 2002). Its unique catalytic properties have led to an increase in the demand and use of this metal, particularly as a vehicle exhaust catalyst (VEC) with an increased amount of converters being fitted with rhodium only (Gómez *et al*., 2000; Merget and Rosner, 2001; Matthey, 2012). In addition, it is used in various other industries, such as chemical, electronic and petroleum industries, as well as production of jewellery and glass. It is also used in dentistry and in medicine for its anti-carcinogenic abilities (Wiseman and Zereini, 2009; Zereini *et al*., 2012).

The increased demand for PGMs has led to increased occupational exposure of workers to these metals in refineries and catalytic industries and increased concentrations of PGMs in the environment (Wiseman and Zereini, 2009). Occupational exposure may occur via three routes, namely ingestion, inhalation and dermal exposure (Sartorelli *et al*., 2012). The majority of the research conducted on PGMs focused on platinum as airborne particulate matter and the exposure to this metal via inhalation (Gómez *et al*., 2000; Gómez *et al*., 2001). Despite dermal exposure becoming an increasingly popular topic of research, major shortcomings still exist in the literature with regard to dermal exposure, and especially the possibility of permeation of toxic substances through the skin. Very little published information is available with regard to the dermal exposure, permeation and toxicity of PGMs and especially that of rhodium (Bocca and Forte, 2009; Wiseman and Zereini, 2009).

With regard to the toxicity of rhodium, several cases have been reported for occupational exposure to rhodium causing hypersensitive reactions such as allergic contact dermatitis and urticaria (Bedello *et al*., 1989; De La Cuadra and Grau- Massanés, 1991; Goossens *et al*., 2011). Some researchers found that rhodium is able to act as a sensitising agent in the salt form and others have reported it to cause contact sensitisation due to the use of prostheses and dental amalgams (Foti *et al*., 2002; Bocca and Forte, 2009; Goossens *et al*., 2011; Sartorelli *et al*., 2012). Other researchers, however, did not find enough evidence to support this statement (Yajun and Xiaozheng, 2012).

A general lack of data exists with regard to the occupational dermal exposure of rhodium. The extent to which workers are dermally exposed to this substance, whether in metal or salt form, has not yet been published and many inadequacies still exist in this regard. The potential of rhodium to permeate through the skin has not yet been investigated and no published information is available. This is an important area of research, as the skin permeating ability of

hazardous substances is crucial when investigating the health risks associated with such substances, especially where dermal exposure in the workplace is a possibility. Investigating permeation of xenobiotics provides a manner in which the diffusion characteristics of hazardous substances can be identified (Tanojo e*t al*., 2001). This may provide an indication of the acceptable level of exposure, if any at all, to hazardous substances in the working environment (Byford, 2009).

The suspicion that rhodium, in the form of rhodium trichloride ($RhCl_3$), may be able to permeate through the skin and the influence that pH of synthetic sweat might have on such permeation was the main motivation for this study. $RhCl_3$ is considered to be a soluble rhodium compound and it may possibly have electrochemical reactions with sweat, as demonstrated for other metals (Palacios *et al*., 2000; Hostýnek *et al*., 2006). Sweat acts as an electrolyte, causing electrochemical oxidation, which leads to the formation of metal ions (Hostýnek *et al*., 2006). One of the factors influencing the *in vitro* permeation of a metal through the skin is the ability of that metal to undergo oxidation and form ions. The ability of a metal to form soluble compounds or ions is a critical factor to consider when investigating *in vitro* permeation of metals. Some researchers consider the formation of ions to be detrimental to the permeation process. Guy and Hadgraft (1989) found metal ions to permeate the skin poorly, due to their inability to partition through the stratum corneum. This is supported by Smith (1990) who considered ionised molecules to permeate through the skin much less rapidly than non-ionised molecules. Tanojo *et al*. (2001) found that nickel ions forming in the presence of synthetic sweat, may be bound to fatty acids, peptides or other counter ions in the stratum corneum, which may increase the retention of the metal. Hostýnek *et al*. (2003) found aluminium (III) to form complexes with the skin, leading to the formation of insoluble salts. In direct contradiction to this, researchers found that metals forming ions will permeate the skin more readily than non-ionised metal molecules (Hostýnek *et al*., 2006; Larese Filon *et al*., 2007). This has been attributed to the smaller size of the ions (Tanojo *et al*., 2001). Metals such as nickel, cobalt, chromium, silver and gold have all been found able to permeate through the skin due to the formation of permeable ions (Tanojo *et al*., 2001; Larese Filon *et al*., 2004; Larese Filon *et al*., 2007; Larese Filon *et al*., 2009; Larese Filon *et al*., 2011; Larese Filon *et al*., 2012).

For some metals, ionisation takes place more rapidly in an acidic environment, and a decrease in pH would cause an increase in the release of ions from that metal and, therefore, higher permeation will take place (Tanojo *et al*., 2001; Larese Filon *et al*., 2007). Initially it was found that chromium did not permeate the skin, due to its inability to oxidise at a pH of 6.5 (Larese Filon *et al*. 2007). In a study that followed, it was found that chromium permeated more readily through the skin at a lower pH, due to the ability of the metal to undergo oxidation at a pH of 4.5 (Larese Filon *et al*., 2008). Ek *et al*. (2004) reported that a decrease in the environmental pH would lead to an increase in the solubility of rhodium. As the permeation of metals is dependent

on its solubility, this might suggest that more permeation of rhodium would occur in an acidic environment.

The *in vitro* permeation of rhodium through the skin and the clinical effects thereof have not yet been demonstrated. The influence of the pH of the synthetic sweat on the permeation of metals, specifically for rhodium, has not been investigated. It is unclear whether a pH of 4.5 or a pH of 6.5, as used in previous studies, should be used when conducting such experiments. With regard to rhodium, the processes of oxidation and ionisation in synthetic sweat, as described for other metals, remain yet to be proven by investigation. Therefore, the aim of this study was to investigate the *in vitro* permeation of rhodium, applied as $RhCl_3$ through the skin via the Franz diffusion cell method at different pH values of 4.5 and 6.5 and to determine whether any differences exist between the permeation of rhodium at these pH values.

3.4 Materials and methods

3.4.1 Chemicals

All chemicals used were of analytical grade. Ammonia (32%), hydrochloric acid (37%) and potassium di-hydrogen phosphate were purchased from Merck, South Africa. Sodium chloride urea, lactic acid (88 - 92%) and sodium phosphate dibasic was purchased from Sigma Aldrich, South Africa. Nitric acid (65%) and hydrochloric acid (33%) was purchased from De Bruyn Spectroscopic Solutions, South Africa and hydrogen peroxide (50%) and acetone was purchased from Associated Chemical Enterprises, South Africa. Rhodium trichloride ($RhCl_3$) was obtained from Johnson Matthey and sponsored by Anglo American Technical Solutions, South Africa. All solutions were prepared with ultrapure water from a Millipore purification system (milliQ).

A physiological solution used as receptor solution was prepared by dissolving 2.38 g sodium phosphate dibasic (Na_2HPO_4), 0.19 g potassium di-hydrogen phosphate (KH_2PO_4) and 9 g sodium chloride (NaCl) in 1000 ml milliQ water. The pH of this solution was adjusted to 7.35 with hydrochloric acid (HCl). Synthetic sweat was prepared as the donor solution, consisting of 0.5% NaCl, 0.1% urea and 0.1% lactic acid in 1000 ml milliQ water with the pH adjusted to either 4.5 or 6.5 with ammonia. Before use the pH values of the donor solutions were tested.

3.4.2 Preparation of skin

Caucasian female abdominal skin was obtained from various hospitals as biological waste from surgeries (abdominoplasty), with approval from the NWU Ethics Committee (ethics number NWU-00114-11-A5) and after informed consent was given by the patients. The age of the patients ranged from 39 to 42 years old. Full thickness human abdominal skin (intact dermis and epidermis) was used, after being frozen at -20 ^{o}C for no longer than six months before use.

Frozen skin was allowed to thaw after which the subcutaneous fat was removed with a scalpel. Circular pieces of skin with a diameter of 24 mm were cut with an iron punch. The thickness of the skin was measured with a Vernier calliper and did not exceed 1 mm.

Each circular piece of skin was mounted separately on the receptor compartment of a Franz cell, with the stratum corneum facing upwards, towards the donor compartment. The skin was wedged between the donor and receptor compartments, with vacuum grease (Dow Corning, USA) applied to provide a seal. The compartments were held together with a clamp. The mean exposed diffusion area was 1.015 cm^2.

Skin integrity was tested with 0.9% NaCl before and after each experiment by measuring the transcutaneous electrical resistance with a conductometer (Tinsley LCR Databridge Model 6401) operating at a frequency of 1 kHz. Two stainless steel electrodes were connected to the conductometer, each of which was placed in either the donor or receptor compartment of the Franz cell. The resistance was expressed as kilo ohm (kΩ) and the cells with the best value ranging between 10–80 kΩ, and within a range of 8 kΩ of each other, were acceptable for use. Resistance values below or outside this range were considered as an indication that the skin was either damaged, or the diffusion system was set up incorrectly.

3.4.3 Preparation of *in vitro* diffusion system

In this study, Franz diffusion cells were used to establish the *in vitro* permeation of rhodium. The temperature of the receptor compartment was maintained by immersing the compartment under water at 37 ^oC in order to simulate the normal core body temperature. The contents of the receptor compartment were continuously stirred with a magnetic stirrer.

This experiment was conducted twice. The first study was conducted on skin from two separate donors, aged 42 years and the second on skin from two new donors, both aged 39 years. At time 0, the donor compartments of the experimental cells were filled with 1 ml of donor solution. This donor solution contained 0.04306 g of 34.83 % $RhCl_3$ dissolved in 50 ml synthetic sweat. The blank cells were prepared in the exact same manner, with the exception that no $RhCl_3$ was introduced in the synthetic sweat used in the donor compartment. The receptor compartments were filled with 2 ml receptor solution. The donor compartment was covered with a piece of parafilm and a cap, while the sampling port of the receptor compartment was covered with parafilm only. This was done to prevent loss of the solutions via evaporation.

At selected intervals of 8, 12 and 24 hours, 2 ml of the receptor solution were removed for analysis. The receptor compartment was rinsed with 2 ml fresh receptor solution, which was also added to the same vial for analysis. Thus, 4 ml receptor solution was removed into a vial for analysis at each time interval. The solution was immediately replaced with 2 ml fresh

physiological solution. Care was taken to prevent air bubbles in the receptor compartment. During standardisation tests, it was found that the amount of rhodium that permeated before six hours was close to the analytical detection limit and did not contribute to the steady state of the rhodium permeation. It was, therefore, decided that sampling before 8 hours would be unnecessary. It was chosen to start sampling after 8 hours in order to simulate real working conditions in occupational settings where a work shift is on average 8 hours long. At the last interval (after 24 hours) the receptor solution was removed and the receptor compartment was again rinsed and removed for analysis. The donor solution was removed for analysis as well and the donor compartment was rinsed four times with 1 ml synthetic sweat. This was done to ensure that no rhodium remained on the skin surface and any rhodium detected in the skin was in the skin and not on the skin surface. In total, 5 ml of donor solution was removed for analysis. After testing the skin integrity, as previously described, skin was dismounted from the cell system and placed into a vial to be digested for analyses.

3.4.4 Digestion of skin

Prior to analysis each piece of skin was digested. This was done by adding each piece of skin to a glass beaker and rinsing it with acetone. The beaker was heated and the acetone was allowed to evaporate. Nitric acid (HNO_3) was added, followed by hydrogen peroxide (H_2O_2). Each of the acids was allowed to evaporate before adding the next one. The HNO_3 and H_2O_2 were used to dissolve the tissue and destroy the organics in the sample. *Aqua regia* and HCl was added separately and also allowed to evaporate to ensure that any PGMs that could have been oxidised by the destruction of organic matter were returned to a stable state. The sample was made up to 10 ml with 0.07 M HCl for analyses.

3.4.5 Analyses

Several different types of samples, each with different expected concentrations of rhodium were analysed. This included the donor solutions, the physiological receptor solutions and the solutions resulting from skin digestion. Three different calibration matrices were needed to analyse all these sample types. Calibration standards made up in 0.07 M HCl were used for the skin samples. Calibration standards made up in synthetic sweat were used to analyse donor solutions and calibration standards made up in physiological solution were used to analyse receptor solutions. This is done in order to ensure that the matrix of the calibration standards matches the samples. Fresh calibration standards were prepared for each method.

The donor solutions with high expected rhodium concentrations were analysed using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (Spectr Analytical ARCOS). Calibration standards of 1, 2, 3, 4, 10 and 20 parts per million (ppm) rhodium were used with an internal standard of 4 ppm yttrium. ICP-OES can detect rhodium concentrations in the order of

20 parts per billion (ppb) or 20 µg/L in a solution, whereas Inductively Coupled Plasma Mass Spectrometry (ICP-MS) can detect rhodium concentrations in the order of 2 part per trillion (ppt) or 2 ng/L or even lower in a solution. Therefore, receptor solutions with low expected concentrations of rhodium were analysed using ICP-MS (Thermo Scientific Element XR). Receptor solutions were spiked with 10 ppb indium as an internal standard and made up to 5 ml with 0.07 M HCl prior to analysis in order to ensure that enough solution was available for repeat analyses.

The analyses for skin solutions were performed using ICP-OES and the same calibration standards of 1, 2, 3, 5, 10 and 20 ppm rhodium, as used for the donor solutions. The skin solutions were spiked with 4 ppm yttrium to correct for volume inaccuracies.

3.4.6 Data and statistical analyses

The cumulative mass of $RhCl_3$ that permeated through the skin was plotted against time. Curve fitting was applied to this data, as described by Diez-Sales *et al*. (1999). The following equation (Eq. 1) was used to fit the data:

$$Q\,(t) = AKhC_v \left[D\frac{t}{h^2} - \frac{1}{6} - \frac{2}{\pi^2} \sum_{n=1}^{\infty} \frac{(-1)^n}{n^2} \exp\left(\frac{-D^2\pi^2 t}{h^2}\right) \right] \qquad \textit{(Eq. 1)}$$

In this equation, *Q(t)* represents the amount of the substance that permeated the skin within time (*t*). *K* represents the partition coefficient of the substance between the vehicle and the skin and *h* is the diffused path length. *D* is the diffusion coefficient of the substance in the skin and C_v is the actual concentration of the substance in the donor compartment. As *t* approaches infinity, the exponential term becomes negligible and the equation is simplified to the following (Eq. 2):

$$Q\,(t) = AKhC_v \left[D\frac{t}{h^2} - \frac{1}{6} \right] \qquad \textit{(Eq. 2)}$$

The values of *K* and *D* are not known. The products *K x h* and D/h^2 were replaced by α and β, which is determined by fitting Eq. 2 to permeation plots. These plots were experimentally obtained by curve fitting the graphs by using EasyPlot (Spiral Software). The intercept of this curve with the x-axis represents the lag time in hours (h), which is the minimum time needed to reach a constant flux.

The permeability coefficient (k_p) was determined using Eq. 3 and Eq. 4 to calculate the flux *(J).*

$$k_p = \frac{KD}{h} \; (= \alpha\beta) \qquad \textit{(Eq. 3)}$$

$$J = k_p C_v \qquad \textit{(Eq. 4)}$$

Flux, abbreviated as *J*, is the parameter that allows the calculation of K_p, which represents the permeability coefficient. The flux represents the amount of metal permeated per area and unit time, and is presented in this study as ng/cm^2/h at a steady-state equilibrium. The cumulative concentration that permeated at selected time intervals is presented as ng/cm^2.

Data analyses were performed using Excel for Windows and Statistica 12.0 (Statsoft Inc.). The descriptive statistics (mean and standard error of means) were determined. An independent t-test was performed to determine whether any statistically significant differences exist between the permeation of $RhCl_3$ at different pH values. T-test was conducted with values that were Box Cox transformed as the original data were skewed. Data differences with $p \leq 0.05$ were considered to be statistically significant.

3.5 Results

This study investigated the ability of rhodium, applied as $RhCl_3$ to permeate through the skin, as well as whether any statistically significant differences exist between the permeation of rhodium at a pH of 4.5 and 6.5. The Franz diffusion cell method was used and the mass of rhodium that permeated the skin was measured at different time intervals of 8, 12 and 24 hours. The reported data is summarised in Table 1. It was found that rhodium was able to permeate through the skin at both pH values. Over the 24 hour period, the cumulative mass of rhodium that permeated through the skin at both pH values increased with prolonged exposure time. This is depicted in Figure 1, which indicates the cumulative mass of rhodium permeating per skin area at a pH of 4.5 and a pH of 6.5 plotted against time. This figure also depicts that permeation at a pH of 4.5 was between 1.43 and 1.83 times higher than that at a pH of 6.5 over the 24 hour period.

The cumulative mass of rhodium that permeated through the skin after eight hours was higher at a pH of 4.5 than at a pH of 6.5. Although the difference between these permeation values were not statistically significant, it tended towards being significant ($p = 0.06$). After 12 hours, the cumulative mass of rhodium that permeated through the skin was statistically significantly higher for pH 4.5 than for 6.5 ($p = 0.02$). After 24 hours the cumulative mass of rhodium that permeated through the skin was higher for pH 4.5 than for pH 6.5, although this difference was not statistically significant ($p = 0.15$).

Table 1: Summary of rhodium permeated through and retained in the skin at pH 4.5 and pH 6.5.

	pH 4.5	pH 6.5	p
	Mean ± SEM	Mean ± SEM	
Number of cells (n)	10	15	
Cumulative mass permeated 8h (ng/cm^2)	0.71 ± 0.10	0.46 ± 0.10	0.06
Cumulative mass permeated 12h (ng/cm^2)	1.56 ± 0.24	0.85 ± 0.13	**0.02**
Cumulative mass permeated 24h (ng/cm^2)	3.48 ± 0.65	2.43 ± 0.38	0.15
Mass in skin (ng/cm^2)	1428.68 ± 710.46	1029.90 ± 449.12	0.13
Percentage in skin (%)	2.27 ± 1.13	1.61 ± 0.71	0.19
Mass diffused (ng/cm^2)	0.88 ± 0.17	0.62 ± 0.10	0.15
Percentage diffused (%)	3.29×10^{-4} ± 6.18×10^{-5}	2.25×10^{-4} ± 3.6×10^{-5}	0.13
Flux ($ng/cm^2/h$)	0.16 ± 0.03	0.12 ± 0.02	0.22
Lag time (h)	4.07 ± 0.53	5.03 ± 1.09	0.88

Values presented in red are statistically significant ($p \leq 0.05$)

At a pH of 4.5, the percentage of rhodium that remained in the skin (2.27%) was exponentially higher than the percentage that diffused through the skin (0.0003%). The same was observed for a pH of 6.5 with the percentage remaining in the skin being 1.61% and the percentage that diffused through the skin 0.0002%. Furthermore, 1.39 times more rhodium remained in the skin at a pH of 4.5 than at a pH of 6.5. The lag time for both pH values was less than six hours. The lag time for permeation at pH 4.5 was 0.8 times shorter (4.07 hours) than the lag time at a pH of 6.5 (5.03 hours).

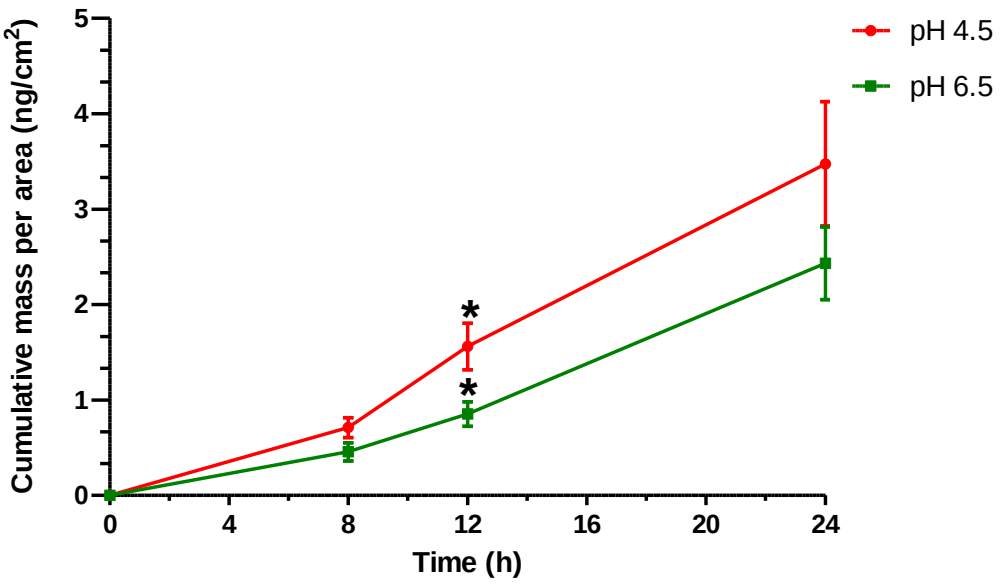

Figure 1: Cumulative mass of rhodium that permeated per area of skin at pH of 4.5 (n = 10) and 6.5 (n = 15) (Mean ± SEM). * Significant difference (p = 0.02).

3.6 Discussion

This study proved that rhodium, applied as $RhCl_3$ is able to permeate *in vitro* through full thickness human skin. The results indicated that the permeation of rhodium is influenced by pH, as the permeation at a pH of 4.5 was statistically significantly higher than at a pH of 6.5 after 12 hours.

Workers that are in contact with the salt compounds of rhodium on a daily base are at risk of being dermally exposed to this metal. These include workers in the mining industry where PGMs are extracted, refineries and other chemical industries (Boscolo *et al*., 2004; Goossens *et al*., 2011). Following dermal exposure, permeation of rhodium may occur, as proven in this study, where rhodium was able to permeate through the skin at a pH of 4.5 and 6.5. Workers that are continuously exposed to this metal are at an even greater risk of permeation, as this study indicated a cumulative increase in the permeation of rhodium with prolonged exposure time for both pH 4.5 and 6.5. An increase of 2.19 times in permeation at pH 4.5 and 1.85 times at pH 6.5 was observed from eight to 12 hours. This is significant for workers working longer than the average eight hours as even more permeation may take place. This also applies to workers that are cumulatively exposed to rhodium for longer than 12 hours. This exposure may not be continuous, as there is the possibility of workers going home and not washing, which prolongs the exposure to rhodium.

In general the skin surface pH of workers is considered to be acidic, ranging from pH 4 to 5.5 or even lower due to physical activity, as the evaporation of sweat leads to a decrease in pH (Larese Filon *et al*., 2007; Larese Filon *et al*., 2008; Byford, 2009). This study demonstrated that permeation occurs at both pH values of 4.5 and 6.5, but the permeation is higher at a pH of 4.5. The flux, which represents the rate of permeation through the skin, also indicated this, being 1.3 times higher at a pH of 4.5 than at a pH of 6.5. Larese Filon *et al*. (2008) demonstrated that chromium was only able to permeate the skin when in contact with a lower pH value of 4.5, rather than a pH of 6.5, as previously investigated (Larese Filon *et al*. 2007). Larese Filon *et al*. (2009) stated that a decrease in pH of one unit would lead to a 10 – 100 times increase in permeation of certain substances. In this study it was found that the cumulative mass of rhodium that permeated through the skin after eight hours was 1.5 times higher at a pH of 4.5 than at a pH of 6.5. After 24 hours, the cumulative mass of rhodium that permeated was 1.4 times higher at a pH of 4.5 than at a pH of 6.5, although these differences were not statistically significant. Therefore, an increase in permeation was observed with an increase in pH of 2 units, although not as drastic as proposed by Larese Filon *et al*. (2009a). A statistically significant difference occurred after 12 hours, with the permeation of rhodium being 1.83 times higher for pH 4.5 than for pH 6.5 ($p = 0.02$). Therefore, an acidic working environment would most likely lead to an increase in the permeation of rhodium as demonstrated in this study. This is supported by Ek *et al*. (2004), stating that a decrease in the environmental pH would lead to an increase in the solubility of rhodium.

Considering the results for other metals, permeation of metals is enhanced by its solubility and ability to form free ions via oxidation (Hostýnek *et al*., 2006). Certain metals undergo ionisation more easily in an acidic environment, such as a lower pH, and would demonstrate increased permeation across the skin (Larese Filon *et al*., 2007). Tanojo *et al*. (2001) stated that free nickel ions permeated the skin faster than non-ionised nickel molecules, due to the smaller size of the ions and an increase in the formation of ions caused an increase in the permeation of the metal. Other metals for which this phenomenon has been demonstrated include cobalt and chromium (Larese Filon *et al*., 2004; Larese Filon *et al*., 2007). Although this study did not demonstrate the ability of rhodium to form permeable ions, it did confirm an increase in permeation with a decrease in pH, as found by these researchers and might suggest that an increase in ionisation occurs with a decrease in pH.

The mass of rhodium that remained in the skin was 1623 times higher than the mass that diffused through the skin. More rhodium was, therefore, retained in the skin than permeated through the skin in a period of 24 hours. The accumulation of rhodium was higher at a pH of 4.5 than at 6.5 with 1.38 times more rhodium remaining in the skin at a pH of 4.5. It is not unlikely that the work shift of some workers may exceed eight hours, as is the case for workers that work overtime. This may cause the accumulation of rhodium in the skin to have a cumulative

effect with more rhodium being retained in the skin and perhaps more rhodium consequentially diffusing through the skin. Since rhodium can accumulate in the skin, subsequent exposure over a number of days may increase the permeation through the skin.

Tanojo *et al*. (2001) attributed the accumulation of nickel in the skin to the formation of nickel ions, which are bound to peptides, fatty acids and other counter ions in the stratum corneum which led to increased retention of the metal. Hostýnek *et al*. (2003) explained that certain metals such as aluminium (III) have been found to form complexes with the skin and form insoluble salts, which would increase the accumulation in the skin. Considering that $RhCl_3$ is a soluble rhodium compound, it is improbable to act in the same manner; however, no other mechanisms have been proposed by which rhodium may accumulate in the skin or permeate through it (Palacios *et al*., 2000). When taking the mechanisms proposed by Jepps *et al*. (2013) for other substances into consideration, rhodium may be systemically distributed through the body, causing sensitisation reactions. Constant exposure of workers to $RhCl_3$ for long periods of time might pose a risk or increased accumulation of rhodium in the skin, creating a concentration gradient between the stratum corneum and the dermis. Considering the investigation of permeation in previous studies, the accumulation of rhodium in the skin may possibly lead to rhodium being distributed to the vascular network and the lymphatic system, from where it may either be distributed throughout the body and exert health effects or it may be cleared from the body via the lymphatic system (Ngo *et al*., 2009; Jepps *et al*., 2013). Several substances, such as copper have been proven to be distributed to the blood stream after diffusing through the skin, where it may influence metabolism and general health (Larese Filon *et al*., 2004; Ngo *et al*., 2009; Ghafourian *et al*. 2010). Hostýnek *et al*. (2006) demonstrated that copper may exert clinical effects in the body, after accumulating in the stratum corneum. Although some clinical effects, such as sensitisation have been demonstrated for rhodium, it has not been confirmed whether it is due to accumulation in the skin or distribution through the body. Rhodium has been reported for causing contact dermatitis, urticaria and skin lesions due to dermal exposure, as well as inhalation in occupational settings (Bedello *et al*., 1987; De La Cuadra and Grau-Massanés, 1991; Goossens *et al.,* 2011). The clinical effects of rhodium, after permeating the skin, was not investigated in this study, but it has been demonstrated that rhodium does diffuse through the skin and it should not be excluded that it may be distributed through the body from here, as reported for other metals (Tanojo *et al*., 2001)

The lag time for both pH values was less than six hours with the lag time for pH 4.5 being the shortest. The relatively short lag time indicates the time that is needed for rhodium to permeate through the skin. The lag time for pH 4.5 was only 0.8 times shorter than for pH 6.5, indicating that although higher permeation occurred at pH 4.5, the steady state can be reached within a few hours, even at a higher pH. On average, a work shift is eight hours long and this provides enough time for rhodium to permeate through the skin of a worker that is exposed to $RhCl_3$ in

the workplace, as indicated by the lag time. Lag time does not give an indication of the time needed for rhodium to penetrate into the skin. Therefore, the time needed for rhodium to penetrate into the skin may possibly be less than six hours. If a worker is exposed to rhodium for less than six hours, it is not impossible that permeation has already occurred and that rodium has accumulated in the skin during that time. Without further investigation it should, therefore, not be wrongfully assumed that a worker may be exposed to rhodium compounds, as long as this exposure does not exceed six hours, as it has not been excluded that accumulation in the skin can occur before this time.

When comparing the *in vitro* permeation of rhodium with that of other metals, both at a pH of 4.5 and 6.5, several differences in the experimental conditions were observed, such as the concentration of the metal to which the skin was exposed, as well as the time intervals at which the receptor fluid was removed (Larese Filon *et al*., 2004; Larese Filon *et al*., 2007; Larese Filon *et al*., 2009b; Larese Filon *et al*., 2011). It is, therefore, difficult to compare the permeation of rhodium to that of other metals due to fundamental differences in experimental conditions.

3.7 Conclusion

This study has demonstrated that rhodium applied in the salt form, $RhCl_3$, is able to permeate through intact human skin *in vitro* in the presence of synthetic sweat at both a pH of 4.5 and 6.5. An increase was found in the cumulative mass of rhodium that permeated at both a pH of 4.5 and 6.5 with prolonged exposure time. A decrease in the pH of synthetic sweat led to a non-significant increase in the permeation of the $RhCl_3$; however, a significantly higher mass of rhodium permeated after 12 hours at a pH of 4.5. The continuous dermal exposure of workers to rhodium salt compounds pose the risk of rhodium permeating through the skin and even more so in an acidic workplace or workers with a skin surface pH of 4.5 or less.

Following dermal exposure, more rhodium accumulated in the skin than diffused through it. The accumulation of rhodium occurred more readily at a pH of 4.5 than at a pH of 6.5. The mechanisms by which this accumulation occurs, has not yet been demonstrated, but it is possible that rhodium may exert health effects, such as sensitisation after accumulating in the skin.

The lag time for permeation of rhodium at a pH of 4.5 and a pH of 6.5 was less than six hours, indicating enough time for permeation to occur in a worker who has an average work shift of eight hours. The lag time at a pH of 4.5 was shorter than at a pH of 6.5, once again demonstrating that rhodium permeated more readily at a lower pH.

Rhodium should be included in risk asessments as a potential health hazard. Emphasis should be placed on avoiding skin contamination to this metal. Preventative measures should be taken

in the workplace to minimise dermal exposure of workers to rhodium salts. These should include implementing engineering controls, such as enclosing the process to separate the worker from the source of exposure. Administrative regulations can be used, such as shorter work shifts and regular rotation of workers at a process where dermal exposure to rhodium can occur. Through the selection and use of adequate type of personal protective equipment, such as impermeable gloves and overalls, the skin contact with rhodium can be minimised.

3.8 References

Bocca B, Forte G. (2009) The epidemiology of contact allergy to metals in the general population: prevalence and new evidences. Open Chem Biomed Meth J; 2: 26 – 34.

Byford T. (2009) Environmental Health Criteria 235: Dermal Absorption. Int J Environ Stud; 66: 662 – 788.

Cawthorn RG. (1999) The platinum and palladium resources of the Bushveld complex. S Afr J Sci; 95: 481- 489.

Diez-Sales O, Copovi A, Casabo CG, Herraez M. (1999) A modelistic approach showing the importance of the stagnant aqueous layers in *in vitro* diffusion studies, and *in vitro – in vivo* correlations. Int J Pharm; 77: 1 – 11.

Ek KH, Morrison GM, Rauch S. (2004) Environmental routes for platinum group elements to biological materials – a review. Sci Total Environ; 334 – 335: 21 – 38.

Foti C, Amoruso A, Cassano N, Vena GA. (2002) Contact sensitisation to nickel from rhodium-plated 'nickel-free' earrings. Contact Dermatitis; 46: 309.

Ghafourian T, Samars EG, Brook JD, *et al*. (2010) Modelling the effect of mixture components on permeation through the skin. Int J Pharm; 398: 28 - 32.

Gómez MB, Gómez MM, Palacios MA. (2000) Control of interferences in the determination of Pt, Pd and Rh in airborne particulate matter by inductively coupled plasma mass spectrometry. Anal Chem Acta; 404: 285 – 294.

Gómez B, Gómez M, Sanchez JL, *et al*. (2001) Platinum and rhodium distribution in airborne particulate matter and road dust. Sci Total Environ; 269: 131 – 144.

Goossens A, Cattaert N, Nemery B, *et al*. (2011) Occupational allergic contact dermatitis caused by rhodium solutions. Contact Dermatitis; 64: 158 – 184.

Guy RH, Hadgraft J. (1989) Chapter 6: Structure-activity correlations in percutaneous absorption. In Bronaugh RL, Maibach HI, editors. Percutaneous absorption: Methods – Methodology – Drug Delivery. 2nd ed. New York: Marcel Dekker, Inc. p. 95 – 109. ISBN 0-8247-8036-1.

Hanson KM, Behne MJ, Barry NP, *et al*. (2002) Two-photon fluorescence lifetime imaging of the stratum corneum pH gradient. Biophys J; 83: 1682 – 1690.

Hostýnek JJ. (2003) Factors determining percutaneous metal absorption. Food Chem Toxicol; 41: 327 – 345.

Hostýnek JJ, Dreher F, Maibach HI. (2006) Human stratum corneum penetration by copper: *In vivo* study after occlusive and semi-occlusive application of the metal as powder. Food Chem Toxicol; 44: 1539 – 1543.

Jepps OG, Dancik Y, Anissimov YG, Robers MS. (2013) Modeling the human skin barrier – towards a better understanding of dermal absorption. Adv Drug Deliv Rev; 65: 152 – 168.

Larese Filon F, Maina G, Adami G, *et al*. (2004) *In vitro* percutaneous absorption of cobalt. Int Arch Environ Health; 7: 85 – 89.

Larese Filon F, Gianpiero A, Venier M, *et al*. (2007) *In vitro* percutaneous absorption of metal compounds. Toxicol Lett; 170: 49 – 56.

Larese Filon F, D'Agostin F, Crosera M, *et al*. (2008) *In vitro* percutaneous absorption of chromium powder and the effect of skin cleanser. Toxicol In Vitro; 22: 1562 – 1567.

Larese Filon F, D'Agostin F, Crosera M, *et al*., (2009) Human skin penetration of silver nanoparticles through intact and damaged skin. Toxicology; 225: 33 -37.

Larese Filon F, Crosera M, Adami G, *et al*. (2011) Human skin penetration of gold nanoparticles through intact and damaged skin. Nanotoxicology; 5: 493 - 501.

Larese Filon F, Crosera M, Timeus E, *et al*. (2012) Human skin penetration of cobalt nanoparticles through intact and damaged skin. Toxicol In Vitro; 27: 121 – 127.

Matthey J. (2012) Platinum interim review: Other platinum group metals. Available at: http://www.platinum.matthey.com/media/1393522/platinum_2012_interim_review.pdf; Accessed: 5 July 2012.

Merget R, Rosner G. (2001) Evaluation of the health risk of platinum group metals emitted from automotive catalytic converters. Sci Total Environ; 270: 165 – 173.

Ngo MA, O'Malley M, Maibach HI. (2009) Percutaneous absorption and exposure assessment of pesticides. J Appl Toxicol; 30: 91 – 114.

Palacios MA, Gómez M, Moldovan M, Gómez B. (2000) Assessment of environmental contamination risk by Pt, Rh and Pd from automobile catalyst. Microchem J; 67: 105 – 113.

Sartorelli P, Montomoli L, Sisinni AG. (2012) Percutaneous penetration of metals and their effects on skin. Prevent Res; 2: 158 – 164.

Smith KL. (1990) Chapter 2: Penetrant characteristics influencing skin absorption. In Kemppainen BW, Reifenrath WG, editors. Methods for skin absorption. Boca Raton: CRC Press. p. 23 – 34. ISBN 0-8493-4651-7.

Tanojo H, Hostýnek JJ, Mountford HS, Maibach HI. (2001) *In vitro* permeation of nickel salts through human stratum corneum. Acta Derm Venereol; 21: 19 – 23.

Wiseman CLS, Zereini F. (2009) Airborne particulate matter, platinum group elements and human health: a review of recent evidence. Sci Total Environ; 407: 2493 – 2500.

Yajun W, Xiaozheng LE. (2012) Health risk of platinum group elements from automobile catalysts. Procedia Eng; 45: 1004 – 1009.

Zereini F, Alsenz H, Wiseman CLS, *et al*. (2012) Platinum group elements (Pt, Pd, and Rh) in airborne particulate matter in rural vs. urban areas of Germany: concentrations and spatial patterns of distribution. Sci Total Environ; 416: 261 – 268.

Chapter 4: Concluding Chapter

Chapter 4: Concluding Chapter

4.1 Conclusion

Rhodium is one of the platinum group metals (PGMs) and due to its unique catalytic properties an increased amount of this metal is being used in vehicle exhaust catalysts (VEC's) (Cawthorn, 1999; Gómez *et al*., 2000). In workplaces where rhodium is produced or utilised, workers are at risk of being dermally exposed to rhodium in either the metallic form or its salt compounds (Boscolo *et al*., 2004; Goossens *et al*., 2011). Previous studies on the *in vitro* permeation of metals have reported that metals such as nickel, cobalt and chromium are able to permeate through the skin, after undergoing oxidation and forming permeable ions (Tanojo *et al*., 2001; Larese Filon *et al*., 2004; Larese Filon *et al*., 2007; Larese Filon *et al*., 2009; Larese Filon *et al*., 2011; Larese Filon *et al*., 2012). For some metals, this ionisation takes place more rapidly in an acidic environment and a decrease in the pH would cause an increase in the release of ions from that metal (Larese Filon *et al*., 2007). Prior to this study, it has not been established whether rhodium is able to permeate through the skin and the influence that pH would have on permeation.

The aim of this study was to determine whether rhodium is able to permeate through the skin using the Franz diffusion cell method and to determine whether any differences exist between the *in vitro* permeation of rhodium at a pH of 4.5 and a pH of 6.5.

This study has proven that rhodium, applied as $RhCl_3$, is able to permeate through intact human skin *in vitro* in the presence of synthetic sweat at both a pH of 4.5 and 6.5. At a pH value of 4.5, as well as 6.5, the cumulative mass of rhodium that permeated through the skin increased with prolonged exposure. A decrease in the pH of synthetic sweat led to a non-significant increase in the permeation of the rhodium; however, a significantly higher mass of rhodium permeated after 12 hours at a pH of 4.5. There was also a higher flux at a pH of 4.5 than at a pH of 6.5. From this, the conclusion can be made that continuous dermal exposure of workers to rhodium salts poses the risk of rhodium permeating through the skin and even more so in an acidic working environment or workers with a skin surface pH of 4.5 or less. The lag time for permeation of rhodium at a pH of 4.5 and a pH of 6.5 was less than six hours. This relatively short lag time is an indication of the strong potential of rhodium to permeate through skin and again confirms that rhodium permeates more readily at a lower pH. In a working environment, this would provide enough time for rhodium permeation to occur during a work shift of eight hours. It should, however, not be assumed that exposure to rhodium for less than six hours is safe.

This study demonstrated that rhodium has the ability to permeate through the skin, but even more so to accumulate in the skin, regardless of pH. The mass of rhodium that was retained in

the skin after 24 hours was exponentially higher than the mass of rhodium that diffused through the skin at both a pH of 4.5 and 6.5. The accumulation of rhodium in the skin may be an indication of its ability to exert health effects, such as sensitisation due to systemic distribution throughout the body. When taking pH into account, more rhodium accumulated in the skin at a pH of 4.5 than at a pH of 6.5. A pH of 4.5 may cause more accumulation of rhodium in the skin, perhaps leading to quicker distribution through the body and a more rapid onset of sensitisation.

Larese Filon *et al*. (2009) stated that a decrease of one unit in pH would lead to a 10 to 100 fold increase in permeation through the skin. The hypothesis of this study was stated that the *in vitro* permeation of rhodium at a pH of 4.5 would be significantly higher than at a pH of 6.5. This study demonstrated that a higher cumulative mass of rhodium permeated through the skin and a higher mass of rhodium accumulated in the skin at a pH of 4.5 than 6.5. The flux was higher and the lag time shorter at a pH of 4.5 than 6.5. The hypothesis is, therefore, partially accepted, as the only statistically significant difference between permeation at pH 4.5 and 6.5 was the statistically significantly higher permeation at pH 4.5 after 12 hours.

4.2 Recommendations for occupational settings

The ability of rhodium to permeate through the skin, as demonstrated in this study, should raise concerns with regard to its ability to act as a health hazard in occupational settings. Rhodium has been found able to cause sensitisation in the salt form, but not as a metal compound (Goossens *et al*., 2011). Therefore, skin contact with salt compounds of rhodium should be prevented in the workplace as far as possible. With regard to preventative strategies, control of rhodium exposure can be achieved by applying engineering controls in the workplace. Ideally hazardous operations should be conducted in entirely enclosed systems, but not all processes lend themselves to this approach. This is done in order to separate the worker from the source of exposure or to reduce the number of employees exposed to rhodium. If engineering controls are not feasible or not effective in reducing exposure to rhodium, administrative controls should be implemented. These include scheduling reduced work periods in in a contaminated area or at a hazardous process in order to minimise the exposure of the worker to rhodium.

To further reduce the exposure, works schedules can be adjusted whereby workers alternate between jobs assignments in order to ensure that no worker is overexposed to rhodium. Further administrative controls include appropriate work practices, proper maintenance of the working area and especially the equipment used, and general cleanliness and personal hygiene. It is important that equipment be kept as clean as possible and hazardous residues should regularly be removed or cleaned from equipment before use. If possible, equipment should be automatically cleaned without worker involvement. Personal hygiene includes adequate washing facilities where workers are able to wash exposed skin promptly. In work places where dermal exposure to rhodium occurs, it should perhaps be necessary to provide

pH-neutral soap for workers to wash with in order to prevent further alteration of the skin surface pH.

The last control method to be implemented is the provision and proper use of adequate personal protective equipment (PPE). Workers should receive thorough training with regard to the use of PPE. During the selection of PPE, various factors should be taken into consideration, such as the size, comfort, restriction of mobility and the ease by which it is put on or removed. PPE should be effective in protecting against exposure to rhodium and should be resistant against permeation and penetration of the rhodium compounds present in the workplace, as well as degradation. PPE chosen for protection against rhodium should include impermeable gloves, overalls and masks. This equipment should be worn continuously. Workers should be careful to avoid contamination of the gloves especially during the donning and doffing of gloves.

4.3 Recommendations for future studies

It is recommended that more studies be conducted on the influence of pH on the *in vitro* permeation of metals, especially the PGMs. Although some studies have been conducted to determine the ionisation and permeation of metals at different pH values, these studies have not been conducted simultaneously and experimental differences are, therefore, not excluded (Larese Filon *et al*., 2007; Larese Filon *et al*., 2008). The release of rhodium ions in synthetic sweat at different pH values can be investigated and dissolution studies can be conducted to determine whether rhodium undergoes oxidation in the presence of synthetic sweat, in order to either confirm or deny the ability of rhodium to form ions. This would provide a better understanding on the mechanism by which rhodium permeates through the skin. The effect of pH on the ionisation of rhodium should be investigated. It is necessary to obtain more experimental data on rhodium and rhodium-containing products to provide more information on the ability of rhodium ions to pass through the skin and the physiological conditions required to do so. Such studies could be done for other PGMs as well and in particular for the lesser-known metals, such as iridium, osmium and ruthenium.

Future studies should be done with the aim of reducing uncertainties surrounding the physiological conditions in which *in vitro* permeation studies should be conducted. This includes specifically the pH of the synthetic sweat that should be used. It has been established that the pH of the skin surface ranges between 4.5 and 6 (Hanson *et al*., 2002; Larese Filon *et al*., 2007). In a working environment where workers undergo physical activity, the pH value can be 4.5 or even lower (Larese Filon *et al*., 2007; Larese Filon *et al*., 2008). It is, therefore, recommended that future *in vitro* permeation studies are conducted using synthetic sweat with a pH of 4.5, since it has been demonstrated in this and other studies that permeation is more likely to occur at this pH. Other factors influencing the permeation of metals should be

considered and used as criteria when investigating the permeation, such as the charge and the reactivity of the molecule.

The accumulation of rhodium in the skin, and the mechanisms involved should be investigated. The time needed for accumulation to occur can be investigated by the Franz diffusion cell method. Pieces of skin can be removed from the diffusion system for analysis at different time intervals. As this was not the aim of this study, the skin was only removed after 24 hours. Tape stripping methods can be used to determine in which layer of the skin the accumulation of rhodium takes place.

The consequential effects of rhodium following dermal exposure should be investigated in order to determine whether rhodium is distributed through the skin or cleared from the body. More investigations should be done on the possibility of rhodium to cause sensitisation in order to confirm or deny the ability of this metal to act as an allergen.

In general, the work force in the mining, refinery and catalytic industries where dermal exposure to PGMs, and specifically rhodium, may occur, is mostly male. It is, therefore, recommended that further studies are done in order to investigate any gender-specific differences that may occur with regard to the permeation of rhodium through the skin, as well as for other metals. However, the availability of skin from male donors is limited and such studies might not be feasible at this stage.

4.4 Limitations

This study was subjected to a number of limitations. In normal working conditions, it is unlikely for skin to be exposed to sweat (synthetic or natural) for 24 hours, as a general work shift is on average only eight hours long. The hydration of the skin for 24 hours with synthetic sweat versus the actual exposure conditions in the working environment may create a bias for the permeation of rhodium. The mass of rhodium that was initially applied to the skin is much higher than the mass that is expected to be present in the workplace. This mass was chosen in order for the rhodium obtained in the results to be higher than the detection limit of the analytical method. For the duration of the 24 hours in this study, the skin was static. In an occupational setting, however, the exposed skin would be continuously stretching and bending, causing it to be in a constant state of motion. Care was taken not to include any damaged skin in this study, with stretch marks or visible diseases. In the workplace the skin of workers may be damaged through cuts and abrasions, which could lead to much higher permeation values. The *in vitro* permeation of metals, especially those suspected or known to have adverse health effects on the body should be investigated in order to provide more information on the toxicology of such metals.

4.5 References:

Boscolo P, Di Giampaolo L, Reale M, *et al*. (2004) Different effects of platinum, palladium and rhodium salts on lymphocyte proliferation and cytokine release. Ann Clin Lab Sci; 34: 299 – 306.

Cawthorn RG. (1999) The platinum and palladium resources of the Bushveld complex. S Afr J Sci; 95: 481- 489.

Gómez MB, Gómez MM, Palacios MA. (2000) Control of interferences in the determination of Pt, Pd and Rh in airborne particulate matter by inductively coupled plasma mass spectrometry. Anal Chim Acta; 404: 285 – 294.

Goossens A, Cattaert N, Nemery B, *et al*. (2011) Occupational allergic contact dermatitis caused by rhodium solutions. Contact Dermatitis; 64: 158 – 184.

Hanson KM, Behne MJ, Barry NP, *et al*. (2002) Two-photon fluorescence lifetime imaging of the stratum corneum pH gradient. Biophys J; 83: 1682 – 1690.

Larese Filon F, Maina G, Adami G, *et al*. (2004) *In vitro* percutaneous absorption of cobalt. Int Arch Environ Health; 7: 85 – 89.

Larese Filon F, Boeniger M, Giovanni M, *et al*. (2006) Skin absorption of inorganic lead (PbO) and the effect of skin cleansers. J Occup Environ Med; 48: 692 – 699.

Larese Filon F, Gianpiero A, Venier M, *et al*. (2007) *In vitro* percutaneous absorption of metal compounds. Toxicol Lett; 170: 49 – 56.

Larese Filon F, D'Agostin F, Crosera M, *et al*. (2009) Human skin penetration of silver nanoparticles through intact and damaged skin. Toxicol; 225; 33 – 37.

Larese Filon F, Crosera M, Adami G, *et al*. (2011) Human skin penetration of gold nanoparticles through intact and damaged skin. Nanotoxicol; 1-9.

Larese Filon F, Crosera M, Timeus E, *et al*. (2012) Human skin penetration of cobalt nanoparticles through intact and damaged skin. Toxicol In Vitro; 27: 121 – 127.

Tanojo H, Hostýnek JJ, Mountford HS, Maibach HI. (2001) *In vitro* permeation of nickel salts through human stratum corneum. Acta Derm Venereol; 21: 19 – 23.

Chapter 5: Appendix