In vitro skin permeation of selected platinum group metals

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But He said to me, "My grace is sufficient for you, for My power is made perfect in weakness." Therefore I will boast all the more gladly of my weaknesses, so that the power of Christ may rest upon me.

2 Corinthians 12:9

Sy antwoord was: "My genade is vir jou genoeg. My krag kom juis tot volle werking wanneer jy swak is." Daarom sal ek baie liewer oor my swakhede roem, sodat die krag van Christus my beskutting kan wees.

2 Korintiërs 12:9

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Summary

Title: *In vitro* skin permeation of selected platinum group metals.

Background: Platinum group metal (PGM) mining and refining is a large constituent of the mining sector of South Africa and contributes significantly to the gross domestic product. The PGMs include the rare metals platinum (Pt), palladium (Pd), rhodium (Rh), ruthenium (Ru), iridium (Ir) and osmium (Os). During the refining process workers are potentially exposed to various chemical forms of the PGMs via the respiratory and dermal exposure routes. Historically, emphasis has been on respiratory exposure while the extent of skin exposure is still unknown. Among the different forms of PGMs, the salts are potential sensitisers, with platinum being a known respiratory sensitiser. Workers occupationally exposed to platinum and rhodium have reported respiratory as well as skin symptoms. However, it is unknown if these metals in the salt form are permeable through human skin, and whether dermal exposure could contribute to sensitisation. Evidence regarding differences between African and Caucasian skin anatomy and structure, as well as permeation through skin is contradictory, and no information is available on metal permeation through African skin. The *in vitro* diffusion method has been utilised successfully in occupational toxicology to demonstrate that metals such as chromium, cobalt and nickel, to name a few, permeate through human skin. The permeability of platinum and rhodium has not been investigated previously.

Aims and objectives: The research aim was to obtain insight into the permeability of platinum and rhodium through intact human skin and to provide information needed to determine the potential health risk following dermal exposure to these metals. The specific objectives included: (i) to critically review the *in vitro* diffusion method that is used to determine the permeability of metals through human skin, (ii) to investigate the permeation of potassium tetrachloroplatinate (K_2PtCl_4) and rhodium chloride ($RhCl_3$) as representative PGM salts through intact human skin over a 24-hour period, (iii) to evaluate the difference in permeability of platinum and rhodium through intact human skin, (iv) to evaluate the difference in permeability of platinum through intact African and Caucasian human skin.

Methods: Abdominal skin obtained after cosmetic procedures was obtained from five female Caucasian and three female African donors between the ages of 28 and 52 with ethical approval from the North-West University. Full thickness skin tissue was mounted in a vertical Franz diffusion cell. Skin integrity was tested by measuring the electrical resistance across the skin before and after conclusion of the experiments, using a Tinsley LCR Data bridge Model 6401. The donor solution of 32.46 mg K_2PtCl_4 in 50 ml of synthetic sweat (pH 6.5), and 43.15 mg $RhCl_3$ in 50 ml of synthetic sweat (pH 6.5) was prepared. The donor solution was applied to the stratum corneum side of the skin

and physiological receptor solution (pH 7.35) was added to the receptor compartment. The concentration of the metals in the receptor solution was determined by high resolution inductively coupled plasma-mass spectrometry after extraction at various intervals during the 24 hours of the study. After completion of the study, the skin was rinsed four times to remove any platinum or rhodium remaining on the skin surface. The skin was digested using hydrogen peroxide, nitric acid and hydrochloric acid during different steps to determine the mass of the metals remaining in the skin by inductively coupled plasma-optical emission spectrometry.

Results: The comparison of published *in vitro* skin permeation studies involving metals is impeded by the variations in the experimental design and dissimilarity in the reporting of results. Differences in experimental design included, most noticeably, the use of various donor and receptor solutions, different temperatures wherein the receptor compartment was placed, differences in skin thickness and variations in exposed skin surface areas. The metals considered in the review, namely chromium, cobalt, gold, lead, mercury, nickel, platinum, rhodium and silver, permeate through intact human skin under physiological conditions. Large variations in the permeability results were observed, with the notable differences in methodology as the probable reason. Results obtained from the *in vitro* experiments indicate that platinum and rhodium permeated through intact Caucasian skin with flux values of 0.12 and 0.05 ng/cm^2/h, respectively. The cumulative mass of platinum (2.57 ng/cm^2) that permeated after 24 hours of exposure was statistically significantly ($p = 0.016$) higher than rhodium permeation (1.11 ng/cm^2). The mass of platinum (1 459.47 ng/cm^2) retained in the skin after 24 hours of exposure was statistically significantly ($p < 0.001$) higher than rhodium retention (757.04 ng/cm^2). The comparison of permeability between two different racial groups indicates that platinum permeated through the skin of both racial groups with the flux through African skin found as 1.93 ng/cm^2/h and 0.27 ng/cm^2/h through Caucasian skin. The cumulative mass of platinum permeated after 24 hours of exposure was statistically significantly ($p = 0.044$) higher through African skin (37.52 ng/cm^2) than Caucasian skin (5.05 ng/cm^2). The retention of platinum in African skin (3 064.13 ng/cm^2) was more than twice the mass retained in Caucasian skin (1 486.32 ng/cm^2).

Conclusions: The *in vitro* diffusion method is an applicable method to determine skin permeability of metals. However, the experimental design and format of data reporting should be standardised to enable comparison of results from different studies. Platinum and rhodium permeated through intact human skin, with platinum permeation significantly higher. African skin was significantly more permeable by platinum than Caucasian skin. Both platinum and rhodium were retained inside the skin after 24 hours of exposure, possibly forming a reservoir which could contribute to continued permeation through the skin even after removal thereof from the skin. Platinum and rhodium permeated through full thickness skin and thereby could possibly contribute to local skin symptoms

such as dermatitis and urticaria found in occupationally exposed workers. By permeating through the upper layers of the skin, these metals could potentially reach the viable epidermis and contribute to sensitisation.

Key words: metal skin permeation, platinum, rhodium, platinum group metals, skin sensitisation, *in vitro* skin permeation, dermal exposure, Franz diffusion cell.

Opsomming

Titel: *In vitro* vel deurlaatbaarheid van geselekteerde platinumgroepmetale.

Agtergrond: Die mynbou- en raffineringsaktiwiteite van platinumgroepmetale (PGM) maak ’n groot deel van die mynbousektor van Suid-Afrika uit en dra aansienlik by tot die bruto binnelandse produk. Die PGM sluit die seldsame metale platinum (Pt), palladium (Pd), rodium (Rh), rutenium (Ru), iridium (Ir) en osmium (Os) in. Gedurende die raffineringsproses word werkers potensieel blootgestel aan verskeie chemiese vorms van die PGM deur respiratoriese- en velblootstelling. Histories het die fokus meer op respiratoriese blootstelling geval terwyl die omvang van velblootstelling steeds onbekend is. Onder die verskillende vorme van PGM, is soute potensiële sensitiseerders, met platinum reeds bekend as ’n respiratoriese sensitiseerder. Werkers met beroepsblootstelling aan platinum en rodium het al respiratoriese- en velsimptome gerapporteer. Dit is egter onbekend of hierdie metale in die sout vorm deurlaatbaar is deur menslike vel en of velblootstelling kan bydra tot sensitisering. Die literatuur met betrekking tot verskille tussen Afrikaan en Kaukasiese vel-anatomie, velstruktuur en veldeurlaatbaarheid is teenstrydig, en geen inligting is beskikbaar oor metaaldeurlaatbaarheid deur Afrikaan-vel nie. Die *in vitro* diffusiemetode is suksesvol gebruik in beroepstoksikologie om te demonstreer dat metale soos chroom, kobalt en nikkel, om ’n paar te noem, deurlaatbaar is deur die menslike vel. Die deurlaatbaarheid van platinum en rodium is egter nog nie voorheen ondersoek nie.

Doelstellings en doelwitte: Die navorsingsdoelstelling is om insig te verkry in die deurlaatbaarheid van platinum en rodium deur intakte menslike vel en om inligting in te samel wat nodig is vir die bepaling van die potensiële gesondheidsrisiko na velblootstelling aan hierdie metale. Die spesifieke doelwitte sluit in: (i) om ’n kritiese oorsig te gee van die *in vitro* diffusiemetode wat gebruik is om die deurlaatbaarheid van metale deur menslike vel te toets, (ii) om die deurlaatbaarheid van kaliumtetrachloroplatinaat (K_2PtCl_4) en rodiumchloried ($RhCl_3$) as verteenwoordigende PGM soute deur intakte menslike vel oor ’n 24-uur periode te ondersoek, (iii) om die verskil in die deurlaatbaarheid van platinum en rodium deur intakte menslike vel te ondersoek, (iv) om die verskil in die deurlaatbaarheid van platinum deur intakte Afrikaan- en Kaukasiese menslike vel te ondersoek.

Metodes: Abdominale vel wat verwyder is gedurende kosmetiese prosedures is verkry vanaf vyf vroulike Kaukasiese en drie vroulike Afrikaan-skenkers tussen die ouderdomme van 28 en 52 met die etiese goedkeuring van die Noordwes-Universiteit. Voldikte velweefsel is gemonteer in ’n vertikale Franz diffusie-sel. Velintegriteit is getoets deur die elektriese weerstand oor die vel te meet voor en na afloop van die eksperimente deur die gebruik van ’n Tinsley LCR Data Bridge Model 6401. Die

skenkeroplossing van 32,46 mg K_2PtCl_4 in 50 ml sintetiese sweet (pH 6,5), en 43,15 mg $RhCl_3$ in 50 ml sintetiese sweet (pH 6,5) is voorberei. Die skenkeroplossing is aangewend aan die stratum korneum kant van die vel en fisiologiese reseptoroplossing (pH 7,35) is bygevoeg in die reseptorkompartement. Die konsentrasie van die metale in die reseptoroplossing is bepaal deur hoë-resolusie induktiewe gekoppelde plasma-massa spektrometrie na onttrekking by verskillende intervalle gedurende die 24 uur van die studie. Na voltooiing van die studie is die vel vier keer afgespoel om enige platinum of rodium wat op die veloppervlak agtergebly het, te verwyder. Die vel is verteer deur die gebruik van waterstofperoksied, salpetersuur en soutsuur gedurende verskillende stappe om die massa van metale wat in die vel agtergebly het te bepaal deur induktiewe gekoppelde plasma-optiese emissie spektrometrie.

Resultate: Die vergelyking van gepubliseerde *in vitro* veldeurlaatbaarheidstudies met metale is belemmer deur die variasie in die eksperimentele ontwerp en verskille in die aanbieding van resultate. Verskille in eksperimentele ontwerp sluit in die gebruik van verskeie skenker- en reseptoroplossings, verskillende temperature waarin die reseptorkompartement geplaas is, verskille in die veldikte en in die blootgestelde veloppervlak-areas. Die metale wat in die oorsig oorweeg is, naamlik chroom, kobalt, goud, lood, kwik, nikkel, platinum, rodium en silwer beweeg deur intakte menslike vel onder fisiologiese kondisies. Groot verskille is opgemerk in die deurlaatbaarheidsresultate, met noemenswaardige verskille in metodologie as die mees waarskynlike rede. Resultate verkry uit die *in vitro* eksperimente dui aan dat platinum en rodium deurlaatbaar is deur intakte Kaukasiese vel, met flukswaardes van 0,12 en 0,05 ng/cm^2/u, onderskeidelik. Die kumulatiewe massa van platinum (2,57 ng/cm^2) wat deurgelaat is na 24 uur van blootstelling was statisties betekenisvol ($p = 0.016$) hoër as rodiumdeurlaatbaarheid (1,11 ng/cm^2). Die massa van platinum (1 459,47 ng/cm^2) wat in die vel agtergebly het na 24 uur van blootstelling was statisties betekenisvol ($p < 0.001$) hoër as die rodiumretensie (757,04 ng/cm^2). Die vergelyking van deurlaatbaarheid tussen die twee verskillende rassegroepe dui aan dat platinum deurlaatbaar was deur die velle van beide rassegroepe, met die fluks deur Afrikaan-vel as 1,93 ng/cm^2/u en as 0,27 ng/cm^2/u deur Kaukasiese vel. Die kumulatiewe massa van platinum wat deurgelaat is na 24 uur van blootstelling is statisties betekenisvol ($p = 0.044$) hoër deur Afrikaan-vel (37,52 ng/cm^2) as deur Kaukasiese vel (5,05 ng/cm^2). Die retensie van platinum in Afrikaan-vel (3 064,13 ng/cm^2) was meer as twee keer die massa van die retensie in Kaukasiese vel (1 486,32 ng/cm^2).

Gevolgtrekking: Die *in vitro* diffusiemetode is ’n toepaslike metode om die veldeurlaatbaarheid van metale te bepaal. Die eksperimentele ontwerp en formaat van data rapportering moet egter gestandaardiseer word om die vergelyking van die resultate van verskillende studies te vergemaklik.

Platinum en rodium is beide deurlaatbaar deur intakte menslike vel, maar platinum se deurlaatbaarheid is beduidend hoër. Afrikaan-vel is beduidend meer deurlaatbaar vir platinum as Kaukasiese vel. Beide platinum en rodium is teruggehou in die vel na 24 uur van blootstelling en het moontlik 'n reservoir gevorm wat kan bydra tot voortgesette deurlaatbaarheid deur die vel selfs na die verwydering daarvan vanaf die vel. Platinum en rodium is deurlaatbaar deur volle dikte vel en kan so moontlik bydra tot lokale velsimptome soos dermatitis en urtikarie wat gevind word onder beroepsblootgestelde werkers. Metale wat deur die boonste lae van die vel kan dring kan potensieel die lewende epidermis bereik en so bydra tot sensitisering.

Sleutelterme: metaal vel deurlaatbaarheid, platinum, rodium, platinumgroepmetale, velsensitisering, *in vitro* veldeurlaatbaarheid, velblootstelling, Franz diffusie-sel.

Preface

This thesis is submitted in article format and written according to the requirements of the NWU manual for postgraduate studies and conforms to the requirements preferred by the appropriate journals. The thesis is written according to UK English spelling, with exception of institutional or organisational names and references that were used as is. Three articles and three conference contributions are included in this thesis:

Article I: *In vitro* permeation of metals through human skin: A review.

Article II: *In vitro* permeation of platinum and rhodium through Caucasian skin.

Article III: *In vitro* permeation of platinum through intact African and Caucasian skin.

Appendix A:

Oral presentation: *In vitro* percutaneous absorption of a platinum salt through African and Caucasian skin.

Oral presentation: *In vitro* percutaneous absorption of a platinum salt through intact Caucasian skin: preliminary results.

Poster presentation: *In vitro* permeation of platinum and rhodium through intact Caucasian skin.

For uniformity, the reference style required by the journal *Toxicology in Vitro* is used throughout the thesis, with the exception of Chapter 5, which is written according to the guidelines of *Toxicology Letters*. Details on the requirements of the reference style can be found at the beginning of Chapters 3 and 5 of this thesis.

For the purpose of this thesis the term *race* is used to define a specific population based on genetic similarities, where racial divisions are based on differences in skin colour and physical features (Anand S.S., 1999. Using ethnicity as a classification variable in health research: perpetuating the myth of biological determinism, serving socio-political agendas, or making valuable contributions to medical sciences? Ethn. Health. 4, 241-244).

The contributions of the listed co-authors and their consent for use in this thesis are given in Table 1. The relevant editors or publishers granted permission for the use of the published material, and proof is given in Annexure A.

Table 1: Contributions of the different authors and consent for use.

Author	Contributions of co-authors	Consent*
A Franken	Responsible for the planning of the experimental method and design of the study under supervision. Responsible for data collection by performing experimental studies. Responsible for data analysis and interpretation of results. First author of articles included in Chapters 3 to 5, Responsible for conference presentations and responsible for writing the thesis.	
JL Du Plessis	As Promoter planned and designed the study in collaboration with the candidate (student) and other promoters. Assisted with data interpretation and supervised the writing of the conference presentations, articles and thesis.	
FC Eloff	As Co-promoter assisted in planning the study in collaboration with the candidate and other promoters. Assisted with data interpretation and supervised writing of the conference presentations, articles and thesis.	
J Du Plessis	As Co-promoter assisted in planning the study in collaboration with the candidate and other promoters. Assisted with data interpretation and provided subject specific guidance. Supervised writing of the conference presentations, articles and thesis.	
CJ Badenhorst	Gave a critical review of the articles included in Chapter 4 and 5 as a co-author, as well as of the conference presentations. Arranged the sponsorship of the PGM salts.	
A Jordaan	Responsible for TEM analysis and assistance with data interpretation, and gave a critical review of the article included in Chapter 4.	
CJ Van der Merwe	Gave a critical review of the conference presentations.	
C Ramotsehoa	Gave a critical review of the conference presentations.	
A Van der Merwe	Gave a critical review of the conference presentations.	
PJ Laubscher	Gave a critical review of the conference presentations.	

* I declare that I have approved the chapter/article(s) and that my role in the study as indicated above is representative of my actual contribution, and that I hereby give my consent that it may be published as part of the thesis of Miss. A. Franken.

The outline of the thesis is as follows:

- Chapter 1 – General introduction with background, research aims and objectives, and hypotheses.
- Chapter 2 – A literature study on topics relevant to this thesis.
- Chapter 3 – Article I entitled: *In vitro* permeation of metals through human skin: A review, submitted to *Toxicology in Vitro* for publication.
- Chapter 4 – Article II entitled: *In vitro* permeation of platinum and rhodium through Caucasian skin, published in *Toxicology in Vitro*.
- Chapter 5 – Article III entitled: *In vitro* permeation of platinum through African and Caucasian skin, submitted to *Toxicology Letters* for publication.
- Chapter 6 – The conclusion with recommendations, limitations and recommendations for future studies.
- Appendix A – Conference contributions, two oral presentations, and one poster presentation.
- Appendix B – Permission for use of copyright material and the declaration of language editing.

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

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List of units

%	percent/percentage
°C	degrees Celsius
cm^2	square centimetre
cm/h	centimetre per hour
g/mol	gram per mol
h	hour
km	kilometre
µg	microgram
µg/l	microgram per litre
µg/ml	microgram per millilitre
µm	micrometre
$µg/cm^2$	microgram per square centimetre
$µg/m^3$	microgram per cubic metre
$µg/cm^2/h$	microgram per square centimetre per hour
mg	milligram
mg/ml	milligram per millilitre
mg/l	milligram per litre
ml	millilitre
mm	millimetre
ng	nanogram

List of units continued

nm	nanometre
ng/l	nanogram per litre
ng/ml	nanogram per millilitre
ng/cm^2	nanogram per square centimetre
ng/cm^3	nanogram per cubic centimetre
ng/cm^2/h	nanogram per square centimetre per hour
nm	nanometre
pg/m^3	picogram per cubic metre

List of abbreviations

≤	less than or equal to
<	less than
>	more than
ANCOVA	analysis of covariance
Ag	silver
Au	gold
aq	aqueous
CO	carbon monoxide
CO_2	carbon dioxide
Co	cobalt
$CoCl_2$	cobalt chloride
Co-57	cobalt isotope
Cr	chromium
Cr_2O_7	dichromate
$CrCl_3$	chromium chloride
$CrCl_3.6H_2O$	chromium chloride hexahydrate
$Cr(NO_3)_3$	chromium nitrate
$Cr(NO_3)_3.9H_2O$	chromium nitrate nonahydrate
$Cr(SO_4)_3$	chromium sulphate
Cr(III)	trivalent chromium
Cr(VI)	hexavalent chromium

List of abbreviations continued

Cu	copper
$CuCl_2$	copper chloride
$Cu(CO_2CH_3)_2$	copper acetate
CuPC	copper pyrrolidone
$CuSO_4$	copper sulphate
$C_{16}H_{30}NiO_4$	nickel di-octanoate
$C_{16}H_{36}Pb$	tetrabutyl lead
Derm	dermatomed skin (split thickness)
D	dermis
E	epidermis
Eds.	editors
EDETOX	Evaluations and Predictions of Dermal Absorption of Toxic Chemicals
et al.	*et alii* (and others)
FFP3	filtering face piece 3
$FeSO_4$	iron sulphate
FT	full thickness
GDP	gross domestic product
GHK-$Cu(Ac)_2$	glycyl-$_L$-histidyl-$_L$-lysine cuprate diacetate
h	hour
HC	hydrocarbons
HCl	hydrochloric acid
Hg	mercury

List of abbreviations continued

$HgCl_2$	mercury chloride
Hg-203	mercury isotope
HNO_3	nitric acid
H_2O_2	hydrogen peroxide
HSE	Health and Safety Executive
IARC	International Agency for Research on Cancer
ICP-OES	inductively coupled plasma-optical emission spectrometry
ICP-MS	inductively coupled plasma-mass spectrometry
IgE	immunoglobulin E
IL-2	interleukin 2
Ir	iridium
ISO	International Organization for Standardization
KH_2PO_4	potassium di-hydrogen phosphate
K_2CrO_7	potassium dichromate
K_2PtCl_4	potassium tetrachloroplatinate
LOD	limit of detection
MDHS	methods for the determination of hazardous substances
MDI	diphenyl methane diisocyanate
MHC	major histocompatibility complex
MRC	South African Medical Research Council
$NaBH_4$	sodium borohydride
NaCl	sodium chloride

List of abbreviations continued

Na_2CrO_4	sodium chromate
Na_2HPO_4	disodium hydrogen phosphate
$(NH_4)_2PtCl_6$	ammonium hexachloroplatinate
NIOSH	National Institute for Occupational Safety and Health
ND	not detectable
Ni	nickel
$NiBr_2$	nickel bromide
$NiCl_2$	nickel chloride
$NiCl_2.6H_2O$	nickel chloride hexahydrate
NiDO	nickel(II) soap
$Ni(NO_3)_2.6H_2O$	nickel nitrate hexahydrate
$Ni(CH_3COO)_2.4H_2O$	nickel acetate tetrahydrate
NiI_2	nickel iodide
$NiSO_4$	nickel sulphate
$NiSO_4.6H_2O$	nickel sulphate hexahydrate
Ni-63	nickel isotope
NO_x	nitrogen oxide
NRF	National Research Foundation of South Africa
OECD	Organisation for Economic Co-operation and Development
OEESC	Occupational and Environmental Exposure of Skin to Chemicals
OELs	occupational exposure limits
Os	osmium

List of abbreviations continued

p	p-value
Pb	lead
$Pb(CH_3CO_2)_2$	lead acetate
PbO	lead oxide
PGM	platinum group metal
PGMs	platinum group metals
pH	hydrogen ion concentration
Pd	palladium
Pt	platinum
PPE	personal protective equipment
PVC	polyvinyl chloride
Rh	rhodium
$RhCl_3$	rhodium chloride
Rh_2O_3	rhodium oxide
RS	receptor solution
Ru	ruthenium
SAIOH	Southern African Institute for Occupational Hygiene
SC	stratum corneum
SCCNFP	Scientific Committee on Cosmetic Products and Non-Food Products
SEM	standard error of means
Sk	skin notation
Sen	sensitisation notation

List of abbreviations continued

ss	synthetic sweat
Ti	titanium
TiO_2	titanium dioxide
TEM	transmission electron microscopy
TEWL	transepidermal water loss
TM	trademark
u	unpublished
vs	versus
Zn	zinc
ZnO	zinc oxide

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Chapter 1: General introduction

1.1 Introduction

The PGMs include the rare metals platinum (Pt), palladium (Pd), rhodium (Rh), ruthenium (Ru), iridium (Ir) and osmium (Os) (Ravindra *et al.*, 2004). In South Africa the platinum group metals (PGMs) mining and refining industry is one of the largest components of the mining sector, contributing 22% of the country's mining exports in 2012 (Chamber of Mines, 2014). South Africa is the leading producer of platinum and rhodium supplying 72.6% of the primary global platinum and 79.8% of the primary rhodium in 2012 (Chamber of Mines, 2013a). The wide range of applications for PGMs has led to an increase in its demand, especially in the use of catalytic converters. South Africa produced 13% of the world's platinum catalytic converters in 2012 (Chamber of Mines, 2013b). During this time the PGM mining sector contributed approximately 2.2% to the gross domestic product (GDP) of South Africa, whereas mineral sales contributed 5.2% (Chamber of Mines, 2014). In 2012 the platinum mining sector employed 197 847 workers, of which approximately 40% were African males and 34% African females, according to the national workforce distribution (Department of Labour, 2012; Chamber of Mines, 2013b).

The mining of platinum group metals and subsequent smelting, refining or recycling processes exposes workers to these metals on a daily basis. Secondary industries such as catalyst manufacturers, electronic industries and jewellery fabrication utilise these precious metals, which also leads to occupational exposure (Kielhorn *et al*., 2002). One of the potential hazards during precious metal refining is dermal exposure to various platinum or rhodium salts together with other metals and other contaminants, such as acids. Studies have indicated the sensitisation properties of PGM salts in the mining industry, as well as secondary industries, especially catalyst production plants. These studies have shown that platinum salts are allergens that affect both the respiratory system and the skin (Cleare *et al*., 1976; Linnett and Hughes, 1999; Cristaudo *et al*., 2005). During the refining process these metals are found in numerous chemical forms of which halide complexes are the most commonly found and are the biggest concern, as it is particularly these complexes containing chloride or bromide that are capable of eliciting reactions (Cleare *et al*., 1976; Niezborala and Garnier, 1996; Linnett and Hughes, 1999; Cristaudo *et al*., 2005). Rhodium sensitivity in the refining industry is less known than platinum salt sensitivity. However, rhodium salts are considered to be sensitisers with studies reporting respiratory symptoms as well as skin related symptoms such as urticaria and contact dermatitis in occupationally exposed subjects (Bedello *et al.,* 1987; De La Cuadra and Grau-Massanés, 1991). The skin related symptoms such as contact dermatitis reported after rhodium exposure is suggested to be an allergic reaction where a type IV hypersensitivity reaction is associated with exposure (Kusaka, 1993; Adams, 2006). Workers

sensitised to PGMs have shown both respiratory and skin symptoms, but it is unknown whether respiratory exposure or a combination of respiratory and dermal exposure may have been involved in sensitisation and the possible elicitation of the skin symptoms (Santucci *et al.,* 2000; Cristaudo *et al*., 2005; Kiilunen and Aitio, 2007; Goossens *et al.,* 2011). Maynard *et al.* (1997) suggest that the dermal route could contribute to platinum sensitisation, since workers were being sensitised even though respiratory exposure was below the occupational exposure level (OEL).

No published literature regarding the extent of worker dermal exposure to PGMs exists, and no information is available on the skin permeation of PGMs. In the absence of dermal OELs the only legislative parameter available to indicate risk of dermal exposure is skin notations (Sk), cautioning against potential skin absorption of substances. A sensitisation notation (Sen) is used to indicate that a substance is capable of causing respiratory sensitisation. In the South African legislation applicable to mining and general industries, soluble platinum salts have a respiratory OEL - recommended limit and sensitisation notation. Platinum dust as respirable particles and rhodium (soluble and insoluble) are not listed with sensitisation notations (Department of Labour, 1995; Department of Mineral Resources, 1996). Neither platinum nor rhodium is listed with a skin notation, therefore no legislative warning against dermal exposure and potential dermal permeation is provided.

The *in vitro* diffusion method using a Franz type diffusion cell can be employed to determine the permeability of a substance through the skin. This method utilises human skin that is clamped between two compartments. Skin samples are often obtained from the abdominal area after surgical removal. The substance of interest is applied in a donor solution to the stratum corneum (SC) side, and the dermis side is exposed to a receptor solution. The mass of the contaminant that permeates through the skin is determined by analysing the receptor solution at specific time intervals. This method has been used in occupational toxicology to determine the skin permeation of metals such as chromium, cobalt, gold, lead, mercury, nickel, silver, titanium and zinc (Sartorelli *et al.,* 2003; Larese Filon *et al.,* 2006; Cross *et al.,* 2007; Larese *et al.,* 2007; Mavon *et al.,* 2007; Larese Filon *et al.,* 2009; Larese Filon *et al.,* 2011). However, the permeability of platinum group metals (PGMs) has not been investigated before.

Numerous studies have found contradictory evidence of differences between African and Caucasian skin regarding skin anatomy and structure, as well as permeation through the skin (Weigand and Gaylor, 1974; Wedig and Maibach, 1981; Guy *et al.,* 1985; Kompaore *et al.,* 1993). Although the SC is equally thick in African and Caucasian skin, Weigand and Gaylor (1974) describe more cell layers in the SC of African skin. This suggests that African skin is more compact with greater intercellular cohesion. African skin is suggested to be more resistant with less susceptibility to chemical irritants than Caucasian skin, as well as to show faster recovery after barrier disruption by tape stripping (Weigand

and Mershon, 1970; Weigand and Gaylor, 1974; Frosch and Kligman, 1977; Reed *et al.,* 1995). A study done by Sinha *et al*. (1978) showed no significant differences in the percutaneous absorption of a corticosteroid. In contrast, a study on topical anaesthetics showed less effectiveness in African volunteers, and a study using dipyrithione showed 34% less absorption in African volunteers (Wedig and Maibach, 1981; Hymes and Spraker, 1986). However, no information is available on metal permeation through the skin of different racial groups and literature on structural differences dates back to before 1993.

No published information is available on the permeation of platinum or rhodium through intact human skin. Furthermore, no published information is available on the potential difference in permeation of metals through African and Caucasian skin. Considering the majority of potentially exposed South Africa mining workers are African, it is important to also include African skin in research on PGM permeation. Workers are potentially exposed to PGMs on a daily basis, and for that reason it is imperative to establish whether these PGMs can permeate through the skin and if there is a difference in permeation between African and Caucasian skin. For the purpose of this thesis potassium tetrachloroplatinate (K_2PtCl_4) and rhodium chloride ($RhCl_3$) were utilised in the *in vitro* skin permeation experiments as representatives of platinum and rhodium salts. This thesis aims to give a critical review of published literature regarding *in vitro* metal permeation through human skin. It furthermore aims to provide insight into the *in vitro* skin permeation of platinum and rhodium, the potential difference in permeability between the two PGMs and the potential difference in permeation between African and Caucasian skin. This information will provide the platinum mining industry with valuable information that may be of use in risk assessments and implementation of control measures to prevent and reduce future exposure.

1.2 Research aims and objectives

1.2.1 General research aim

The general research aim is to gain insight into the permeability of platinum and rhodium through intact human skin and to provide information that may be useful to determine the potential health risk following dermal exposure to these metals.

1.2.2 Specific objectives

The specific objectives are:

i. to critically review the *in vitro* diffusion method that is used to determine the permeability of metals through human skin;
ii. to investigate the permeation of potassium tetrachloroplatinate (K_2PtCl_4) and rhodium chloride ($RhCl_3$) as representative PGM salts through intact human skin over a 24-hour period;
iii. to evaluate the difference in permeability of platinum and rhodium through intact human skin, and;
iv. to evaluate the difference in permeability of platinum through intact African and Caucasian human skin.

1.2.3 Hypotheses

(i) Published *in vitro* skin permeation studies reported the permeation of nickel, cobalt and chromium through intact skin. Based on the permeability of these metals the permeation of platinum and rhodium through intact human skin is hypothesised.

(ii) Published *in vitro* skin permeation studies with nickel, chromium and cobalt reported significant differences in the permeability of these metals. Based on the difference in charge it is hypothesised that platinum and rhodium permeation differ statistically significantly from each other.

(iii) Published literature report significant differences in skin structure, barrier function and permeation of drugs between African and Caucasian skin, which suggests that African skin is less permeable. It is therefore hypothesised that platinum has a statistically significantly lower permeation through African skin.

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Chapter 2: Literature study

This chapter critically addresses literature relevant to this thesis. The discussion first addresses platinum group metal (PGM) mining and refining, demand and the contribution of this industry to the South African economy. This will be followed by a discussion of their physicochemical properties and potential for occupational PGM exposure. The skin structure and barrier function, as well as potential health effects of PGMs will be discussed with emphasis on sensitisation. Finally, the mechanisms of skin permeation and factors potentially influencing permeation will be presented. A critical review of the *in vitro* skin permeation of metals presented as an article submitted for potential publication is included in Chapter 3 of this thesis.

2.1 PGM mining, demand and uses

Platinum group metals (PGMs) are the second largest export revenue generator for South Africa after gold. In 2012 PGM mining contributed 21.9% of the total mineral exports, which amounts to 2.2% of South Africa's total gross domestic product (GDP) (Chamber of Mines, 2014). South Africa is the leading producer of platinum and rhodium, supplying 72.6% of the primary global platinum production and 79.8% of the primary rhodium production in 2012 (Chamber of Mines, 2013).

Platinum, palladium and rhodium are in high demand for the production of automotive emission control catalysts (autocatalysts). These three precious metals are often used in combinations for treating emissions from engines and are included in catalysts to remove pollutants from emissions that arise as a result of incomplete combustion (Seymour and O'Farrelly, 2012). The pollutants carbon monoxide (CO), hydrocarbons (HC) and nitrogen oxide (NO_x) are converted to carbon dioxide (CO_2), water and nitrogen (Ravindra *et al.,* 2004).

The PGMs are used in various applications as a result of their high mechanical strength and good ductility (Ravindra *et al.,* 2004). In addition to the use in autocatalysts, platinum is also used in catalysts to control pollution from construction, agricultural and other diesel engines. Platinum has been in demand for the use in jewellery for more than ten years, whereas the demand for investment opportunities increased during the last six years (Chamber of Mines, 2013). Platinum is also used in chemical, electrical and industrial applications, as well as petroleum refining applications. In addition, platinum has been approved for the treatment of cancer in humans (Seymour and O'Farrelly, 2012). Palladium is also used in jewellery manufacturing or investment opportunities and in industrial applications such as petroleum refining catalysts or medical applications for dental restorations. In addition to the use in autocatalysts, rhodium is used in chemical application and the glass sector. Ruthenium is widely used in the electrical industry, with a smaller demand in the electrochemical and

chemical industry. The demand for iridium is mainly in the electrical and electrochemical industries (Chamber of Mines, 2013; Johnson Matthey, 2013).

2.2 The South African workforce

The general employed workforce of South Africa consists of 73.4% Africans, 12.8% Caucasian, 10.9% Coloured and 3.2% Indian or Asian (Statistics South Africa, 2014). The general workforce consists of approximately 40% African males, and 34% African females, which is in stark contrast to the 6.2% Caucasian males and 4.6% Caucasian females (Department of Labour, 2013).

According to the latest figures published by Statistics South Africa (2014), the mining industry of South Africa employs 75 000 women and 344 000 men. In this industry 62.6% of employees are African males and 8.9% are African females (Department of Labour, 2013). Therefore, more than 260 000 general mining employees are estimated to be African males. The platinum mining industry constituted approximately 37.7% of all mining employees in South Africa in 2013 (Chamber of Mines, 2013).

2.3 Platinum group metals (PGMs)

Platinum group metals consist of platinum (Pt), palladium (Pd), rhodium (Rh), ruthenium (Rh), osmium (Os), and iridium (Ir). For the purpose of this thesis only platinum and rhodium were selected for inclusion in the experiments as both these metal salts have sensitisation potential. In addition the platinum mining industry expressed concern about possible sensitisation to these two metal salts during refining. Therefore, the emphasis will fall on platinum and rhodium, while the other precious metals are not discussed in detail. These six metals are often found together in nature with larger deposits of platinum, palladium and lesser deposits of ruthenium, rhodium, iridium and osmium. The PGM containing ore occur in association with nickel, copper, iron and sulphides. The most significant PGM deposits are found in South Africa, Zimbabwe, Russia, the United States of America, Canada and China (Seymour and O'Farrelly, 2012; Johnson Matthey, 2013).

PGMs are extremely resistant to corrosion by alkalis, salts and diluted acids, and quite resistant to more concentrated acids. However, these metals are soluble in *aqua regia* as a result of oxidising conditions. Platinum and rhodium are especially utilised in applications where resistance to oxidation at high temperatures are required. The PGMs, but particularly platinum and palladium, show catalytic properties for various chemical reactions, making these metals popular for use in various applications as discussed in Section 2.1 of this chapter (Seymour and O'Farrelly, 2012).

2.3.1 Platinum

In South Africa, platinum is extracted from ore with a platinum content of 50-60% (Seymour and O'Farrelly, 2012). Platinum is a silver-grey metal with a high melting point of 1 769 °C. Platinum has six stable isotopes and usually occurs in a valence state of +2 or +4, but can also occur in the valence state of +1, +3, +5 or +6 (Lykissa and Maharaj, 2006; Killunen and Aitio, 2007; Seymour and O'Farrelly, 2012). Platinum is found in different complex salts, most commonly bound to chloride, dichloride, dioxide or sulphate (Goering, 2001). The crystal structure of platinum, face-centred cubic, gives it soft, ductile properties and makes it resistant to oxidation and high temperature corrosion. However, it is its catalytic properties that have led to the high demand for platinum (Seymour and O'Farrelly, 2012).

2.3.2 Rhodium

Rhodium is also a silvery metal with a melting point of 1 960 °C. Rhodium only has one stable isotope (Rh-103), and usually occurs in a valence state of +3. It is found in a face-centred cubic structure similar to platinum, and is often used to harden platinum and palladium (Seymour and O'Farrelly, 2012).

2.4 PGM refining and recycling processes

2.4.1 Refining

Platinum group metals are recovered by a multistage refining process, which depends on the composition of the ore and the refinery (Seymour and O'Farrelly, 2012). After extraction the ore is crushed and concentrated by flotation, whereafter smelted to extract the metals during different processes. The refining process starts with base metal refining where nickel, copper, cobalt, iron and sulphur are extracted by leaching processes. Thereafter the precious metals, such as platinum and rhodium are extracted either by precipitation and dissolution steps or solvent extraction (Cramer, 2001). The precipitation and dissolution technique adds *aqua regia*, consisting of hydrochloric and nitric acid, to produce a solution containing the PGMs as chlorides. Platinum reacts with *aqua regia* to form chloroplatinic acid. Ammonium chloride is then added, which precipitates ammonium hexachloroplatinate ($(NH_4)_2PtCl_6$), and ammonium hydroxide and hydrochloric acid is added to precipitate palladium. The insoluble rhodium, iridium, ruthenium and osmium are obtained last in the process. Rhodium is extracted as rhodium sulphate, which is leached out with water. In contrast, the solvent extraction technique uses chlorine to bring the PGMs into solution and this process can be used to extract individual metals from a mixture. During solvent extraction the PGM concentrate is dissolved in a hydrochloric acid-chlorine solution, which forms soluble chloride ions of each of the metals. From this step, base metals are extracted. Ammonium chloride is used to precipitate platinum as ammonium

hexachloroplatinate, and rhodium is recovered last by precipitation or ion exchange (Seymour and O'Farrelly, 2012).

2.4.2 Recycling

PGMs are recycled from autocatalysts, electronics or jewellery where the PGMs are dissolved in *aqua regia,* forming chloro-complexes of platinum, palladium and rhodium (Seymour and O'Farrelly, 2012).

2.5 Exposure to PGMs

The use of platinum group metals in a wide variety of applications and industries has led to occupational exposure during the mining, refining and manufacturing of metals and metal products, but also environmental exposure. Platinum, palladium and rhodium are largely used in catalytic converters of vehicles, and therefore environmental exposure has increased as a result of exhaust emissions (Hultman, 2007).

2.5.1 Occupational exposure

Occupational exposure to various forms of the PGMs is probable during primary production such as mining and refining or during secondary use where these metals are used in chemical processes or production of metal containing material. During the different steps of production or manufacturing workers could be exposed through inhalation and/or the skin (dermal route) and/or ingestion (oral route). The risk of occupational exposure is highest for soluble platinum and other PGM compounds during refining processes and catalyst production (Killunen and Aitio, 2007). Occupational exposure may also occur in clinical settings where platinum is used as a chemotherapeutic agent for treatment of cancer (Goering, 2001).

Historically, occupational exposure has focused on respiratory exposure to dust, aerosols or vapour as a result of the high prevalence of respiratory symptoms. Inhalation of substances was considered as the most important pathway of exposure. The dermal exposure route has often been overlooked when evaluating occupational exposure and the impact of substances on the body (Schneider *et al.,* 1999; Semple, 2004).

Studies reporting allergic responses or sensitisation to platinum as a result of respiratory exposure also listed skin symptoms such as urticaria and contact dermatitis. However, dermal exposure was not considered. Therefore, it is unknown whether dermal exposure contributed to the skin symptoms reported or if dermal exposure could contribute to sensitisation (Calverley *et al.,* 1995; Niezborala and Garnier, 1996). Maynard *et al.* (1997) suggest that dermal exposure could contribute to sensitisation after investigating short term exposure to airborne soluble platinum. The results indicated that

respiratory exposure levels were significantly below the OEL, but there was opportunity for skin contact with platinum salts. The authors conclude that sensitisation either occurred at airborne levels below the OEL, or dermal exposure could be an alternative exposure route leading to sensitisation.

2.5.1.1 Respiratory exposure

Respiratory exposure to platinum and palladium have been reported for various occupational settings, whereas reports on rhodium exposure is limited (Baker *et al.,* 1990; Maynard *et al.,* 1997; Merget *et al.*, 2002; Violante *et al.,* 2005; Cristaudo and Picardo, 2007). Respiratory exposure in the workplace can occur as a result of direct emission of particulates into the air, by re-suspension of particles settled on surfaces or clothing by cleaning (Schneider *et al.,* 1999).

Respiratory exposure to platinum has been quantified in different industries with historical data on platinum respiratory exposure in occupational settings ranging from 1976 to 1997 as summarised by Killunen and Aitio (2007). In refinery settings average exposure results obtained from different studies ranged from 0.08 to 27.1 $\mu g/m^3$; during catalyst production the average exposure results ranged from 0.004 to 438 $\mu g/m^3$, and in the metal coating industry ranged from 0.017 to 0.079 $\mu g/m^3$ (Killunen and Aitio, 2007). Maynard *et al.* (1997) reported maximum short term exposure in a refinery setting as 25.96 $\mu g/m^3$, 10.82 $\mu g/m^3$ in a catalyst production setting and 1.10 $\mu g/m^3$ in the metal coating industry.

Cristaudo and Picardo (2007) investigated PGM exposure levels during the assembly of catalysts and recycling of metals by personal and area sampling. Rhodium respiratory exposure ranged between 0.001 and 0.003 $\mu g/m^3$ in area samples and 0.001 and 0.0035 $\mu g/m^3$ in personal samples. Information on rhodium respiratory exposure in other occupational settings is lacking despite the sensitisation potential of rhodium salt (Merget *et al.,* 2010).

2.5.1.2 Dermal exposure

In the past dermal exposure was overlooked as a route of exposure contributing to total body burden. Only recently has interest increased in the dermal exposure route as potential entry route for toxic compounds (Ngo and Maibach, 2010). The interest in skin exposure has led to studies investigating dermal exposure by removal methods such as wipe sampling, fluorescent tracer methods or interception methods (Lidén *et al.,* 2006). These methods determine the mass of the contaminant deposited on the skin, or retained on the skin after the period of exposure. However, the substances could potentially permeate into and through the skin during and after exposure; therefore dermal exposure could be underestimated by the dermal surface sampling methods. Overestimation of exposure may also occur when material not in contact with the skin, which is not relevant to skin exposure, is removed (Schneider *et al.,* 1999). Even though the skin has been recognised as a potential entry route, data on

occupational dermal exposure is still limited. Wipe sampling methods for skin surface sampling have been successfully utilised for metals such as nickel, chromium and cobalt (Lidén *et al.,* 2006; Lidén *et al.,* 2008; Du Plessis *et al.,* 2010). Even though surface sampling methods are available, the control of occupational dermal exposure is impeded by the lack of quantitative limits indicating safe or acceptable exposure levels.

As a result of dermal exposure a metal could potentially interact with the skin by firstly permeating through the skin and then contributing to systemic loading, secondly the metal may induce local effects such as irritation on the skin or influence barrier function. Lastly, the metal can induce an allergic skin reaction as a result of an immune system response. These interactions may occur simultaneously and there is concern that dermal exposure may contribute to respiratory symptoms reported. Dermal exposure may contribute to the development of systemic sensitisation, and respiratory symptoms will manifest with subsequent inhalation exposure of the sensitiser (Semple, 2004).

Schneider *et al.* (1999) propose a multiple compartment conceptual model of the pathways leading to dermal exposure from the source to the surface of the skin. This model indicates six compartments, namely the source, air, surface contamination, outer clothing contaminant layer, inner clothing contaminant layer and the skin contaminant layer. Skin exposure can occur by direct deposition on the skin from the air, deposition on the skin from splashes, by transfer from a contaminated surface or by submersion into the substance.

PGM dermal contact exposure can occur in a refinery setting during the different refining processes. However, the risk of dermal exposure is highest in the solvent extraction or dissolution and precipitation process where the PGM concentration is the highest. During refining the platinum salts are handled in a dry form that is released as a dust or in a wet process where it can be released as a droplet or a spray (Hunter *et al.,* 1945). This process is often enclosed, with selected openings where dermal exposure could occur when taking process samples or cleaning equipment. Additionally, exposure could occur when digging glove boxes, dropping hoppers, during maintenance on ventilation systems or during shut down periods where the equipment is flushed or dismantled. Dermal exposure will depend on the refining system and equipment and will differ between plants (Barnard, 2014). The use of respiratory protection, such as an airstream helmet, full face mask with double filters or a sealed mask with compressed air supply, is usually enforced in high risk areas, with little emphasis on dermal protection (Calverley *et al.,* 1995).

Dermal contact to PGMs can occur in catalyst manufacturing plants where intermediate products are produced, such as dust, granules, pellets or beads, or in the production of the finished products such as the catalysts. The substrates used in the catalysts are impregnated with PGMs by automated machines or

manually, depending on the systems used. In addition, dermal contact can take place in recycling plants where PGMs are recycled from used catalysts or other products (Cristaudo *et al.,* 2005).

2.5.2 Environmental exposure

Platinum, palladium and rhodium have been found in road dust, soil and rivers as a result of settling out of airborne emissions (Apostoli *et al.,* 2007). PGMs are released in vehicle exhaust emissions when these metals are released from the surface of the catalyst by oxidative or reductive conditions, high temperatures and mechanical abrasion. PGM aerosol particles emitted into the air range between 5 and 20 nm, with the majority between 0.1 to 10 nm. Particles larger than 10 µm settle out as a result of gravity, whereas particles smaller than 10 µm remain airborne for extended periods (Kalavrouziotis and Koukoulakis, 2009). PGMs could also be emitted into the environment as airborne particles, or into waste water from industrial plants manufacturing or utilising these metals (Pyrzynska, 2000). PGMs are also found in various water ecosystems, including rain, ground and drinking water, as well as river and ocean sediments. These PGMs are mostly released in the metallic form and is inert. However, a significant fraction of these metals may be bio-available in the environment (Wiseman and Zereini, 2009). The metallic form of PGMs can be altered when they are deposited in the environment, where particles could become soluble and end up in the food chain. Platinum exposure can therefore also occur through dietary intake in cases where platinum becomes biologically available (Ravindra *et al.,* 2004).

Platinum is also released from hospitals to a lesser extent as it is used as treatment for cancer and patient excretions containing platinum is released into hospital sewage. The contribution of this exposure route is less significant in comparison with vehicle exhaust emissions. Sewage is mostly contaminated with platinum from road run-off originating from traffic (Ravindra *et al.,* 2004).

Platinum has been found in the human body fluids of non-occupationally exposed people, mainly as a result of PGM emissions by vehicle exhaust catalysts (Ravindra *et al.,* 2004). Catalysts often contain smaller quantities of rhodium and therefore information on rhodium exposure as a result of vehicle exhaust catalysts are limited (Merget and Rosner, 2001). As a result of environmental contamination, communities could be exposed to airborne particle PGMs through the respiratory system, or through ingestion of contaminated water or food. This environmental exposure could result in bio-accumulation in the body of non-occupationally exposed communities (Ravindra *et al.,* 2004). Dermal exposure from environmental contamination is less likely to contribute to total body burden in comparison with inhalation and ingestion exposure.

2.6 The skin

2.6.1 Anatomy

The skin consists of different layers and appendages, such as sweat glands, hair follicles and sebaceous glands. The layers are divided into three regions, namely the epidermis, the dermis and the subcutaneous fat. The epidermis comprises approximately 5% of the full thickness skin and is divided into four or five layers, depending on the anatomical site. Thick skin, such as on the palms of the hands and soles of the feet, contain five layers, whereas thin skin contains four layers. The four layers of thin skin are the stratum germinativum, stratum spinosum, stratum granulosum and the stratum corneum (SC) (Martini and Bartholomew, 2003; Kielhorn *et al.,* 2006). For the purpose of this thesis only the epidermis, and in particular, the SC will be discussed in detail.

The epidermis contains keratinocytes that are formed in the stratum germinativum layer and differentiate as they migrate outwards to produce the SC to replace skin cells that are shed from the surface (Martini and Bartholomew, 2003; Barry, 2007). In the stratum spinosum new cells continue to divide and add thickness to the epidermis, and in the stratum granulosum the cells start to produce keratin (Martini and Bartholomew, 2003). During keratinisation the epidermal cells mature into flattened squamous cells to form the SC (Proksch and Brasch, 2011). Desquamation takes place in the epidermis where new keratinocytes are formed every 17 to 71 days, depending on the anatomical site, and the outermost cells are shed from the surface (Kielhorn *et al.,* 2006). The epidermal layer also contains melanocytes and Langerhans cells that are important for skin protection (Barry, 2007). The Langerhans cells are dendritic cells located in the stratum spinosum where they function as immunological cells. These cells are initial receptors for the cutaneous immune response by recognising local antigens and migrating through the epidermis into the dermis and into the lymphatic system to present the antigen to T lymphocytes (Monteiro-Riviere, 2010).

The SC is the outermost layer of the epidermis, ranging between 10 and 20 µm and consisting of corneocytes that have lost the nucleus and their capacity for metabolic activity (Kielhorn *et al.,* 2006; Proksch and Brasch, 2011). The SC is usually between 15 and 20 cell layers thick. The corneocytes are mainly comprised of keratin, and are connected to each other by corneodesmosomes. Corneocytes are enclosed in a cell envelope that provides binding sites for the non-polar lipids that surround these cells (Kielhorn *et al.,* 2006). The corneocytes are surrounded by a lipid matrix consisting of ceramides, cholesterol and free fatty acids organised as lamellar lipid layers (Wiechers, 2008; Proksch and Brasch, 2011).

The dermis consists of a connective tissue fibrous protein matrix, which is surrounded by mucopolysaccharides (Barry, 2007). Inside this matrix are blood vessels, sensory nerves and lymphatics

and also the inner segments of sweat glands and pilobaceous units. This layer provides nutritional support for the epidermis, which has no blood supply, and also removes waste products. This layer is important for storage of water and provides flexibility and strength (Kielhorn *et al.,* 2006; Barry 2007). Underneath the dermis is a layer of subcutaneous fat that provides mechanical cushioning and a thermal barrier and is also important in the synthesis and storage of chemicals (Barry 2007).

The skin surface contains an average of 40-70 hair follicles and 200-250 sweat ducts per square centimetre. The sweat glands are coiled tubes emerging from a coiled ball approximately 100 μm in diameter located in the dermis. The eccrine glands secrete sweat to assist in heat control or as a result of emotional stress. The apocrine sweat glands are 10 times larger than eccrine glands and their secretions contain proteins, lipids, lipoproteins and saccharides. Sebaceous glands are holocrine glands producing sebum, which acts as a lubricant and maintains an acidic condition on the skin surface. These glands are most numerous and largest on the face, inside the ear, the midline of the back and anogenital surfaces (Kielhorn *et al.,* 2006; Barry 2007).

2.6.2 Function

This section provides a brief description of the function of the skin. This skin acts as a barrier, but it is not an impenetrable barrier and it can be an important route of exposure because of the large surface area (Kielhorn *et al.,* 2006). In combination with the mucosal linings, the skin protects the internal body from environmental factors such as pollution, humidity and ultraviolet radiation and limits the passage of substances into and out of the body. The skin also provides a valuable mechanism to regulate body temperature by preventing or enabling heat loss through the skin or by sweating. The skin is easily damaged through mechanical, chemical, or biological attack or by radiation and is able to repair itself by a rapid turnover of skin and formation of new skin cells (Kielhorn *et al.,* 2006; Barry, 2007).

The barrier function of the skin presides primarily in the non-viable SC. This provides a physical barrier against the external environment and also prevents the loss of water and nutrients (Kielhorn *et al.,* 2006). The inside barrier regulates transepidermal water loss (TEWL), whereas the outside barrier protects against penetration of substances (Proksch and Brasch, 2011). The lipid layers of the SC are especially important for the barrier function as all substances permeating through the skin have to cross the lipid bilayers between the keratinocytes (Wiechers, 2008).

2.6.3 Barrier function parameters

The barrier properties of the skin can be evaluated by parameters such as TEWL, SC hydration and skin surface pH. TEWL is the diffusion of condensed water through the SC, while SC hydration is the water content of the SC (Gabard *et al.,* 2006; Imhof *et al.,* 2009). Skin surface pH is an indication of the

hydrogen ion (H^+) concentration in the watery solution present on the skin surface (Agache, 2004). If any of the parameters deviate from normal ranges, the barrier function of the skin could be influenced, and therefore the permeability of the skin could be altered. For example, a disturbed skin barrier is marked by an elevated TEWL value and is frequently correlated with a low SC hydration (Proksch *et al.,* 2008). Changes in the skin surface pH can influence the dissolution or partitioning of chemicals that come into contact with the skin and thereby influence skin permeability (Stefaniak *et al.,* 2013). Impairment of the skin barrier integrity could lead to enhanced permeation of chemicals through the skin, and also enable permeation of larger molecules that are normally not permeable through the skin (Kezic and Nielsen, 2009).

2.6.4 Racial differences in skin anatomy and barrier function parameters

Berardesca and Maibach (2003) conclude that African and Caucasian skin is equal in thickness; despite an increased number of cell layers with greater intercellular cohesion in African skin. African skin has increased lipid content, a decreased amount of ceramides and increased desquamation. African skin also has increased resistance to stripping and an increased recovery after stripping, as well as increased electrical resistance. The majority of literature regarding racial differences in skin anatomy, barrier function parameters, and protective characteristics are contradictory and was published decades ago.

Some studies indicate no significant difference in the thickness of the SC between African and Caucasian skin, although significant variation was found between individuals (Thomson, 1955; Freeman *et al.,* 1962; Gambichler *et al.,* 2006). However, Weigand *et al.* (1974) describe African skin as having more SC cell layers with greater intercellular cohesion. This was confirmed by a higher number of tape strips needed to remove the SC of African skin when compared to Caucasian skin, as well as African skin measuring twice the electrical resistance (Johnson and Corah, 1963; Weigand *et al.,* 1974). La Ruche and Cesarini (1992) confirm equivalent SC thickness and greater compaction and lipid content in African skin, reporting twenty cell layers in African skin compared to sixteen layers in Caucasian skin. Muizzuddin *et al.* (2010) also confirm the stronger barrier of African skin with stronger cohesion of cell layers. Therefore, the increased number of cell layers in African skin is more compact with a higher density in the SC, since the thickness is equal for African and Caucasian skin (Kompaore *et al.*, 1993; Rawlings, 2006). According to Reinertson and Wheatley (1959) the greater intercellular cohesion of African skin can be explained by the higher lipid content. It is proposed that a SC with more cell layers and greater cohesion between cell layers will provide a better barrier against penetration of substances through the primary barrier of the skin, providing African skin with a superior barrier against permeation (Kompaore *et al.,* 1993). In contrast with higher overall lipid content of the SC, lower levels of ceramides were found in African skin, which is characteristic of dry skin (Sugino *et al.,* 1993; Muizzuddin *et al.,* 2010).

Published literature on racial differences in skin barrier function parameters such as TEWL, SC hydration and skin surface pH of African and Caucasian skin are contradictory. A number of studies found higher baseline TEWL values for African skin compared to Caucasian skin, indicating a weaker barrier, although not all differences were significant (Wilson *et al.,* 1988; Kompaore *et al.,* 1993; Berardesca *et al.,* 1998). In contrast, lower TEWL values were reported for African skin when measured on the legs, cheeks and forearms (Warrier *et al.,* 1996; Muizzuddin *et al.*, 2010). No significant differences were found *in vivo* during baseline TEWL measurements between African and Caucasian skin (Berardesca *et al.,* 1991; Reed *et al.,* 1995; Grimes *et al.,* 2004; Fotoh *et al.,* 2008). Wesley and Maibach (2003) concluded in a review that the majority of evidence indicates that TEWL is higher in African skin when compared to Caucasian skin. SC hydration differences between races are inconclusive, with some authors indicating no significant difference in SC hydration and others indicating higher SC hydration levels of African skin (Berardesca and Maibach, 1988; Berardesca *et al.,* 1991; Manuskiatti *et al.,* 1998; Fotoh *et al.,* 2008). Berardesca *et al.* (1991) suggest that the increased cellular cohesion and increased lipid content of African skin resulted in higher water retention in the skin, which explains the higher SC hydration. Higher SC hydration was found on African cheeks, but no significant differences were found on the forearms and legs (Warrier *et al.,* 1996). Findings on skin surface pH are also contradictory as Fotoh *et al.* (2008) report a significant difference in the cutaneous pH, with a higher pH value measured in African skin, whereas Warrier *et al.* (1996) found a significantly lower pH in African skin measured on the cheek, and a non-significant lower pH measured on the legs. Contrasting these findings, Berardesca *et al.* (1998) and Grimes *et al.* (2004) found no significant differences in pH measured on the forearm under basal conditions. However, after three tape strips the pH of African skin decreased significantly (Berardesca *et al*., 1998). The different sites used for these barrier function parameters as well as the different study designs and instruments used, may provide possible explanations for the contradictory findings (Darlenski and Fluhr, 2012).

Gunathilake *et al.* (2009) ascribe the enhanced barrier function of darker skin to increased epidermal lipid content, increased lamellar body production and reduced acidity, which leads to enhanced lipid processing. African skin is also suggested to have a stronger barrier with high resistance to mechanical damage based on the higher maturation index, indicating a high amount of covalently bound proteins in the corneocytes (Muizzuddin *et al.,* 2010). This enhanced skin barrier found in African skin provides better protection against permeation of any substance that comes into contact with the skin, which could contribute to less sensitisation in this group (Fluhr *et al.,* 2008).

It is important to note that this literature dates back to before 2010, with most of it dating back to before 1998. The findings were based on results obtained from one to 119 test subjects for each racial group, with significant disparity between the numbers of test subjects compared with each other. For example,

Muizzuddin *et al.* (2010) compared skin barrier property results between 73 African and 119 Caucasian test subjects, and Gambichler *et al.* (2006) compared epidermal thickness between 12 African and 71 Caucasian test subjects. An important statement on the lipid content of the SC was based on one African and three Caucasian test subjects (Reinertson and Wheatley, 1959). It is imperative that research on anatomical and functional differences between racial groups be conducted and includes larger sample sizes.

2.7 PGM health effects

Exposure to PGMs has numerous health effects, depending on the duration and concentration of exposure, as well as a person's predisposition to allergic reactions. Adverse health effects include irritation, sensitisation and genotoxicity by induction of oxidative stress and damage (Boscolo *et al.,* 2004; Cristaudo *et al.,* 2005). For the purpose of this thesis only sensitisation leading to respiratory and skin symptoms or conditions, as well as other skin reactions will be discussed. The carcinogenicity of metallic platinum and rhodium is unknown, and data on carcinogenicity of cis-platin is insufficient to evaluate the carcinogenicity in humans (IARC, 1981). Sensitisation skin conditions such as urticaria and allergic contact dermatitis, as well as irritant skin conditions such as eczema will be discussed in Section 2.7.4 of this chapter.

2.7.1 Sensitisation

The immunologically mediated reaction to a substance ensuing after previous sensitisation to the substance is often referred to as hypersensitivity or an allergic reaction (Eaton and Klaassen, 2003). Skin sensitisation is an immunological reaction resulting in a local immune response as a result of topical exposure to an allergen. A subsequent encounter with the allergen elicits cutaneous inflammation along with other symptoms recognised as allergic contact dermatitis. Once an individual is sensitised to a particular substance, subsequent exposure to even low levels of the allergen will elicit the immunological reaction. Skin irritation is a direct inflammatory response at the site of exposure as a result of local damage of the skin (Basketter and Kimber, 2011). Skin irritation is distinct from sensitisation since the reaction is proportional to the dose applied (Cohen and Rice, 2003). These events are not always connected, as a substance can cause skin irritation, but not sensitisation. Some substances, however, can cause both events, causing local inflammation at the site of exposure and also sensitisation where subsequent exposures will elicit an immunological reaction (Basketter and Kimber, 2011).

An allergic reaction of the skin will depend on an individual's barrier function, and also the influence of the site of elicitation, moisture, temperature, season, as well as environmental and endogenous factors

on the permeation of the allergen (Schaefer *et al.,* 2011). The retention of metals in the skin for extended periods can lead to prolonged antigen formation in the skin, thereby eliciting an allergic reaction (Lacy *et al.,* 1996).

Respiratory sensitisation or respiratory hypersensitivity is characterised by initial sensitisation of the respiratory tract, and a reaction provoked by subsequent exposure. The hypersensitivity reaction is characterised by rhinitis, conjunctivitis and asthma. Respiratory reactions are almost immediate after exposure to the allergen. However, hypersensitivity to chemicals may be of late-onset, suggesting the possibility of an additional hypersensitivity reaction other than IgE mediated (Kimber and Wilks, 1995). Kimber and Dearman (2002) acknowledge that inhalation exposure represents the most common and most important route of respiratory sensitisation in the workplace, but nonetheless suggest that dermal contact to substances may effectively cause respiratory sensitisation. Rattray *et al.* (1994) report respiratory sensitisation in guinea pigs resulting from topical or intradermal exposure to diphenyl methane diisocyanate (MDI), and no sensitisation by inhalation exposure. After a subsequent inhalation challenge the sensitised guinea pigs exhibited pulmonary responses. The authors conclude that skin contact to allergens such as MDI may be an important cause of respiratory sensitisation. Dearman *et al.* (1998) found an IgE mediated immediate hypersensitivity reaction in mice after topical application of platinum salts. The results were consistent with elevated total serum IgE levels and specific IgE responses of sensitised workers to respiratory challenge. Furthermore, histological examination of cutaneous lesions caused by ammonium hexachloroplatinate indicated eosinophils which are also characteristic of respiratory hypersensitivity reactions. The results indicated that the cytokine production elicited by platinum salts was similar to the reaction elicited by the organic respiratory allergen MDI. The results indicated that mice topically exposed to platinum salts showed an immunological response similar to respiratory sensitisation in workers. Consequently, occupational respiratory sensitisation is not limited to inhalation exposure, and dermal contact could contribute to sensitisation (Kimber and Dearman, 2002).

2.7.2 Mechanism of sensitisation

2.7.2.1 Type I hypersensitivity

This reaction is an immediate hypersensitivity reaction mediated by Immunoglobulin E (IgE) production. After the initial exposure to the allergen, IgE is produced and binds to local tissue mast cells and also circulates in the blood where it binds to mast cells at distant sites. After re-exposure to the allergen, the antigen (allergen bound to a protein) binds to IgE antibodies on mast cells, which causes degranulation of mast cells resulting in the release of mediators and cytokines, which in turn leads to synthesis and release of more cytokines by eosinophils, basophils and macrophages. These mediators promote vasodilatation,

bronchial constriction and inflammation. Clinical manifestations can include urticarial skin reactions, rhinitis and conjunctivitis to asthma and anaphylaxis. The reaction may begin within minutes of re-exposure to the antigen, therefore it is referred to as an immediate reaction (Kaplan *et al.,* 2013).

2.7.2.2 Type IV hypersensitivity

Type IV hypersensitivity is divided into three classes: contact hypersensitivity, tuberculin-type hypersensitivity and hypersensitivity pneumonitis. For the purpose of this thesis contact hypersensitivity is discussed.

Allergic contact dermatitis is a delayed type hypersensitivity (type IV) initiated by skin exposure followed by epidermal pathology (Kaplan *et al.,* 2013). This reaction is divided into two phases: the sensitisation or induction phase requires four days to several weeks, while the elicitation or effector phase reaction develops within one to four days (Rustemeyer *et al.,* 2011). Metal ions are small in size and incomplete antigens, and referred to as haptens. These haptens have to bind to endogenous peptides in the body to form full antigens that are capable of eliciting an immune response (Budinger and Hertl, 2000; Eaton and Klaassen, 2003). The mechanism can be explained in six steps:

a) Sensitisation phase

i) The hapten (allergen) binds to the skin compound such as the major histocompatibility complex (MHC) proteins after permeating through the skin (Rustemeyer *et al.,* 2011). Metal ions such as nickel form stable metal-protein complexes by coordination bonds before binding to the MHC protein (Budinger and Hertl, 2000).

ii) The hapten-protein complex is attached to the antigen presenting Langerhans cells, which are activated by the hapten. The Langerhans cells mature and travel via the afferent lymphatics to the regional lymph nodes where they settle as interdigitating cells in the paracortical T-cells.

iii) T-cells recognise and bind to the allergen-MHC molecule complex, thereby activating the allergen-specific memory T-cells.

iv) Activated T-cells start producing growth factors, including interleukin 2 (IL-2), whereafter an autocrine cascade follows. The receptors for IL-2 are upregulated and proliferation of specific T-cells occurs in the draining lymph nodes.

v) The specific T-cells are released via the efferent lymphatics into the blood and start to circulate, and most of the cells are able to migrate into peripheral tissue. In the absence of further contact with an allergen, the prevalence of these cells decreases in subsequent weeks. However, it never returns to the low levels of unsensitised individuals (Rustemeyer *et al.,* 2011).

b) Elicitation phase

vi) After subsequent contact with the allergen, even very low doses, the Langerhans cells present the hapten-carrier complex to memory T-cells. The activated T-cells secrete local cytokines and chemokines that have a proinflammatory action, causing the arrival of more inflammatory cells and thereby causing further release of local mediators. This leads to a gradually developing eczematous reaction resulting in erythema, papules and vesicles reaching its maximum after 18-72 hours and then declines (Burns-Naas *et al.,* 2003; Rustemeyer *et al.,* 2011).

In summary, contact hypersensitivity causes the accumulation of allergen specific memory T-cells in the epidermis and these are reactivated after encountering allergen-presenting cells after subsequent contact with the allergen. These T-cells release proinflammatory cytokines, which initiate the inflammatory process resulting in visible erythema, induration and vesicle and papules formation of the skin. This reaction is delayed with symptoms only occurring after 18 hours, in contrast to immediate allergic reactions that develop within minutes (Burns-Naas *et al.,* 2003; Rustemeyer *et al.,* 2011)

2.7.3 Allergenicity of platinum and rhodium

Occupational sensitisation as a result of respiratory exposure to platinum has been widely reported for precious metal refineries, as well as secondary industries (Bolm-Audorff *et al.,* 1992; Niezborala and Garnier, 1996; Maynard *et al.,* 1997; Cristaudo *et al.,* 2005). Rhodium sensitisation is less known and literature is limited. Platinum salt sensitisation (previously known as platinosis) has been diagnosed by company doctors using symptoms such as asthma, rhinitis, urticaria and eczema together with skin tests (Niezborala and Garnier, 1996).

Cleare *et al.* (1976) indicate the halogenated salts, specifically tetrachloroplatinate and hexachloroplatinate, as the major sensitisers forming immunogenic complexes with proteins. The allergenicity of platinum is related to the charge and reactivity of the compound. Ammonium and potassium hexachloroplatinate and potassium and sodium tetrachloroplatinate represent the most powerful sensitisers (Cristaudo *et al.,* 2005). Occupational exposure to halogenated platinum occurs in refineries and catalyst plants, therefore the potential for sensitisation to platinum salts in these settings is elevated (Merget *et al.,* 2000). During platinum treatment and refining the chlorinated compounds have lower stability and higher reactivity than the element alone, which increases the sensitisation potential (Boscolo *et al.,* 2004). In the past rhodium was considered a poor sensitiser, but recently the sensitisation potential of rhodium salts have been assumed to be equal to platinum, causing bronchial and skin sensitisation (Adams, 2006).

Workers in a precious metal refinery have shown platinum salt sensitisation, listing respiratory symptoms such as asthma, immediate or delayed bronchospasm, wheezing or tightness of the chest and coughing. Numerous studies reported sensitisation in refinery workers as classified by positive reactions to skin prick tests with platinum salts (Venables *et al.,* 1989; Baker *et al.,* 1990; Calverley *et al.,* 1995). Workers who were sensitised to platinum salts were from all the areas of the refinery, excluding the administrative offices (Baker *et al.,* 1990). Workers from a catalyst production plant were also sensitised to platinum salts as diagnosed by skin prick tests. However, in comparison to refinery workers, catalyst production workers had lower incidence rates of sensitisation, possibly as a result of mechanisation (Merget *et al.,* 2000). Cristaudo *et al.* (2005) also confirmed sensitisation during catalyst manufacturing and recycling and indicated that clinical manifestations involved both the respiratory system and the skin. Platinum refinery and catalyst production plant workers have indicated occupational asthma persisting even after removal from exposure (Adams, 2006).

The allergenicity of platinum halide salts is predominantly a type I hypersensitivity reaction associated with an increase in total serum IgE and causing allergic asthma (Cleare *et al.,* 1976; Murdoch, *et al.,* 1986; Baker *et al.,* 1990). However, a type IV reaction has also been indicated in some patients, occurring within 24 hours (Goering, 2001; Hultman, 2007). In conjunction with respiratory symptoms, dermal symptoms such as dermatitis, urticaria and eczema have also been found in sensitised refinery workers (Pepys *et al.,* 1972; Murdoch *et al.,* 1986; Calverley *et al.,* 1995). These studies do not differentiate between immediate and delayed reactions when reporting symptoms. However, Pepys *et al.* (1972) found late bronchial reactions in two individuals who had negative reactions to skin prick tests, with no explanation given. The bronchial reactions after inhalation tests reached a maximum after 90 minutes and four hours respectively. Immediate reactions were noted in eight other workers, where the reaction started in 10 minutes and resolved within one hour. Considering the delayed reaction reported by Pepys *et al.* (1972) and the dermal symptoms listed for sensitised workers as listed before, the possibility exists that sensitisation to platinum salts may also result from a Type IV hypersensitivity reaction. Adams (2006) suggests that a delayed-type of hypersensitivity is possible, but it has not been proven by patch testing. A delayed reaction was also described for sensitised workers exposed to platinum dichloride (Nakayama and Ichikawa, 1997). Kimber and Wilks (1995) suggest the possibility of an additional hypersensitivity reaction other than IgE mediated, therefore sensitisation to platinum salts may be a combination of immediate and delayed hypersensitivity reactions.

Occupational exposure to rhodium causes a type I hypersensitivity reaction, leading to allergic asthma, and also a type IV hypersensitivity reaction of the skin, leading to contact dermatitis (Kusaka, 1993; Adams, 2006). The low sensitisation potential was indicated by Murdoch *et al.* (1986) where only six

positive prick tests were found from 306 workers in a refinery setting, with no positive tests in the absence of a platinum salt. In catalyst manufacturing and recycling factories positive skin prick tests were found for only two out of 23 workers tested (Cristaudo *et al.,* 2005). Respiratory symptoms associated with rhodium exposure include asthma and rhinitis, with numerous studies reporting dermal symptoms from exposure to rhodium (Bedello *et al.,* 1987; De la Cuadra and Grau-Massanés, 1991; Merget *et al.,* 2010; Goossens *et al.,* 2011). Goossens *et al.* (2011) reported two cases of rhodium salt sensitisation. In the first case, a jeweller working with rhodium solutions developed itchy, scaly skin lesions that were sometimes vesicular on all the fingertips. The symptoms persisted for several years and the lesions only healed after absence from work for more than a week. Laboratory testing indicated elevated serum IgE levels. The patient showed positive results to patch tests with cobalt chloride and rhodium chloride. In the second case an operator at a precious metal refinery listed eczematous lesions on his hands, face, wrist and forearms, and did not report any respiratory symptoms. After accidental contamination with Rh_2O_3 powder on his arms, he immediately developed erythema eruption considered as a burn. After subsequent contamination the worker developed vesicles on his forehead that spread over his face. The patient showed positive results to patch tests with palladium chloride, cobalt chloride and rhodium chloride. Goossens *et al.* (2011) conclude that rhodium as a salt is a sensitiser, but not as a metal. De la Cuadra and Grau-Massanés (1991) also indicate possible sensitisation to rhodium in a jeweller plating metals with rhodium. The worker developed dermatitis of the hands that only healed after absence from work for several days. Patch tests indicated positive reactions to rhodium (De La Cuadra and Grau-Massanés, 1991).

2.7.4 Skin reactions

Skin reactions such as contact urticaria, contact dermatitis and eczema have been reported after occupational exposure to platinum salts (Cristaudo *et al.,* 2005). The sensitisation skin reactions such as urticaria and allergic contact dermatitis, as well as irritation skin reactions such as eczema are discussed briefly below.

2.7.4.1 Urticaria

In occupational settings workers often develop contact urticaria as a result of skin contact with chemicals. These allergens elicit an IgE mediated immunological reaction, causing hives through the immediate type I hypersensitivity reaction. The hives (wheal) appear within minutes, and may appear red with itching and stinging. As a result of degranulation of the mast cells, histamine and other vasoactive substances are released, causing the skin symptoms. The hives generally disappear within a few hours or a few days (Rice and Mauro, 2013). Contact urticaria is divided into non-immunologic and

immunologic urticaria. Non-immunologic urticaria occurs without previous exposure, remains localised and does not cause systemic symptoms, whereas an immunologic urticaria reaction spread beyond the site of contact (Ngo and Maibach, 2010). It is unclear whether urticaria caused by platinum exposure is an immunologic or non-immunologic reaction.

2.7.4.2 Eczema

Eczema is an inflammatory skin condition observed in a variety of skin conditions with diverse causes. The term is used for a group of medical conditions including allergic and contact dermatitis. It is characterised by itching and soreness with signs of dryness, erythema, papulation, scaling and vesiculation. The terms dermatitis and eczema are often used as synonyms (Berth-Jones, 2010).

2.7.4.3 Contact dermatitis

Contact dermatitis is a cutaneous reaction to skin irritation or allergy and is the largest category of occupational compensated skin disease. Contact dermatitis can be categorised into irritant and allergic dermatitis and involves an inflammatory process with symptoms such as erythema (redness), induration (thickening), scaling (flaking) and vesiculation (blistering) at the site of contact. The specific type of dermatitis cannot be distinguished by the symptoms since the clinical presentation can be similar and the symptoms may occur simultaneously (Rice and Mauro, 2013).

Irritant dermatitis develops as a result of direct skin contact with an irritant, and the intensity of the reaction is proportional to the exposed dose (Cohen and Rice, 2003; Rice and Mauro, 2013). Dermal penetration of the irritant causes direct cellular injury by localised inflammation (Ngo and Maibach, 2010). These substances will cause an adverse reaction to anyone if the concentration is sufficient. Strong irritants can cause chemical burns, resulting in scarring, whereas mild irritants damage the skin barrier and thereby facilitate permeation of substances through the barrier (Rice and Mauro, 2013).

Allergic dermatitis is a delayed hypersensitivity reaction involving the immune system. The person is firstly sensitised to the substance after permeation into the lipid barrier of the skin, thereafter a subsequent exposure to very small doses of the substance will result in the elicitation of an allergic reaction. The reaction occurs after a latency period of half a day to several days (Rice and Mauro, 2013).

2.8 Permeation through the skin

2.8.1 Mechanisms of permeation

Substances can permeate through the skin by means of three possible pathways:

i) The transcellular pathway where the substance is transferred through the corneocytes. For a substance to pass through the skin by means of this pathway it has to move through the cell membranes of the corneocytes and also through the lipid matrix in the intercellular space (Kielhorn *et al.,* 2006). This pathway is also possible for larger molecules, since corneocytes undergoing desquamation are relatively permeable (Schaefer *et al.,* 2011).

ii) The intercellular pathway where the substance moves around the corneocytes, through the lipid matrix in the intercellular spaces (Kielhorn *et al.,* 2006).

iii) The appendageal pathway where the substance bypasses the corneocytes, but enters the hair follicles, sweat glands and sebaceous glands (shunts) (Kielhorn *et al.,* 2006). Earlier literature indicates that the contribution of hair follicles to skin permeation is minimal, as only 0.1-1% of the skin area is covered by the follicles. However, this is contradicted by Otberg *et al.* (2004), who report different hair follicle distribution and densities in different body regions (Otberg *et al.,* 2004; Ngo *et al.,* 2010). In areas with greater density and size of hair follicles, such as the scalp, this route could play a significant part in skin permeation (Kielhorn *et al.,* 2006). Therefore this route of permeation has been reconsidered to contribute to skin permeation as the appendages may provide a mechanism for larger molecules and ions to penetrate the skin and may function as reservoirs. The unique structure of the corneocytes in these structures also provides less of a barrier to substances entering the follicle (Otberg *et al.,* 2004; Ngo *et al.,* 2010).

Some substances may accumulate in the skin by binding to structures within the SC, thereby forming a reservoir (Barry, 2007). These substances are retained in the skin instead of passing through the skin entirely, and can be released at a later time. In addition to the SC, the viable epidermis and dermis could act as reservoirs, thereby creating a skin depot. The duration and formation of the reservoir is dependent on the type of substance, as well as skin temperature and relative humidity the skin is exposed to (Kielhorn *et al.,* 2006). Skin depot formation was suggested for pesticide and lead since permeation continued after the skin was washed (Zenzian, 2003; Larese Filon *et al.,* 2006).

2.8.2 Factors influencing permeation

There are a wide variety of factors that can influence the permeation of a substance through the skin, for the purpose of this thesis only a few relevant factors are discussed briefly. These factors can influence permeation *in vivo* as a result of occupational exposure, or during *in vitro* experimental studies. These factors are divided into three categories namely exposure, substance and skin related factors.

2.8.2.1 Exposure related factors

The exposure related factors will not be discussed in detail in this chapter as it forms part of Chapter 3 of this thesis. These factors include the duration of exposure, the exposure concentration or in *in vitro* experiments the concentration applied to the skin, and the area of skin exposed. Other exposure related factors that will not be addressed in this thesis include the type of task, use of protective clothing, personal hygiene and contaminated clothing.

2.8.2.2 Substance related factors

The substance related factors will not be discussed in detail in this chapter as it forms part of Chapter 3 of this thesis. These factors include the presence of other materials on the skin, molecular weight, pH and ionisation and oxidation state of the substance. Other substance related factors that will not be addressed in this thesis include solubility and structure.

2.8.2.3 Skin related factors

2.8.2.3.1 Age of skin

The age of the skin donor can influence skin permeation, with lower permeation through skin from aged subjects (Roskos *et al.,* 1989). In contrast, the barrier integrity of aged skin could be reduced since the skin has diminished blood supply, diminished surface lipid content and reduced hydration of the skin (Hostýnek, 2003).

2.8.2.3.2 Anatomical site of exposure

The thickness of the skin can influence permeation both *in vivo* and *in vitro*. *In vivo* the thickness of the skin varies according to anatomical site, for instance soles of the feet and palms are thicker, whereas the skin of the scrotal area is thinner and permeation is higher through the scrotal area than through the skin on the legs and abdomen (Boman and Maibach, 2000). Permeation is the highest through the scrotum, followed by the jaw angle and forehead with the lowest permeation for the foot arch, ankle and palm (Feldmann and Maibach, 1967). A comparison between four anatomical areas *in vivo* found permeation

based on the amount of the radiolabelled salt in the SC ranking as arm ≤ abdomen ≤ postauricular ≤ forehead (Rougier *et al.,* 1987). During *in vitro* experiments the skin from one anatomical site, usually abdominal skin, is used to eliminate anatomical site variability (Kielhorn *et al.,* 2006).

2.8.2.3.3 Thickness of the skin *in vitro*

During *in vitro* experiments Cnubben *et al.* (2002) and Wilkinson *et al.* (2006) reported less permeation through full thickness skin than through the epidermis only and dermatomed skin respectively. The SC is the main barrier to permeation and once a substance has permeated across this layer it is available to the cutaneous circulation (Kielhorn *et al.,* 2006). However, for the purpose of this thesis full thickness skin was used in experimental studies to simulate actual conditions where metal permeation will occur through all the layers of the skin. The methods used were benchmarked on other leading studies investigating the permeation of metals using full thickness skin (Larese *et al.,* 2007; Larese Filon *et al.,* 2009).

2.8.2.3.4 Density of hair follicles, sweat and sebaceous glands

Substances can permeate through the skin by means of the shunt route, i.e. through hair follicles and the sebaceous glands or through sweat glands; therefore the density of these appendages can influence permeation on the different anatomical areas. Hair follicle density was ranked as forehead > upper arm > back > thorax > forearm > thigh > calf. However, the diameter of the hair follicle orifice is larger in the calf and thigh than in the forehead (Otberg *et al.,* 2004).

2.8.2.3.5 Occlusion

Occlusion of the skin after exposure, such as donning gloves on already contaminated hands, traps the substance on the skin and thereby prevents evaporation or decontamination. Thereby occlusion may increase permeation of the substance as much as five times in comparison to similar non-occluded exposure (Boman and Maibach, 2000). Permeability of the SC is increased five- to tenfold when the skin is occluded (Sartorelli *et al.,* 2000).

2.8.2.3.6 Skin condition

Mechanical damage as a result of friction or abrasion will lead to partial or complete removal of the SC, which is the main barrier of the skin, and consequently improve absorption of substances through the skin (Morgan *et al*., 2003). Larese Filon *et al*. (2009) demonstrate that metal permeation is increased through damaged skin, and conclude that a small injury to the skin barrier can increase skin absorption. Damaged skin may allow larger compounds that would normally not be permeable through intact skin to permeate and damaged skin has an increased absorption rate (Kezic and Nielsen, 2009).

2.8.2.3.7 Skin film liquids

If a metal comes into contact with the skin, it has to dissolve in skin film liquids, such as sweat, to form permeable metal ions. Only metals that dissolve in human sweat and the particles that are in close contact with the stratum corneum will permeate through the skin. Therefore, the bioaccessibility fraction is only the amount of metal that dissolves in skin film liquids, mostly sweat, and is available for permeation through the skin. Under physiological conditions, the pH of skin surface film liquids, including sweat and sebum lipids, range between 4.2 and 6.1 (Stefaniak *et al.,* 2014). The pH of the liquid can also influence the charge as well as the size of ions formed, and can therefore influence permeation. For example the wide range of pH of the sweat can influence the type of chromium ion that is formed after dissolution, as indicated by chromate ions forming at a pH above 6, whereas dichromate or hydrogen chromate ions form at a pH between 2 and 6 (Tandon *et al.,* 1984).

2.8.2.3.8 Skin type (Racial differences)

The majority of literature regarding skin permeation and the difference in permeation between African and Caucasian skin suggests lower permeation through African skin with variations in permeation dependent on the molecule. *In vivo* studies by Hymes and Sprake (1986) found lower efficacy of anaesthetics in African skin. Wedig and Maibach (1981) report less absorption of dipyrithione, and Kompaore *et al.* (1993) found lower permeation of methyl nicotinate in African subjects. In concordance with these findings, nitroglycerin was also less permeable through African skin (Williams *et al.,* 1991). Another study testing the permeation of methyl nicotinate indicated significantly lower permeation in African skin when compared to Caucasian skin (Berardesca and Maibach, 1990). Similar results were described by Leopold and Maibach (1996), although differences were not significant, the authors suggest that an increase in population size would provide significant differences in permeation between skin from different races. *In vivo* permeation of three compounds determined by urinary excretion and tape stripping was less in African skin, although no statistically significant differences were found (Lotte *et al.,* 1993). Sinha *et al.* (1978) also found no significant difference in skin permeation of diflorasone diacetate between African and Caucasian skin. However, the small number of subjects (n = 6) in the study should be taken into account.

The overall indication of lower permeation through African skin is supported by the data of reduced skin irritation responses found in African subjects (Robinson, 1999). Various publications report African skin to be less susceptible to chemical irritants than Caucasian skin (Weigand and Mershon, 1970; Weigand and Gaylor, 1974; Frosch and Kligman, 1977). African subjects required longer exposure to the irritant to develop similar irritant reactions as Caucasian subjects (Weigand and Mershon, 1970). Hicks *et al.* (2003) confirm this by reporting lower irritation for African skin, although

not statistically significant. Weigand and Gaylor (1974) suggest the SC to be the main barrier contributing to these differences, as the difference in irritation reactions between races was not seen when the SC was removed. Alternatively Weigand and Mershon (1970) suggest that the different melanin content of the SC could explain the difference in reactivity, where melanin exerts a protective influence against reactivity. It is, however, important to note that the majority of results are based on visual perception of erythema after irritant exposure where accuracy of assessment could be influenced by darker skin (Fluhr *et al.,* 2008). Studies using laser Doppler velocimetry to determine blood flow also showed African skin to be less susceptible to nicotinates and corticoids respectively (Berardesca and Maibach, 1989; Berardesca and Maibach, 1990). African subjects were found to be more resistant than Caucasian subjects after sensitisation was induced by strong sensitising chemicals (Rostenberg and Kanof, 1941). Berardesca and Maibach (1991) indicate less sensitisation rates to clonidine for African skin than Caucasian skin. In contrast, Guy *et al.* (1985) did not find significant differences between racial susceptibility when methyl nicotinate was applied. In addition to being a more resistant barrier to irritants, African skin recovers faster after barrier disruption by tape stripping (Reed *et al.,* 1995).

One *in vitro* study found no significant difference between the permeation of pesticides through African and Caucasian skin, though this study used foreskin from human newborns (Shehata-Karam *et al.,* 1988). No similar *in vitro* studies using abdominal skin from African donors are available. The literature suggesting a superior barrier for African skin was obtained by biological monitoring, such as urinary excretion and blood concentration, or the induction of a vasodilatory response. Therefore results for metal permeation obtained by *in vitro* methods are not directly comparable to *in vivo* results.

Other skin factors that will not be addressed in this literature study include skin perfusion, temperature and skin metabolism.

2.9 *In vitro* method utilised to determine permeation

The *in vitro* diffusion method has been used successfully to determine skin permeation of metals such as chromium, cobalt, copper, gold, lead, mercury, nickel, silver, zinc, titanium and also nanoparticles of cobalt, gold, silver and zinc. This method utilises human skin donated after surgical procedures, or even from cadavers. Most often abdominal skin is used because of its availability from surgical procedures. However, breast, back or leg skin has also been used. The skin is clamped between two compartments of a diffusion cell where the SC side of the skin is exposed to the donor compartment, and the dermis side is exposed to the receptor compartment. The receptor solution in the receptor compartment is removed at various intervals and analysed for the metal content. After completion of the study the skin is removed and analysed for metal content after chemical digestion. This method was utilised to

determine skin permeation of platinum and rhodium through human skin. A review of available literature on metal skin permeation using human skin for *in vitro* experiments was compiled and is included in Chapter 3.

2.10 Summary of literature study

This literature study described the importance of PGM mining and refining to the South African economy, and contribution to job creation and specifically described the workforce distribution. The potential for workplace exposure together with environmental exposure was discussed, with an emphasis on dermal exposure and subsequently the skin structure and function were discussed briefly. Considering that the majority of the South African workforce are Africans, an integrated summary on racial differences in skin anatomy and barrier function was given. The potential health effects of PGM exposure with focus on sensitisation and dermal reactions were discussed. Finally the factors that could influence skin permeation of substances were described briefly.

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Chapter 3: Article I

Franken, A., Eloff, F.C., Du Plessis, J., Du Plessis, J.L. *In vitro* permeation of metals through human skin: A review. Submitted to the journal Toxicology *in Vitro* to be considered for publication.

3.1 Background

Numerous articles on the *in vitro* permeation of metals have been published using human or porcine (pig) skin. Literature specifically using human skin dates from 1963 to the present, investigating the permeation of chromium, cobalt, copper, gold, lead, mercury, nickel, platinum, rhodium, silver, zinc and titanium. This article is a review of these studies, focussing on the experimental design and results from each study. Experimental design factors such as the thickness of the skin, skin exposure area, intervals of receptor solution extraction, donor and receptor solutions utilised as well as the temperature at which the receptor compartment was placed in were reviewed. This review article was submitted to *Toxicology in Vitro*, the official journal of the European Society of Toxicology *in Vitro,* for consideration for publication.

3.2 Instructions to authors (excerpt)

Toxicology *in Vitro* publishes papers reporting and interpreting original toxicological research involving the application or development of *in vitro* techniques.

Conflict of interest: The journal follows ICMJE recommendations regarding conflict of interest disclosures. All authors are required to report the following information with each submission: All third-party financial support for the work in the submitted manuscript. All financial relationships with any entities that could be viewed as relevant to the general area of the submitted manuscript. All sources of revenue with relevance to the submitted work who made payments to you, or to your institution on your behalf, in the 36 months prior to submission. Any other interactions with the sponsor of outside of the submitted work should also be reported. Any relevant patents or copyrights (planned, pending, or issued). Any other relationships or affiliations that may be perceived by readers to have influenced, or give the appearance of potentially influencing, what you wrote in the submitted work.

Language: Text should be written in good English, American or British usage is accepted. For this thesis British spelling was used throughout.

Manuscript format: Manuscripts should include Introduction, Material and methods, Results and Discussion. The abstract should be a self-contained summary of the objectives, results and significance of the study. The manuscript should include three to five bullet point highlights that convey the core findings of the article. A maximum of six keywords should be included in the manuscript.

Tables: Tables should be intelligible without reference to the text and should be planned to fit the page size of the Journal. The same data may not be reproduced in both a table and a figure. Each table must have a title and on each column there should be a heading that clearly identifies the data therein. Number tables consecutively in accordance with their appearance in the text. For the purpose of this thesis the Figures and Tables are included in the text for readability.

References: References should be consistent using the following style:

Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2010. The art of writing a scientific article. J. Sci. Commun. 163, 51–59.

Strunk Jr., W., White, E.B., 2000. The Elements of Style, fourth ed. Longman, New York, pp. 60-61.

Mettam, G.R., Adams, L.B., 2009. How to prepare an electronic version of your article, in: Jones, B.S., Smith , R.Z. (Eds.), Introduction to the Electronic Age. E-Publishing Inc., New York, pp. 281–304.

3.3 *In vitro* permeation of metals through human skin: A review.

Abstract

During the last few decades the interest in skin permeation of specifically metals has increased. The *in vitro* method of utilising diffusion cells has been used to assess skin permeation of metals such as chromium, cobalt, gold, lead, mercury, nickel, platinum, rhodium, silver, titanium and zinc. Differences in experimental design are immediately evident, most noticeably in the use of various donor and receptor solutions, different temperatures at which the receptor solution was kept, differences in skin thickness and variations in exposed skin surface areas. The metals included in this review, except for titanium and zinc, permeated through intact human skin under physiological conditions. Large variations in the permeability results were observed, with flux values ranging between 0.05 ng/cm^2/h (rhodium) and 38 µg/cm^2/h (copper). Permeability for a particular metal also varied with the notable differences in methodology as the probable reason. These metals were also retained in the skin (chromium: 0.002 to cobalt: 290 µg/cm^2), which could lead to reservoir formation and extended exposure after removal of the metal from the outer surface of the skin. Permeation of metals is most likely influenced by the pH of the donor solution as seen with an increase in chromium permeation at a lower pH. Damaged skin is more permeable than intact skin, as is evident for nickel and cobalt. Recommendations are provided on the standardisation of experimental design and format of data reporting to enable comparison of results from future *in vitro* metal permeation studies.

Keywords

Chromium, Cobalt, Gold, Lead, Mercury, Nickel, Platinum, Rhodium, Silver, Titanium, Zinc, *in vitro* permeation.

Highlights

- Differences in *in vitro* experimental design are evident.
- Large variations in the permeability results.
- Limited comparisons of results from different studies.
- Most metals are permeable through intact human skin.
- Possible reservoir formation in the skin.

The Franz diffusion cell method of using glass chambers for *in vitro* skin permeation studies has been used for decades and was validated in 1975 (Franz, 1975). This method was originally developed for use in the pharmaceutical industry to determine the fraction absorbed and the penetration rates of transdermal medications; and is often used to compare the permeation of different medication formulations (McDougal and Boeniger, 2002; OECD, 2004). This method has been adapted for use in the field of occupational toxicology, where it has been used successfully for almost 50 years to quantify the absorption of various metals and pesticides, to name a few, through human skin and to determine the influence of, among others, damaged skin on permeation.

The skin as a route of exposure has often been overlooked in occupational settings when assessing exposure to hazardous substances, since the respiratory route was traditionally considered as the most important exposure pathway (Schneider *et al.,* 1999). However, for some substances the skin is the predominant route of exposure and in past decades the skin has been recognised as an important exposure route. This recognition has led to the development of various methods to determine dermal exposure, as well as dermal permeation of hazardous substances (McDougal and Boeniger, 2002; Semple, 2004). The dermal exposure route is a complex route as the skin itself can be the target organ following exposure to contaminants. Secondly, the skin could be the portal of entry into the body leading to systemic toxicity, and thirdly the substance could lead to allergic reactions triggering responses in the skin or systemically (McDougal and Boeniger, 2002; Semple, 2004).

The skin is potentially exposed to metals in various manufacturing industries, as well as in mining and refining activities. However, non-occupational exposure can also occur as a result of consumer exposure (e.g. nickel released after handling tools) or environmental exposure (e.g. platinum group metals released into the atmosphere by vehicle exhaust catalysts) (Lidén *et al.,* 1998; Wiseman and Zereini, 2009). In occupational settings the skin can be contaminated by direct deposition on the skin from the air; by transfer from contact with contaminated surfaces, tools or clothing; or by direct contact such as a splash or submersion into the contaminant (Schneider *et al.,* 1999; McDougal and Boeniger, 2002). Dermal exposure can be quantified by removal, interception or fluorescent tracer methods. These measurement methods provide an estimate of the amount of the substance on the skin at the time of the sample collection, whereas the *in vitro* method provide information on the rate of permeation through the skin, which could become systemically available and thereby contribute to total body burden (McDougal and Boeniger, 2002; Semple, 2004). The terms permeation, penetration, and absorption are often used interchangeably in *in vitro* studies to describe the diffusion of a substance through the skin. However, their definitions differ: Permeation is the movement from one skin layer into a second layer, whereas penetration is the entry of a substance into a particular skin layer. Absorption is the uptake of a substance through the skin, often leading to the systemic circulation (Kielhorn *et al.,* 2006).

Numerous studies have thus far determined the permeation of various metals through skin and the retention thereof in the skin, although with noteworthy differences in experimental design and extent of results reported. Evident differences include the type of diffusion cell used, surface area of skin exposed and thickness of skin used, to mention a few. Results reported differed with regard to flux or the permeability coefficient reported, or the concentrations in the receptor solution and skin expressed as unit per square centimetre or percentage of the applied mass. Due to these differences the experimental design and findings of published *in vitro* metal permeation studies through human skin will be critically reviewed. Metals included are chromium, cobalt, gold, lead, mercury, nickel, platinum, rhodium, silver, titanium and zinc. Cobalt, gold, silver and zinc nanoparticles were included in this review, but the discussion is limited to the permeation of metals in general. Studies utilising human skin was reviewed and studies utilising porcine skin was excluded. Furthermore, guidelines regarding the experimental design for future *in vitro* studies are proposed to promote consistent data reporting and enable comparison of results between studies.

In vitro methodology: Apparatus used

Studies included in this review all used a type of diffusion cell with human skin clamped between two compartments. The Franz diffusion cell was used predominantly with only a few studies using the Bronaugh flow through or J-shaped diffusion cells (Bress and Bidanset, 1991; Moody *et al,.* 2009). A typical Franz type diffusion cell is depicted in Figure 1A with a summary of differences related to materials it was manufactured from, temperature of receptor compartment and solutions used in the donor and receptor compartments.

The Franz type cells are manufactured from glass or Teflon and the area of exposed skin ranged in surface area from 0.55 to 10 cm^2 (Figure 1B). The stratum corneum (SC) side of the skin is exposed in the donor compartment containing the donor solution, and the dermis side is exposed to the receptor (receiving) compartment containing the receptor solution. The temperature of the receptor compartment ranges between 32 and 37 °C; with 32 °C corresponding to normal skin temperature and 37 °C to body temperature beneath the skin (Franz, 1975; OECD, 2004). During the experiment the receptor solution is removed at various intervals for analysis of metal content. The different intervals of removal from published studies are depicted in Figure 2. After completion of the experiment the skin is removed and destroyed with acids to determine the metal content in the skin. Alternatively, the skin is tape stripped before digestion and are then analysed separately in order to report metal content in the skin layers.

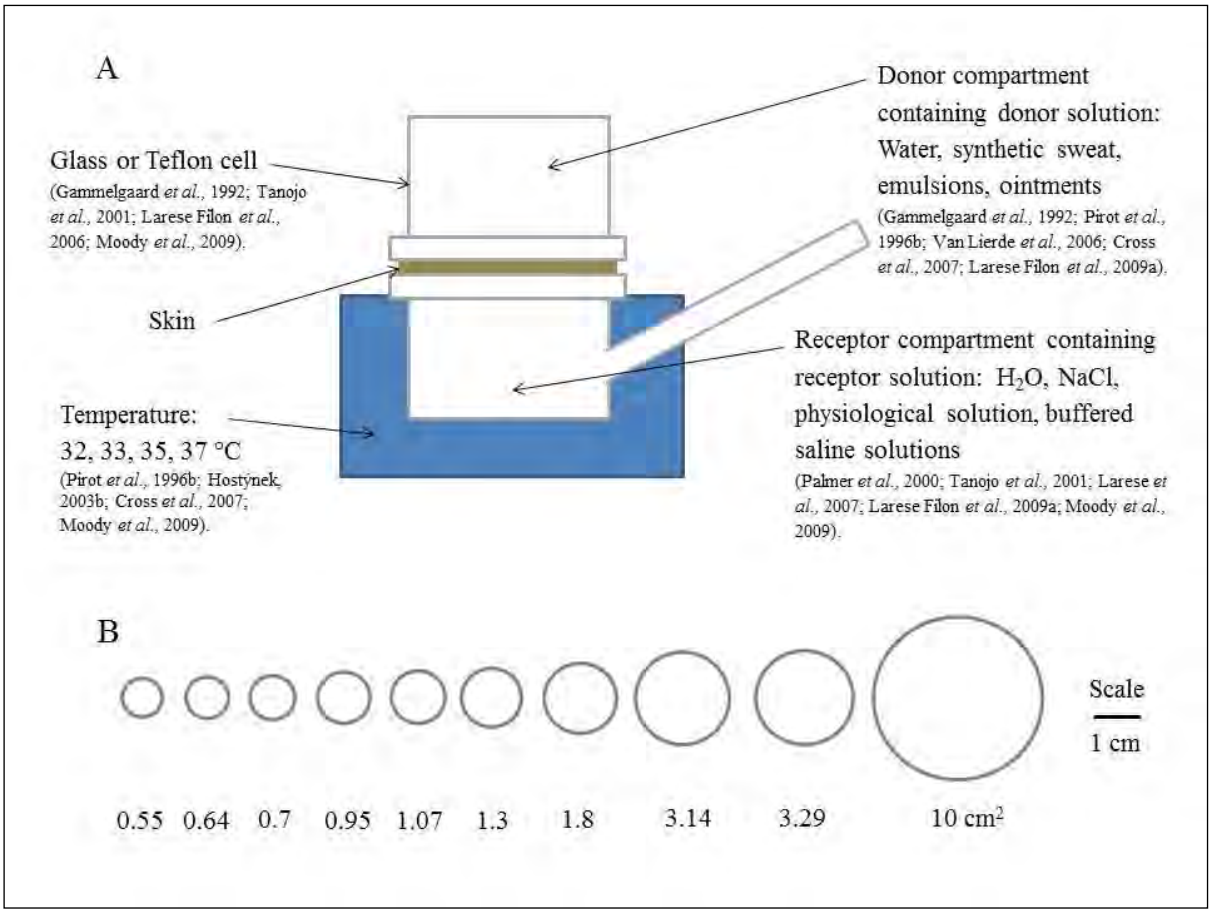

Figure 1: Experimental setup of a Franz diffusion cell. A - Side view of a typical Franz diffusion cell with a summary of experimental setups utilised in published studies. B - Top view, depicting the surface area of exposed skin in the donor compartment used in published studies.

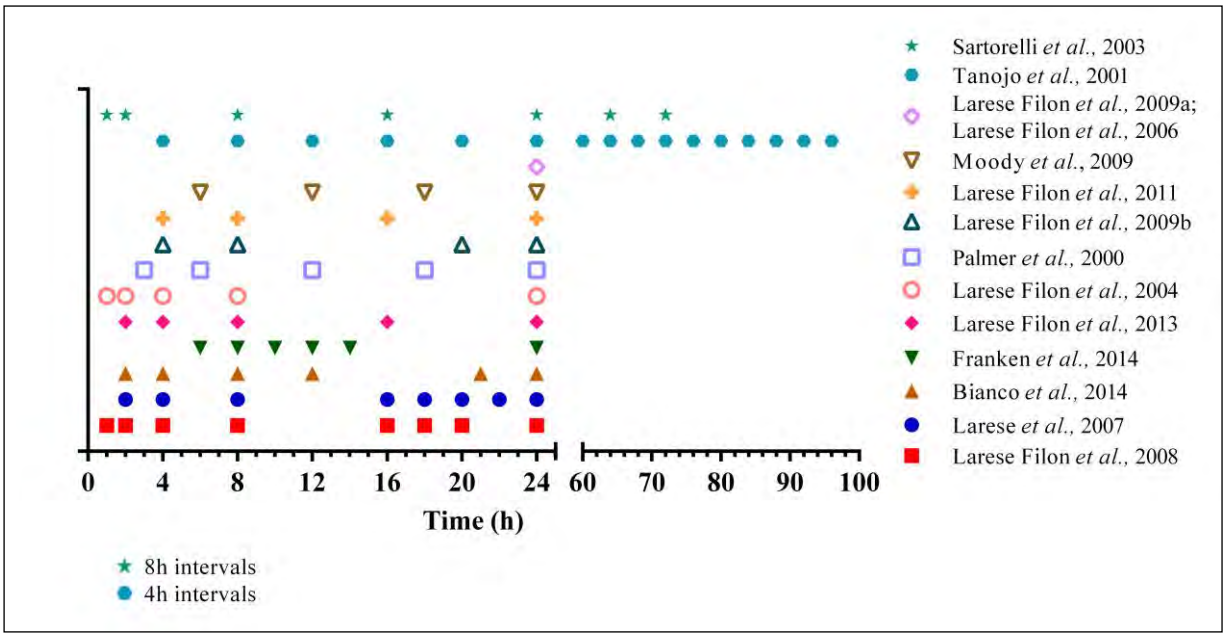

Figure 2: Time intervals (in hours) of receptor solution removal for analysis from 14 published studies.

In vitro methodology: Skin related aspects

After the skin has been clamped between the two compartments of the diffusion cell and before application of the donor solution, the skin integrity is tested. This is done by measuring capacitance (impedance), electrical resistance, electrical conductibility, by measuring the permeation of tritiated water, or by the leak testing. However, this is not reported by all the studies (Gammelgaard *et al.,* 1992; Larese *et al.,* 2007; Larese Filon *et al.,* 2008; Moody *et al.,* 2009; Hostýnek and Maibach, 2010).

The differences in methodology regarding the skin, such as the anatomical area, number of donors, age and gender of donors, as well as origin of skin donations are summarised in Table 1. The skin permeability varies between anatomical areas, for example thinner facial skin is more permeable than skin of the palm and foot (Feldmann and Maibach, 1967; Ngo *et al.,* 2010). The number of and age of donors are rarely reported, but for those reported it ranged between 14 and 71 years. Skin permeation is influenced by the age of the skin donor as aging thins the skin and it undergoes changes in vasculature and collagen fibres that support the skin, thereby compromising skin integrity (Ngo *et al.,* 2010; Ngo and Maibach, 2010). Skin permeability could potentially differ between genders. However, literature regarding gender related differences is conflicting. Several studies reported no significant differences in skin barrier properties or reactivity to skin irritants between males and females, whereas other studies reported significant differences (Lammintausta *et al.,* 1987; Reed *et al.,* 1995; Jacobi *et al.,* 2005; Dao and Kazin, 2007; Darlenski and Fluhr, 2012).

Table 1: Variations in skin related aspects reported in *in vitro* permeation studies.

Methodological aspects	**Variations**	**Reference**
Anatomical area of skin	Abdominal, breast, leg.	Fullerton *et al.* (1988); Hostýnek (2003b); Moody *et al.* (2009); Larese Filon *et al.* (2013).
Number of donors	1, 2, 3, 4, 14.	Fullerton *et al.* (1986); Palmer *et al.* (2000); Larese Filon *et al.* (2006); Hostýnek and Maibach (2010); Larese Filon *et al.* (2013).
Gender of skin donors	Male and female, female only, male only.	Palmer *et al.* (2000); Sartorelli *et al.* (2003); Larese *et al.* (2007); Larese Filon *et al.* (2009a).
Age of skin donors	18, 55-70, 41-71, 45-71, 14-67, 37-52, 21-53.	Hostýnek (2003a); Hostýnek (2003b); Larese Filon *et al.* (2008); Hostýnek and Maibach (2010); Larese Filon *et al.* (2011); Larese Filon *et al.* (2013); Franken *et al.* (2014).
Donations	After surgical procedures, from cadaver/autopsies.	Hostýnek (2003b); Moody *et al.* (2009); Larese Filon *et al.* (2013); Bianco *et al.* (2014)

Metals

The *in vitro* diffusion method has been used to determine the permeation of metals through human skin since 1965. Porcine (pig) skin has also been used as a surrogate for human skin in various *in vitro* studies, but these studies were excluded from this review. The studies on the skin permeation of metals through human skin included chromium, cobalt, copper, gold, lead, mercury, nickel, platinum, rhodium, silver, titanium, zinc and also nanoparticles of cobalt, gold, silver and zinc. These studies will be discussed subsequently, and the main findings is summarised in Table 2. In Table 2 the secondary references are included where the primary article was not obtainable. Dermatomed skin refers to split thickness skin where the SC, epidermis and often a small section of the dermis is included. Full thickness skin refers to skin containing the SC, epidermis and dermis where only the subcutaneous fat was removed.

Chromium (Cr)

Chromium is a known sensitiser causing allergic contact dermatitis in industries such as construction (caused by cement) and leather tanning (Hansen *et al.,* 2003; Lidén *et al.,* 2008). The potential of trivalent chromium to cause sensitisation is low as it readily binds with the skin, thereby reducing the permeation of chromium through the skin. Hexavalent chromium may cause sensitisation as it permeates through the skin layers easily, however hexavalent chromium could also be reduced to trivalent chromium in the skin (Mali *et al.,* 1963; Samitz and Katz, 1963).

The *in vitro* method has been used as early as 1965 in studying the permeation of chromium through human skin (Wahlberg, 1965). Due to the lack of methodological information and comparable results, these studies were excluded from Table 2. The disappearance method was utilised to indicate chromium permeation after application of sodium chromate (Na_2CrO_4) to breast and abdominal skin (Wahlberg, 1965). This method measures the radioactivity of the donor solution by using a scintillation detector, where the counting rate decreases as a function of time, indicating disappearance from the skin surface. Spruit and Van Neer (1966) reported no permeation of chromium after application of 0.14% Cr(III) sulphate solution, although chromium was found in the dermis. After application of 0.9% chromium sulphate, chromium permeated into the salt solution underneath the dermis. Similarly, an increase in chromium diffusion through the skin was found with an increase in concentration (Samitz *et al.,* 1967). They also indicate that more chromium permeated from a chloride solution ($CrCl_3$) than a nitrate ($Cr(NO_3)_3$) or sulphate solution ($Cr(SO_4)_3$), and that permeation from all solutions was lower at a pH of 7 than at a pH of 5 or 9, which was attributed to the lower solubility of Cr(III) near neutrality. Wahlberg (1970) used human breast tissue to determine permeation of sodium chromate (Na_2CrO_4) and chromic chloride ($CrCl_3$) and found that permeation of Na_2CrO_4 increased in a concentration dependent manner.

Table 2: Summary of the experimental design and results from *in vitro* permeation of metals through human skin.

	Author	Form of metal	Concentration applied	Skin thickness ◊	Donor solution	pH of solution	Duration of study (hours)	Permeability coefficient K_p (cm/h)	Flux ($ng/cm^2/h$)	Lag time (hours)	[Receptor solution] ($\mu g/cm^2$)	Content in skin ($\mu g/cm^2$)	Cumulative % permeated	% in skin
Chromium	Fitzgerald and Brooks (1979) in Hostýnek (2003a)	K_2CrO_4	0.00001 - 2.1M	E		7		0.23 - 7 x 10^{-3}						
	Gammelgaard *et al.* (1992) * in Hostýnek (2003a)	$K_2Cr_2O_7$ $CrCl_3.6H_2O$ $Cr(NO_3)_3.9H_2O$	0.034M Cr	FT	aq	4.2 3.0 2.8	190	* 0.43 x10^{-3} 0.041 x10^{-3} 0.03 x10^{-3}	-	-	0.037 N ND	E: 134 D: 12 E: 12.5 D: 1.3 E: 9.6 D: 0.33	-	-
	Gammelgaard *et al.* (1992) in Hostýnek (2003a)	Cr_2O_7	0.125 - 0.5%	FT	-	-	-	0.078 - 0.13 x 10^{-3}	-	-	-	-	-	-
	Fullerton *et al.* (1993)	Cr cement $FeSO_4$ cement	2.1 µg/ml 0.01 µg/ml	FT – breast and abdominal	aq	12.5	48	-	-	-	ND ND	E: 0.003 D: 0.013 E: 0.002 D: 0.034	-	-
	Van Lierde *et al.* (2006)	$CrCl_3.6H_2O$ $Cr(NO_3)_3.9H_2O$ $K_2Cr_2O_7$ $CrCl_3.6H_2O$ $Cr(NO_3)_3.9H_2O$ $K_2Cr_2O_7$	0.034 mol/l	Derm	aq ss	- 5.5	168	-	-	-	< LOD < LOD 0.18 < LOD < LOD 0.10	12.4 15.3 121 2.3 4.3 87	-	-
	Larese *et al.* (2007)	Cr powder † $K_2Cr_2O_7$	2.5 g/50 ml	FT	ss	6.5	24	- 1.52 x 10^{-4}	ND 7 290	ND 12.5	< 0.001 > 90 ‡	-	-	-
	Larese Filon *et al.* (2008)	Cr powder †	2.5 g/50 ml	FT	ss	4.5	24	0.0124	0.84	1.1	0.016	3.19	-	-
	Larese Filon *et al.* (2009a)	Cr powder †	2.5 g/50 ml	FT	ss	4.5	24	-	-	-	0.005	14.4	-	-
Cobalt	Sartorelli *et al.* (2004) in Sartorelli *et al.* (2012)	Co-57	-	-	aq ss	-	24	-	-	-	-	-	1.04 3.30	SC: 27.62 E: 0.37 SC: 41.30 E: 0.23
	Larese Filon *et al.* (2004) ▪ in Larese *et al.* (2007)	Co powder †	2.5 g/50 ml	FT	ss	6.5	24	▪ 3.7 x 10^{-4}	12.3	1.55	-	13.2	-	-
	Larese Filon *et al.* (2009a)	Co powder †	2.5 g/50 ml	FT	ss	4.5	24	-	-	-	0.038	29.6	-	-
	Larese Filon *et al.* (2013)	Co nanoparticles	100 mg/100 ml	FT	ss	4.5	24	-	0.55	7.2	0.009	4.35	-	-
Copper	Pirot *et al.* (1996a) in Hostýnek and Maibach (2006)	$CuSO_4$ $CuSO_4$ $CuCl_2$ $CuCl_2$	20 mg/cm^2	Derm	Petrolatum Gel Petrolatum Gel	-	72	3.2 x 10^{-6} 4.5 x 10^{-6} 1.6 x 10^{-5} 2.3 x 10^{-6}	-	-	< 50	-	-	-
	Pirot *et al.* (1996b) in Hostýnek and Maibach (2006)	CuPC $CuSO_4$ $CuSO_4$ CuPC $CuSO_4$ $CuSO_4$	1 mg/cm^2 1.3 mg/cm^2 0.5 mg/cm^2 1 mg/cm^2 0.9 mg/cm^2 0.5 mg/cm^2	Derm	Water/oil emulsion Ointment	-	72	0.57 x 10^{-4} 0.44 x 10^{-4} 1.2 x 10^{-4} 1.2 x 10^{-4} 0.8 x 10^{-4} 0.9 x 10^{-4}	-	-	-	-	-	-
	Hostýnek and Maibach (2006)	$CuSO_4$ $Cu(CO_2CH_3)_2$	1%	E	aq	-	96	2.73 x 10^{-6} 6.05 x 10^{-6}	-	-	-	-	-	-
	Hostýnek and Maibach (2010) and Hostýnek and Maibach (2011)	GHK-$Cu(Ac)_2$	0.68%	SC E Derm	aq	-	48	55.9 x 10^{-4} 0.003 x 10^{-4} 2.5 x 10^{-4}	38 000 2 1 700	2 7 2	2 110 0.6 136.2	290 61.1 53	19.85 0.006 2	-

Table 2 continued.

	Author	Form of metal	Concentration applied	Skin thickness ◊	Donor solution	pH of solution	Duration of study (hours)	Permeability coefficient K_p (cm/h)	Flux ($ng/cm^2/h$)	Lag time (hours)	[Receptor solution] ($\mu g/cm^2$)	Content in skin ($\mu g/cm^2$)	Cumulative % permeated	% in skin
Gold	Larese Filon *et al.* (2011)	Au nanoparticles	15 µg/cm² 45 µg/cm²	FT	ss	4.5	24	-	- 7.8	-	0.061 0.214	- 1.82	-	-
Lead	Bress and Bidanset (1991) * in Hostýnek (2003a)	$C_{16}H_{36}Pb$ Lead(II) nuolate Lead(II) naphthanate $Pb(CH_3CO_2)_2$ PbO	7.7 mg/cm² (10 mg/1.3cm²)	-	-	-	24	-	* 20 000 4 200 1 000 160 < 30	-	632.3 µg 130.0 µg 29.7 µg 5.0 µg < 1.0 µg	-	-	-
	Larese Filon *et al.* (2006)	PbO powder	5 mg/cm²	FT: Intact Damaged	ss	5.0	24	-	-	-	0.003 0.027	0.32 0.30	-	-
Mercury	Palmer *et al.* (2000)	$HgCl_2$ proprietary formulation (p) $HgCl_2$ aqueous formulation (aq)	290 µg/l	FT	Cream aq	-	24	(p) 0.11 0.027 (aq) 0.284 0.015			33 µg/l 39 µg/l ‡	760 µg/l/g 3900 µg/l/g	0.8 3.7	
	Sartorelli *et al.* (2003)	Hg-203 ($HgCl_2$)	0.0088 nmol/cm³ 0.0607 nmol/cm³	Derm	Buffered solution	4	72	14.42 3.04	0.12 0.18 nmol/cm²/h	-	-	-	-	18.93 44.97
	Moody *et al.* (2009)	Hg-203 ($HgCl_2$)	8.6 µg/ml	Derm - breast	Acetone	-	24	-	0.6	-	-	-	1.4	76.9
Nickel	Fullerton *et al.* (1986) ○ in Hostýnek (2003b)	$NiCl_2$ $NiSO_4$	1.32 mg/ml	FT	aq	5.5	144-239	○ 4.6 x 10^{-5}	-	50	-	-	5-22 1-2	13-43 4-7
	Fullerton *et al.* (1988) * in Hostýnek (2003a)	$NiCl_2$	5%	FT - breast and abdominal	Gel	-	96	* E: 1.5 x 10^{-3} D: 0.23 x 10^{-3} RS: 0.006 ø			0.67	SC: 95.9 E: 19.89 D: 2.89	0.4	SC: 51 E: 10.6 D: 1.6
	Emilson *et al.* (1993) in Hostýnek (2003a)	$NiCl_2$	5%	FT - breast	aq	-	18	5.5 x 10^{-5}	-	-	-	-	-	-
	Hostýnek *et al.* (2000) in Hostýnek (2003a)	$NiCl_2$	1%	E	-	-	-	1.28 x 10^{-5}	-	-	-	-	-	-
	Tanojo *et al.* (2001) ○ in Hostýnek (2003b)	$NiSO_4.6H_2O$ $NiCl_2.6H_2O$ $Ni(NO_3)_2.6H_2O$ $Ni(CH_3COO)_2.4H_2O$	1% w/v Ni (10 mg/ml)	SC - breast and abdominal	aq	-	96	8.5 x 10^{-7} 6.8 x 10^{-7} 5.2 x 10^{-7} 5.2 x 10^{-7} ○ E: 12.8 x 10^{-6}	-	-	-	-	1.09 0.74 0.54 0.02	0.56 0.18 0.95 0.10 (% in SC)
	Hostýnek (2003a)	$NiCl_2$ $C_{16}H_{30}NiO_4$ $NiBr_2$ NiI_2	500 µg/ml	Derm	-	-	-	9.8 x 10^{-3} 1.4 x 10^{-3} < 10^{-2} < 10^{-2}	-	-	-	-	-	-
	Hostýnek and Reifenrath (2002) in Hostýnek (2003a)	$NiCl_2$	0.05%	Derm	-	-	-	9.8 x 10^{-3}	-	-	-	-	-	-

Table 2 continued.

	Author	Form of metal	Concentration applied	Skin thickness ◊	Donor solution	pH of solution	Duration of study (hours)	Permeability coefficient K_p (cm/h)	Flux ($ng/cm^2/h$)	Lag time (hours)	[Receptor solution] ($\mu g/cm^2$)	Content in skin ($\mu g/cm^2$)	Cumulative % permeated	% in skin
Nickel	Hostýnek (2003b)	$NiCl_2$ NiDO	571 µg/ml	Derm - leg	Sodium caprylate solution	-	48	9.8×10^{-3} 1.4×10^{-3}	-	-	-	-	35.7 6.4	0.02 0.6
	Larese *et al.* (2007)	Ni powder †	2.5 g/50 ml	FT	ss	6.5	24	6.1×10^{-4}	16.5	14.56	> 0.15 ‡	-	-	-
	Larese Filon *et al.* (2009a)	Ni powder †	2.5 g/50 ml	FT	ss	4.5	24	-	-	-	0.031	82.3	-	-
	Sartorelli *et al.* (2004) in Sartorelli *et al.* (2012)	Ni-63	-	-	aq ss	-	24	-	-	-	-	-	0.23 0.76	SC: 50.3 E: 0.42 SC: 36.2 E: 0.34
	Moody *et al.* (2009)	Ni-63 ($NiCl_2$)	8.4 µg/ml	Derm - breast	Acetone	-	24	-	0.9	-	-	-	1.8	20.9
Platinum	Franken *et al.* (2014)	K_2PtCl_4	0.3 mg/ml	FT	ss	6.5	24	3.9×10^{-7} (u)	0.12	3.53	0.003	1.5	0.00023	2.24
Rhodium	Franken *et al.* (2014)	$RhCl_3$	0.3 mg/ml	FT	ss	6.5	24	1.8×10^{-7} (u)	0.05	4.41	0.001	0.76	0.0001	1.16
Silver	Larese Filon *et al.* (2009b)	Ag nanoparticles	70 $\mu g/cm^2$ in ethanol absolute diluted 1:10 with SS	FT	ss	4.5	24	-	-	-	0.001	-	-	-
	Bianco *et al.* (2014)	Ag nanoparticles	400 mg/100ml (113$\mu g/cm^2$)	Derm	ss	4.5	24	-	0.2	8.2	-	-	-	-
Titanium	Mavon *et al.* (2007)	TiO_2	2 mg/cm^2	-	Sunscreen formulation	-	5	-	-	-	ND	SC: 50.7 E: 3.0 D: 0.06	-	SC: 94.2 E: 5.6 D: 0.1
Zinc	Pirot *et al.* (1996b) in SCCNF (2003)	ZnO	1.88 mg/cm^2	Derm	Emulsion/ Ointment		72	-	-	-	-	-	0.34	E: 0.5 D: 1.1
	Cross *et al.* (2007)	ZnO nanoparticles	10 $\mu l/cm^2$	E	Sunscreen emulsions	-	24	-	-	-	0.75 ‡ 0.55	-	< 0.01 ‡ < 0.03	-

◊ Skin thickness – abdominal skin unless specified
aq – aqueous
Derm – split thickness dermatomed skin
FT – full thickness
ss – synthetic sweat
ND – not detectable
SC – stratum corneum
E – epidermis
D – dermis
RP – receptor solution
† – not specified
‡ – deduced from graph
LOD – limit of detection
(u) – unpublished
Ø – calculated from disappearance value

Chromium (Cr) continued

Gammelgaard *et al.* (1992) evaluated the permeation of three different chromium salts through full thickness human skin. Chromium permeation was reported after application of potassium dichromate (K_2CrO_7), but not after application of chromium chloride ($CrCl_3$) or chromium nitrate ($Cr(NO_3)_3$). Chromium was detected in the epidermis and dermis after application of all three salts. However, statistically significantly higher concentrations of chromium were retained inside the skin when K_2CrO_7 was applied, indicating higher retention in the skin after exposure to Cr(VI). These results were confirmed by Van Lierde *et al.* (2006) in a similar study. Chromium levels in the skin and permeation thereof increased in a concentration dependent manner (Gammelgaard *et al.,* 1992; Van Lierde *et al.,* 2006). An increase in pH resulted in higher permeation through the skin, as well as retention in the skin after application of K_2CrO_7. The authors attributed their findings to a decrease in the barrier function of the skin caused by the alkaline test solution (Gammelgaard *et al.,* 1992). These studies concluded that Cr(VI) diffused through the skin and Cr(III) not, therefore the bioavailability of Cr(VI) differs significantly from that of Cr(III). The lower permeation of Cr(III) was attributed to a greater rejection of the positive Cr(III) ions by the skin barrier and a stronger affinity for the skin.

In addition Van Lierde *et al.* (2006) investigated the influence of the type of donor solution on permeation. An aqueous donor solution provided higher permeation of chromium than a synthetic sweat solution. These results were attributed to the partial reduction of Cr(VI) to Cr(III) in sweat, which impedes the permeation of chromium.

Fullerton *et al.* (1993) reported retention of chromium in human skin after *in vitro* application of chromium containing cement to the skin. After 48 hours of exposure no chromium could be detected in the receptor solution (< 0.3 ng/ml). However, chromium was retained in the epidermis and dermis. This study aimed to determine if ferrous sulphate added to cement as a reducer of water soluble chromate would influence the retention of chromium in the skin. Higher levels of chromium were retained in the epidermis and dermis after exposure to ordinary cement, and cement reduced with ferrous sulphate when compared with unexposed skin. However, differences were not significant. Even though the cement with added ferrous sulphate had a lower concentration of chromium (0.01 µg/ml), exposure resulted in higher levels of chromium being retained in the dermis when compared with ordinary cement. Ferrous sulphate reduces Cr(VI) to Cr(III), which could contribute to higher retention in the skin as Cr(III) has a stronger affinity for the skin. The study also reported significantly higher retention of chromium in the epidermis and dermis after four repeated applications of cement with added ferrous sulphate over 96 hours of exposure.

The *in vitro* permeation of chromium, as well as that of nickel and cobalt was also investigated by Larese *et al.* (2007). Chromium powder did not permeate the skin even though the cumulative

absorption of chromium was reported graphically as 0.0005 – 0.0009 µg/cm^2 over 24 hours. Flux and lag time could not be calculated due to the low permeation. Polarographic analysis for chromium ions in the donor solution could not detect any chromium ions when using chromium powder. The study concluded that metallic chromium was not oxidised in synthetic sweat with a pH of 6.5, therefore no permeation of chromium occurred under normal physiological conditions. In contrast the permeation of a chromium salt, $K_2Cr_2O_7$, was reported to be very high (Larese *et al.,* 2007).

In a follow-up study Larese Filon *et al.* (2008) investigated the permeation of chromium powder through skin at a pH of 4.5. Synthetic sweat at this lower pH oxidised chromium, since soluble chromium ions (Cr (III)) were present in the donor solution. Chromium permeated through the skin, and flux and lag time could be calculated. Therefore metallic chromium could permeate through the skin if there was oxidation of the chromium by more acidic sweat. This study also evaluated the effect of cleaning the skin with a common detergent. Permeation through the skin was higher if the skin was exposed for 24 hours as opposed to washing the skin after 30 minutes of exposure. In contrast, significantly higher ($p < 0.03$) concentrations of chromium was reported in the skin that was washed after 30 minutes of exposure than that of skin exposed for 24 hours. This suggests that chromium could already pass into the SC during the initial 30 minutes of exposure before cleaning occurred. In addition, the cleansing procedure potentially did not decontaminate the skin entirely, leaving a small amount of chromium on the skin, which could have been oxidised by the addition of synthetic sweat and resulted in further penetration into the skin. This study concluded that chromium presented a low risk of dermal absorption. However, sweat with an acidic pH of 4.5 can oxidise chromium into ions that can then permeate through the skin. The decontamination process was called into question by the authors as cleaning the skin after 30 minutes led to higher concentrations of chromium retained inside the skin (Larese Filon *et al.,* 2008).

Following these studies, Larese Filon *et al.* (2009a) investigated the absorption of metal powders through intact and mechanically damaged skin. The skin was damaged by scratching the skin surface with the tip of a 19-gauge needle, as suggested by Bronaugh and Steward (1985). Synthetic sweat with a pH of 4.5 was used to simulate actual skin surface pH values encountered in the industries (Larese *et al.,* 2007). There was no significant difference in the permeation of chromium between intact (0.005 µg/cm^2) and damaged (0.0046 µg/cm^2) skin, and the higher permeation through intact skin is noteworthy. This was explained in part by the different concentrations of chromium ions in the donor solution. Polarographic analysis reported 0.28 mg/l chromium ions in the donor solution of intact skin and 0.18 mg/l for damaged skin. The authors suggested that only a fraction of the chromium powder was oxidised by synthetic sweat into soluble ions. The percentage of chromium ions in the donor solution was lower than 0.04% for both intact and damaged skin, whereas the percentage of ions in the skin exceeded 99%. The study also reported a significantly higher ($p = 0.011$) chromium content in

damaged skin when compared with intact skin. It was suggested that the high chromium concentration in the skin resulted from chromium ion binding in the skin, which impeded the diffusion of chromium through the skin, which was confirmed by the high percentage of chromium ions retained in the skin. The authors suggested that chromium ions have a strong affinity for epithelial and dermal tissue with which they form complexes, which slows the rate of diffusion (Larese Filon *et al.,* 2009a).

Cobalt (Co)

Wahlberg (1965) reported *in vitro* permeation of cobalt chloride ($CoCl_2$) with higher permeability in the first five hours of exposure than over longer time intervals (up to 48h) investigated. The study utilised the disappearance method (isotope technique) to report the disappearance constant. This study was excluded from Table 2 due to the lack of methodological information and comparable results.

In a study by Larese Filon *et al.* (2004) it was shown that cobalt powder (< 2 µm) suspended in synthetic sweat permeated through human skin. The study compared the use of saline and synthetic sweat (pH 6.5) as solvent and found that cobalt permeation through skin was lower when using saline (0.008 – 0.050 µg/cm^2/h) than synthetic sweat (0.008 – 0.592 µg/cm^2/h). The use of synthetic sweat led to a progressive increase in cobalt permeation with an average flux of 0.0123 µg/cm^2/h. The metal ions in the donor solution were determined as 67 mg/l, and 39 µg/l in the receptor solution by means of polarographic analysis. They confirmed the oxidation of cobalt into soluble ions by synthetic sweat, which led to permeation through the skin. Skin samples were digested to determine cobalt content in the skin.

In a follow-up study Larese Filon *et al.* (2009a) investigated the permeation of cobalt powder through intact and mechanically damaged human skin. The skin was damaged by scratching the skin surface as described for chromium. Through the damaged skin, 3.57 µg/cm^2 cobalt permeated to the receptor solution, which was significantly higher ($p = 0.006$) than the 0.038 µg/cm^2 that permeated through intact skin. A significantly higher ($p = 0.014$) concentration of cobalt was retained in damaged skin (48.7 µg/cm^2 *vs* 29.6 µg/cm^2). This study concluded that mechanical skin injury enhanced the absorption of cobalt through skin and confirmed the oxidation of cobalt into soluble ions by synthetic sweat, which led to permeation through the skin.. This study also adjusted the pH of synthetic sweat to 4.5 and recommended the use of this pH to replicate conditions in the workplace as the actual pH of workers' skin is more acidic, especially during physical activity.

Larese Filon *et al.* (2013) expanded their investigation into the skin permeation of cobalt using cobalt nanoparticles. Synthetic sweat with a pH of 4.5 was used to dissolve the cobalt nanoparticles, which were applied to intact and damaged skin. Cobalt nanoparticles permeated through intact skin, but permeation through damaged skin was significantly higher ($p = 0.001$). The study demonstrated that

cobalt nanoparticles can permeate through skin and reach the epidermis and dermis. The higher surface to volume ratio of the nanoparticles can lead to an increase in the release of metal ions and subsequently higher dermal permeation. Results also indicated retention of cobalt nanoparticles in the skin, with significantly higher ($p < 0.02$) retention in damaged skin. The authors compared their results with two previous studies using cobalt powders, therefore the results were standardised to compensate for different concentrations used. The comparison indicated a tenfold increase in cobalt in the receptor solution of cells exposed to cobalt nanoparticles, and a threefold increase in cobalt nanoparticles in skin.

Copper (Cu)

Pirot *et al.* (1996a) reported permeation of copper through human skin after application of petrolatum or gel formulations containing copper chloride ($CuCl_2$) and copper sulphate ($CuSO_4$). After 72 hours of exposure the epidermis contained two to six times the concentration of copper found in the receptor solution. In a subsequent study the permeation of copper through human skin from five topical formulations containing $CuSO_4$ or copper pyrrolidone (CuPC) was investigated. Copper permeation was reported for all five formulations with no significant differences between them (Pirot *et al.,* 1996b).

In a review by Hostýnek and Maibach (2006) it was concluded that copper compounds and copper applied as a metal can penetrate the SC. If copper in the elemental state comes into contact with the skin, it is oxidised by fluids on the skin surface, resulting in ions capable of penetrating the SC.

Hostýnek and Maibach (2010; 2011) determined the *in vitro* permeation of copper applied as glycyl-$_L$-histidyl-$_L$-lysine cuprate diacetate [GHK-$Cu(Ac)_2$] to assess the potential for its transdermal delivery as an anti-inflammatory agent. The study included isolated SC, epidermis and split thickness skin from male and female cadavers. Results indicated permeation of copper and significant deposition of copper in all three compartments of the skin. The high permeation of copper through the SC was attributed to the absence of underlying skin structures, therefore permeation was not limited by retention in these layers. In addition, a significant mass of copper was retained in the isolated SC, where a 400-fold increase over the baseline was found. The copper accumulated in the skin, thereby forming a skin reservoir that could become systemically available over time. The authors concluded that topical administration of copper in the form of the tripeptide would be an effective topical anti-inflammatory treatment as copper was found to permeate the skin through various pathways. Although this study tested copper permeability for possible treatment, it was included in this review as the results indicated that copper permeated through human skin.

Gold (Au)

Larese Filon *et al.* (2011) reported dose-dependent permeation of gold nanoparticles through human skin. The authors investigated the *in vitro* permeation of gold nanoparticles through intact and mechanically damaged human skin by abrading the skin as described for chromium. They reported significantly higher gold concentrations in damaged skin. By using transmission electron microscopy (TEM) gold nanoparticles were observed in the SC, epidermis and the dermis, and by reaching the dermis these nanoparticles could become systemically available. In contrast to the retention in the skin, there was no significant difference in the permeation through the skin between intact and damaged skin. The authors suggested a strong interaction between nanoparticles and skin components, which delays permeation through skin layers, therefore increasing the retention in damaged skin. The study reported that gold nanoparticles cannot release ions in physiological conditions, therefore the permeation reported was only for nanoparticles and not for ions.

Lead (Pb)

Lead is easily absorbed into the skin, as was shown *in vivo* by Florence *et al.* (1988) and Lilley *et al.* (1988) where dermal exposure to lead resulted in increased levels of lead in sweat and saliva. Stauber *et al.* (1994) used three different analytical techniques to show that lead (as lead nitrate or lead acetate) penetrated through the skin and could be detected in sweat and urine within six hours of the skin application. Lead is initially absorbed rapidly through the sweat glands and hair follicles, and is absorbed slower through the transepidermal route (Stauber *et al.,* 1994). These studies concluded that lead absorption through the skin could contribute significantly to the total body burden, and since the dermal absorption of lead is barely detectable in blood where lead exposure is usually tested, dermal exposure could remain undetected (Lilley *et al.,* 1988; Stauber *et al.,* 1994).

Bress and Bidanset (1991) investigated the *in vitro* permeation of lead and reported permeation of tetrabutyl lead ($C_{16}H_{36}Pb$), lead nuolate, lead naphthanate and lead acetate ($Pb(CH_3CO_2)_2$) through human skin, whereas lead oxide (PbO) did not permeate. The results indicated that tetrabutyl lead was rapidly absorbed through the skin, with 6.3% of the applied dose recovered after 24 hours of exposure.

A subsequent study by Larese Filon *et al.* (2006) investigated the *in vitro* permeation of lead oxide (PbO) powder, as well as the influence of mechanical skin damage on permeation. This study reported that lead oxide can penetrate intact human skin, which is in contrast to results reported by Bress and Bidanset (1991). Larese Filon *et al.* (2006) reported a ninefold increase in penetration through damaged skin. However, there was no significant difference in the retention of lead in the skin between intact and damaged skin that was exposed for 24 hours. The study also investigated the influence of skin decontamination with two types of cleansers after 30 minutes of exposure using the same protocol as for

chromium. Decontamination did not reduce the permeation through the skin, suggesting rapid permeation of lead similar to that reported for chromium (Larese Filon *et al.,* 2008).

Mercury (Hg)

Wahlberg (1965) investigated the *in vitro* permeation of mercury chloride ($HgCl_2$) through human skin by using the disappearance method (isotope technique). Higher absorption of $HgCl_2$ occurred through breast skin than through abdominal skin. The comparison between fresh and stored skin (48 hours at 4 °C) indicated no difference in the absorption rates. In addition, the study reported that absorption measured by analysing the receptor solution was lower that when the disappearance method was used. The authors concluded that $HgCl_2$ reacted with and was stored in the skin and therefore permeation through the skin was gradual. Wester and Maibach (1999) also reported mercury accumulation in the skin and slow absorption into the body. Results indicated 28.5% of mercury in the skin, and 0.07% in the receptor solution. These studies are not included in Table 2 due to the lack of methodological information and comparable results provided.

Palmer *et al.* (2000) reported skin permeation of mercury through human skin after application of complexion lightening lotions containing $HgCl_2$. A glycerol containing formulation was compared with an aqueous formulation where the glycerol had been removed. The results indicated water as an enhancer of mercury permeation as the aqueous formulation indicated higher mercury concentrations in the receptor solution and in the skin. The study concluded that application of the mercury containing lotion resulted in significant and rapid dermal permeation of mercury.

Sartorelli *et al*. (2003) reported skin permeation of mercury after *in vitro* application of $HgCl_2$ to human skin. Significantly lower permeability coefficients were reported when higher concentrations of $HgCl_2$ were applied. In contrast, the percentage of $HgCl_2$ retained in the skin and the cumulative permeation were significantly higher in the cells exposed to the higher concentration of $HgCl_2$. The authors indicated that the solubility of mercury into the SC is a rate-limiting process potentially forming a reservoir in the SC. The concentration applied to the skin can therefore influence the permeability coefficient.

Moody *et al.* (2009) investigated *in vitro* dermal permeation of mercury as Hg-203 in soil, and without soil by using human breast skin. This was done to determine skin permeation after dermal exposure to metal contaminated soil, since people, especially children, may come into contact with soil due to recreational activities. For the purpose of this review only data without soil is reported, which indicated a high total absorption as a result of the high percentage of mercury retained inside the skin. In total 78.3% of the mercury applied to the skin permeated into and through the skin. After soap washing the skin following 24 hours of exposure, 76.9% of mercury was still retained in the skin. The authors

concluded that the skin depot has to be included in exposure estimates on account of the high percentage of mercury retained in the skin.

Nickel (Ni)

Various studies have concluded by means of patch testing that nickel is a sensitising agent causing allergic contact dermatitis in occupational settings, as well as in the general population (Marcussen, 1957; Massone *et al.,* 1991; Shah *et al.,* 1998; Akasya-Hillenbrand and Ozkaya-Bayazit, 2002). Shah *et al.* (1988) reported retail clerks, hairdressers, domestic cleaners, metalworkers and caterers as occupations with a high potential for occupational nickel allergy. Nørgaard (1955) reported permeation of nickel *in vivo* through the skin by using the disappearance method.

These initial studies led to more in-depth *in vitro* skin permeation studies. Fullerton *et al.* (1986) conducted an *in vitro* study on the permeation of nickel chloride ($NiCl_2$) and nickel sulphate ($NiSO_4$) through human breast and leg skin. Significantly higher permeation with skin occlusion was reported, whereafter the remaining studies were carried out under occlusive conditions. They indicated that nickel is capable of permeating through the skin, with an approximate lag time of 50 hours. $NiCl_2$ permeation was 50 times higher than that of $NiSO_4$ and the authors suggested that the higher permeability of $NiCl_2$ may be due to higher cutaneous bioavailability of the salt (Fullerton *et al.,* 1986). Hostýnek (2003a) and Hostýnek (2003b) also reported higher permeability coefficients for $NiCl_2$ when compared with other nickel compounds. In contrast, Tanojo *et al.* (2001) reported a higher percentage of permeation for $NiSO_4$ when compared with $NiCl_2$ when using the SC only. $NiCl_2$ reached the peak for the early stage surge within four hours, whereas $NiSO_4$ reached the peak after 12 hours. This indicated that the maximum permeation occurred in the early hours of the experiment, after which the flux became steady, reaching equilibrium. The difference in permeation was ascribed to differences in water solubility and ionisation properties of the salts.

Fullerton and Hoelgaard (1988) reported considerable nickel binding to the epidermis and a significant mass of nickel in the dermis. Therefore these layers could be local reservoirs where nickel accumulates after permeating through the SC. It also confirmed previous findings that nickel diffusion through the skin is delayed by epidermal binding (Fullerton *et al.,* 1986).

Hostýnek (2003b) reported higher permeation of Ni(II) salt ($NiCl_2$) through dermatomed skin when compared with a Ni(II) soap (NiDO), whereas retention of NiDO in the skin was significantly higher. Higher permeability of both nickel compounds through adolescent skin (14 years) than through elderly skin (67 years) was also found, although significance was not stated. In addition, the authors compared the permeability coefficient of $NiCl_2$ from previous studies, which differed by several orders of magnitude. Permeability through dermatomed skin is the highest (x 10^{-3} cm/h), whereafter full thickness

skin (x 10^{-5} cm/h), the epidermis (x 10^{-6} cm/h), and the lowest permeation through the SC (x 10^{-7} cm/h). This low permeation through the SC was attributed to the lack of appendages, and the holes or shunt routes left by the hair follicles and glands being shut upon hydration and swelling of the corneocytes. Therefore, the method using the SC gives an indication of diffusion across the transcellular or intercellular barrier (Tanojo *et al.,* 2001). In a later study by Larese *et al.* (2007) the permeability coefficient for nickel (from nickel powder) through full thickness skin was reported as 6.1 x 10^{-4} cm/h, which is one order of magnitude higher than the 4.6-5.5 x 10^{-5} cm/h of $NiCl_2$ reported by Hostýnek (2003b). The higher permeability coefficient probably resulted from the 37 times higher concentration of nickel applied to the skin by Larese *et al.* (2007).

Larese *et al.* (2007) conducted *in vitro* percutaneous absorption studies using full thickness skin and reported slow permeation of nickel (from nickel powder) through the skin with a flux of 0.0165 µg/cm^2/h. Polarographic analysis showed that Ni-ions were present in the donor solution at a concentration of 27.1 mg/l confirming the release of Ni-ions as a result of oxidation by synthetic sweat. A follow-up study by the same group investigated the influence of mechanically damaged skin on the percutaneous absorption of nickel powder by abrading the skin with a needle (Larese Filon *et al.,* 2009a). Nickel permeation was significantly higher ($p = 0.004$) through damaged skin than through intact skin. The nickel content retained in damaged skin was also significantly higher ($p = 0.023$).

Moody *et al.* (2009) investigated *in vitro* dermal permeation of nickel as Ni-63 in soil, and without soil by using human breast skin (refer to mercury for more detail) and found a high total absorption as result of a high percentage of nickel retained inside the skin (depot). Comparison of their results to Hostýnek (2003b) indicated a lower percentage of permeation (1.8% *vs* 35.7%) and a higher percentage of retention in the skin (20.9% *vs* 0.02%). The authors suggested the appendageal shunt route as a contributing factor to the difference in results. Human breast skin has lower hair follicle density, and has velus hair that have larger follicles and greater hair follicle density than hair on other anatomical sites.

Silver (Ag)

In vivo studies on the dermal absorption of silver have been published, specifically on silver contained in wound dressings. One such case study has reported absorption of 15 nm silver nanoparticles from a burn wound dressing that led to elevated levels of silver in serum and urine. The study concluded that silver can become systemically available after dermal contact (Trop *et al.,* 2006). Subsequent studies investigated the use of Acticoat™ dressings and the effect thereof on serum silver levels, confirming the permeation of silver through the skin. This silver then becomes systemically available (Vlachou *et al.,* 2007; Moiemen *et al.,* 2011). It is unclear whether silver ions or silver nanoparticles permeated through the skin. However, it is important to note that silver dressings were applied to skin damaged by burns,

and skin-graft areas following burns, therefore permeation would be expected to be higher than through intact skin.

An *in vitro* study by Larese Filon *et al.* (2009b) investigated the permeation of coated silver nanoparticles through intact and mechanically damaged skin by abrading the skin as described for chromium. Silver nanoparticles was 25 ± 7.1 nm in size with a maximum size of 48.8 nm and was dispersed in ethanol absolute and diluted with synthetic sweat at a pH of 4.5. Silver nanoparticles permeated through intact human skin, whereas penetration through damaged skin was five times higher. The authors concluded that silver nanoparticles had lower permeability than nickel and cobalt metal powders. In a recent study, Bianco *et al.* (2014) utilised silver nanoparticles to determine permeation through human skin graft samples. For the purpose of this review, only results obtained with fresh skin is reported. The study reported that 19 nm silver nanoparticles passed the skin barrier and reached the dermis where it was retained. Aggregation of silver nanoparticles occurred in the donor solution after a few hours as a result of the elevated chloride content and an acidic pH.

Titanium (Ti) and Zinc (Zn)

Microscopy studies have found that sunscreen components such as zinc oxide (ZnO) and titanium dioxide (TiO_2) are deposited on the outermost surface of the SC and do not permeate through the skin (Pflücker *et al.,* 2001; Schulz *et al.,* 2002; Mavon *et al.,* 2007; Zvyagin *et al.,* 2008). Two studies utilising scanning electron microscopy reported no permeation of 15-30 nm ZnO nanoparticles through the skin, with remaining nanoparticles on the skin surface or accumulation thereof in skin folds or hair follicles (Roberts *et al.,* 2008; Zvyagin *et al.,* 2008).

Several studies using *in vitro* methods reported ZnO and TiO_2 permeation through human skin to be low or undetectable, with accumulation of the metal particles on the skin surface. The majority of the applied metal particles remained in the upper layers of the SC with particles found in the epidermal compartment located mainly in the furrows or the opened follicle infundibulum (SCCNFP, 2000; SCCNFP, 2003; Cross *et al.,* 2007; Mavon *et al.,* 2007; Durand *et al.,* 2009). Both titanium and zinc were mainly tested as nanoparticles from sunscreen formulations and the authors suggested that the particles formed micron sized aggregates, which reduced permeation (Van der Merwe *et al.,* 2009). The sunscreen formulations containing nanoparticles of zinc and titanium were formulated to stay on the skin in order to protect against solar ultraviolet radiation. In contrast Pirot *et al.* (1996b) reported that ZnO from emulsions or ointments was permeable through human skin with a maximum percentage permeation of 0.34%. The majority of zinc ions were found in the dermis and less in the epidermis. The study concluded that ZnO could become systemically available (Pirot *et al.,* 1996b).

Other metals

Baroli *et al.* (2007) report that iron-based nanoparticles smaller than 10 nm passively penetrated the skin *in vitro* by using diffusion cells and microscopy. The study indicated iron nanoparticles penetration through the SC lipid matrix and the hair follicle orifices reaching the deepest layers of the SC. In exceptional cases iron nanoparticles were also detected in the viable epidermis.

Franken *et al.* (2014) reported low permeation of platinum (Pt) and rhodium (Rh) through human skin (2.3×10^{-4} and 1.0×10^{-4}% permeated respectively), with significant concentrations of the metals retained inside the skin. Permeation of platinum through the skin and retention in the skin was significantly higher when compared with rhodium. The authors concluded that smaller agglomerates of platinum, as observed by TEM, contributed to higher permeation of platinum.

Recommendations for future in vitro skin permeation studies

Standardisation of the methodological aspects such as concentration applied, pH of donor solution and thickness of skin used, as well as the format of data reporting, would enable comparison between results of different studies. The following aspects are recommended for future metal *in vitro* permeation studies utilising human skin:

(i) Use of human skin, as animal skin is generally more permeable than human skin, thereby overestimating the permeation of substances (OECD, 2004). Furthermore, human skin is the 'gold' standard and the best predictor for skin permeation of metals in real life conditions (Sartorelli *et al.,* 2000). (ii) Use of abdominal skin as it generally has a larger surface area than skin obtained from other regions (ex. breast). By using skin only from the abdominal area, variability as a result of differences in skin thickness or follicle density is minimised (Sartorelli *et al.,* 2000). (iii) Use of full thickness skin to simulate actual conditions in the workplace when considering occupational exposure. Thereby disturbance of the barrier function by cutting or separation methods is limited. However, excessive thickness (> 1 mm) should be avoided, and the thickness of skin used should be reported (Sartorelli *et al.,* 2000; OECD, 2004). (iv) Report of the origin of the skin including the age and gender of the donor and the anatomic site the skin was obtained from, as well as methods of skin handling. The potential differences in skin permeability between genders could be eliminated by using skin from only one gender. (v) Inclusion of skin from a number of different donors in experiments to compensate for inter-donor variability that is inherent in biological membranes, such as skin. Skin permeability could be influenced by varying skin properties of different donors, such as skin thickness (Sartorelli *et al.,* 2000). (vi) Of interest would be inclusion of skin from other races, based on suggested anatomical differences between skin of different races. To date only Caucasian skin has been used to determine *in vitro* permeation of metals through human skin. (vii) The use of fresh skin is not always practicable, therefore

skin may be stored at -20 °C for up to four months without loss of barrier function, provided that skin integrity is tested before the skin is used. However, the length of time and the temperature at which it was stored should be reported (Sartorelli *et al.,* 2000; OECD, 2004). (viii) Inclusion of skin integrity testing by measuring electrical resistance before and after experiments. Measurement before commencement of experiments allows for exclusion of damaged skin prior to the experiment, and measurement after conclusion of the experiment ensures no damage or experimental fault occurred (OECD, 2004). If the effect of skin damage on permeation is investigated, skin integrity should be measured after the skin has been damaged to obtain an indication of the extent of damage induced. (ix) To represent the human body it is recommended to use a physiological solution as receptor solution with a salt concentration similar to that found in blood with a pH of 7.35 (Larese Filon *et al.,* 2013). (x) For studies considering occupational exposure, it is recommended to use synthetic sweat as donor solution with a pH between 4.5 and 6.5 as suggested by Larese *et al.* (2007) and Larese Filon *et al.* (2009a), as it is a realistic surrogate of what workers may be exposed to in workplace conditions (Sartorelli *et al.,* 2000). In addition, the physicochemical properties, such as solubility of the metal at a specific pH should be considered. (xi) It is crucial that the composition of the donor solution (synthetic sweat) be standardised since certain components may promote or inhibit oxidisation of metals or influence the reduction of metal ions between valence states. (xii) The metal should be applied as an infinite dose able to reach steady state conditions. (xiii) The receptor solution should be maintained at a constant temperature of 37 °C, to simulate the normal temperature underneath the skin (Franz, 1975). (xiv) Exposure for 24 hours is suggested to allow for steady state conditions and enable flux calculations with five to six interval removals included in order to provide a permeation profile (OECD, 2004). (xv) Inclusion of tape stripping before digestion of the skin to establish the localisation or distribution of the metal within the skin. To remove the SC 15 to 25 strips should be used (OECD, 2004). (xvi) Total recovery of the metal should be calculated from the mass in the receptor solution, mass in the skin, and mass in the solution removed and washed from the skin to ensure proper experimental setup (Sartorelli *et al.,* 2000). (xvii) Results should be reported as flux or permeability coefficient, lag time, total cumulative amount diffused after 24 hours or percentage diffused and the total mass retained in the skin layers or percentage retained.

Conclusion

The *in vitro* methods reviewed are important to predict the risk of dermal exposure and subsequent permeation of metals, thereby enabling agencies and regulatory bodies to set safety standards, since there are currently no regulatory limits for dermal exposure. This review has highlighted large variations in permeability data reported by different studies, resulting from variations in experimental design. Metal concentrations applied to the skin ranged between micrograms and grams, and skin used ranged from dermatomed, SC to full thickness skin. These discrepancies hindered the comparison of results

between different studies, and therefore emphasised the importance of standardising the experimental design to enable comparison between future studies and different studies investigating the same metals.

After the review of the publications reporting on *in vitro* permeation of metals we can conclude that all metals listed in this review, except for titanium and zinc nanoparticles, permeate through human skin. These *in vitro* studies spanning almost 50 years indicate large variation in the permeation results of metals through the skin. For example, the permeability coefficient reported for nickel ranged from 5.2×10^{-7} to 0.006 cm/h. However, differences in methodologies could account for the variation. Flux ranged between 0.05 ng/cm^2/h for rhodium and 38 µg/cm^2/h for copper and permeability coefficients ranged between 5.2×10^{-7} cm/h for nickel and 14.42 cm/h for mercury. Furthermore, these studies reported significant retention of the metals inside the skin, possibly forming reservoirs where internal exposure may continue for extended periods of time, even after external exposure has ended. The highest metal retention was copper with 290 µg/cm^2 found in the SC, whereas the highest concentration in the epidermis was reported for chromium (134 µg/cm^2). Without distinguishing between different layers of the skin, the highest concentration of metal retained in full thickness skin was reported for nickel (82.3 µg/cm^2).

It is evident that permeation of metals is most likely influenced by the pH of the donor solution, and the presence of counter ions such as chloride could influence permeability of metals. Binding of metals to skin components decreases permeability and the valence state of the metal will influence its permeability. In addition, damaged skin is more permeable than intact skin. Recommendations on experimental design and format of data reporting will enable comparison of results from future *in vitro* metal permeation studies.

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Chapter 4: Article II

Franken, A., Eloff, F.C., Du Plessis, J., Badenhorst, C.J., Jordaan, A., Du Plessis, J.L. *In vitro* permeation of platinum and rhodium through Caucasian skin. 2014. Toxicology *in Vitro,* 28:1396-1401.

4.1 Background

The Franz diffusion method has been successfully used to evaluate the permeation of metals such as nickel, cobalt and chromium, to name only a few, through human skin. However, these studies have not included the platinum group metals (PGMs). The mining and refining of PGMs contributes significantly to the economy of South Africa and job creation. Workers refining PGMs or using it during manufacturing of products are potentially exposed to these metals in various forms, including the salt form. The risks involved with respiratory exposure are well known, and therefore this route of exposure is monitored and controlled. In addition, workers are potentially exposed through the dermal route as a result of skin contact to the hands and arms, or secondary contamination to the neck or face. However, this route is less recognised and is rarely monitored or controlled. Platinum is a known sensitiser causing respiratory sensitisation; but rhodium sensitisation is not confirmed. It is unclear if these metals could permeate through the skin, and thereby possibly contributing to worker sensitisation. This article investigated the permeation of platinum and rhodium through human skin and was published in *Toxicology in Vitro*, which is the official journal of the European Society of Toxicology *in Vitro.*

4.2 Instructions to authors (excerpt)

Refer to Chapter 3 for the instructions to authors for the journal Toxicology *in Vitro*.

4.3 *In vitro* permeation of platinum and rhodium through Caucasian skin.

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In vitro permeation of platinum and rhodium through Caucasian skin

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ABSTRACT

During platinum group metals (PGMs) refining the possibility exists for dermal exposure to PGM salts. The dermal route has been questioned as an alternative route of exposure that could contribute to employee sensitisation, even though literature has been focused on respiratory exposure. This study aimed to investigate the *in vitro* permeation of platinum and rhodium through intact Caucasian skin. A donor solution of 0.3 mg/ml of metal, K_2PtCl_4 and $RhCl_3$ respectively, was applied to the vertical Franz diffusion cells with full thickness abdominal skin. The receptor solution was removed at various intervals during the 24 h experiment, and analysed with high resolution ICP-MS. Skin was digested and analysed by ICP-OES. Results indicated cumulative permeation with prolonged exposure, with a significantly higher mass of platinum permeating after 24 h when compared to rhodium. The mass of platinum retained inside the skin and the flux of platinum across the skin was significantly higher than that of rhodium. Permeated and skin retained platinum and rhodium may therefore contribute to sensitisation and indicates a health risk associated with dermal exposure in the workplace.

1. Introduction

In South Africa the platinum group metals (PGMs) mining and refining industry is one of the largest components in the mining sector (Chamber of Mines, 2012). The mining of PGMs include the precious metals platinum (Pt), palladium (Pd), rhodium (Rh), ruthenium (Ru), iridium (Ir) and osmium (Os) (Ravindra et al., 2004). These precious metals have excellent catalytic properties and are resistant to corrosion, resulting in these metals being valuable in various industries, increasing the demand for these metals (Iavicoli et al., 2012). The mining of PGMs and the subsequent smelting, refining or recycling processes results in workers being potentially exposed to these metals, in various forms, on a daily basis. Refinery workers are often simultaneously exposed to the salts of platinum, rhodium and palladium (Kielhorn et al., 2002). Secondary industries such as catalyst manufacturers, electronic industries and jewellery fabrication utilise these precious metals leading to potential occupational exposure in industries other than refineries (Kielhorn et al., 2002; Cristaudo et al., 2005). One of the hazards during metal refining and handling of metals is worker dermal exposure, as verified by Du Plessis et al. (2010, 2013) indicating dermal exposure to nickel and/or cobalt in base metal refineries. No published information is available on the extent of worker dermal exposure to PGM salts, and no *in vitro* information is available on the possibility of skin permeation.

The potent allergenicity of halide platinum salts is well documented, however the allergenicity of rhodium salts is less known (Cleare et al., 1976; Calverley et al., 1995). Platinum salts are well known respiratory sensitisers, although the skin sensitisation potential thereof is less documented. The National Institute for Occupational Safety and Health (NIOSH) pocket guide to chemical hazards identifies the skin as an exposure route and a target organ to soluble platinum as salts, with symptoms including dermatitis and sensitisation of the skin (NIOSH, 2013). Platinum salt sensitivity occurs when haptens combine with serum proteins to form antigens, and is known as a type 1, immunoglobulin IgE-mediated response. Symptoms of platinum salt sensitisation include respiratory symptoms such as tightness in the chest and wheezing, and also dermal symptoms such as eczema and urticaria (Kiilunen and Aitio, 2007). Soluble rhodium salts are considered to be sensitisers, with studies reporting allergic reactions including respiratory symptoms, urticaria and contact dermatitis in occupationally exposed subjects (Santucci et al., 2000; Goossens et al., 2011). NIOSH (2013) acknowledges the skin as a possible exposure route for soluble rhodium; however no possible skin symptoms are

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listed. In the past rhodium was believed to be allergologically safe, and was used to plate other metals to avoid dermatitis in persons sensitised to nickel and cobalt. However two case studies reported dermatitis of workers using rhodium solutions in the jewellery industry (Bedello et al., 1987; De La Cuadra and Grau-Massanées, 1991). De La Cuadra and Grau-Massanées (1991) concluded that rhodium as a salt may be a potential sensitiser, but not as a metal. Studies have concluded that the compounds that are capable of eliciting reactions are a small group of charged compounds containing a reactive ligand system, such as a chloride or bromide. Therefore it is the charge and overall reactivity of a compound that is directly related to its allergenicity (Cleare et al., 1976). During the refining process as well as in secondary industries these metals are found in numerous chemical forms including the most commonly found halide complexes (salts) (Bolm-Audorff et al., 1992; Linnett and Hughes, 1999; Cristaudo et al., 2005).

Previous studies acknowledge platinum as a sensitiser, manifesting in respiratory and dermal symptoms although these studies focused on respiratory exposure (Calverley et al., 1995; Linnett and Hughes, 1999; Merget et al., 2000). The literature suggests sensitisation to occur primarily through respiratory exposure, not taking the dermal route of exposure into consideration. Maynard et al. (1997) however suggested that dermal exposure to these metals could be an alternative route contributing to sensitisation. Their results indicated that respiratory exposure to platinum were below the eight hour and short term occupational exposure limits (OEL), yet exposure resulted in sensitisation to soluble platinum in refinery workers.

Numerous *in vitro* studies have proven that metals are able to permeate through intact human skin, and permeation increases in damaged skin (Larese Filon et al., 2007, 2009). These studies included nickel, cobalt and chromium; however no *in vitro* studies are available for PGMs. This study aimed to establish if a platinum- and rhodium salt are able to permeate through intact Caucasian skin by making use of an *in vitro* diffusion system.

2. Materials and methods

2.1. Chemicals

All chemicals were 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 disodium hydrogen phosphate were purchased from Sigma Aldrich, South Africa. Nitric acid (65%) and hydrochloric acid (33%) were purchased from De Bruyn Spectroscopic Solutions, South Africa. Hydrogen peroxide (50%) and acetone were purchased from Associated Chemical Enterprises, South Africa. Potassium tetrachloroplatinate and rhodium trichloride were obtained from Johnson Matthey and sponsored by Anglo American Technical Solutions, South Africa. Type 1 water used for the solutions was produced with a Millipore purification pack system (milliQ water).

A 0.9% sodium chloride solution was used to test the integrity of the skin. The physiological solution (receptor solution) was prepared by dissolving 4.76 g Na_2HPO_4, 0.38 g KH_2PO_4 and 18 g NaCl into 2 L of milliQ water, the final pH was adjusted to 7.35. The synthetic sweat solution used as the donor solution was prepared with 0.5% NaCl, 0.1% lactic acid and 0.1% urea in milliQ water. For the platinum donor solution 0.03233 g potassium tetrachloroplatinate (K_2PtCl_4) was dissolved in 50 ml synthetic sweat where after the pH was adjusted to 6.5 with ammonia. For the rhodium donor solution 0.04306 g of rhodium (III) chloride ($RhCl_3$) was dissolved in 50 ml synthetic sweat and the pH was adjusted to 6.5. A final concentration of 0.3 mg/ml of each metal was used as the donor solution.

2.2. Preparation of skin membranes

Human abdominal full thickness skin was obtained after surgical abdominoplasty procedures with the approval of the North-West University Ethics Committee (NWU-00114-11-A5). Informed consent was given by the donors. The skin was stored in a freezer at −18 °C for a maximum period of four months. A study done by Harrison et al. (1984) reported that freezing the skin did not affect the absorption of water by comparing frozen and fresh skin segments. On the day of the experiment, the skin was allowed to thaw, and all subcutaneous fat was removed. The skin was cut into circles with a diameter of 2.4 cm and clamped between the two parts of the Franz cell. Skin integrity was tested before and after each experiment using a Tinsley LCR Data bridge (Model 6401). The measurement was taken at 1 kHz in the parallel equivalent circuit using two stainless steel electrodes (Fasano and Hinderliter, 2004). Cells with an electrical resistance below 10 kΩ were rejected, and cells with similar resistance values within one skin donor were chosen for diffusion studies. Skin was obtained from Caucasian women between the ages of 37 and 52. In total skin from four different donors were used, skin from two donors were used to establish platinum permeation, and skin from two donors were used to establish rhodium permeation.

2.3. In vitro diffusion system

Vertical Franz diffusion cells with a receptor compartment of 2 ml were used to perform the percutaneous permeation studies (Franz, 1975). The receptor compartment was maintained at a temperature of 37 °C to simulate the normal temperature below the skin. The physiological solution used as receptor solution was continuously stirred using a magnetic stirrer bar. Each piece of skin was clamped between the two compartments of the Franz cell with a stainless steel clamp. The mean exposed skin area was 1.066 cm^2, and the thickness of the skin was measured as less than 1 mm.

2.4. Platinum permeation

The receptor compartment was filled with 2 ml of physiological (receptor) solution, and the donor compartment was filled with 1 ml of the K_2PtCl_4 dissolved in synthetic sweat. Ten cells were used as experimental cells, and 10 cells as blanks. The receptor solution was removed at 6, 8, 10, 12, 14 and 24 h and placed into clearly marked vials. During each removal the receptor compartment was rinsed with an additional 2 ml of physiological solution, and this rinsing solution was added to the original sample removed. The receptor compartment was rinsed to ensure that all the platinum in this compartment was removed at the specific time interval. After completion of the experiment the donor solution was removed and placed into a vial, and the compartment was rinsed four times with 1 ml of synthetic sweat each time to remove all remaining platinum from the skin. The donor rinsing solution was added to the original donor solution for analysis. After testing the skin integrity the cell was dismantled and the skin was placed into a vial for analysis.

2.5. Rhodium permeation

The above mentioned experiment was repeated by dissolving $RhCl_3$ in synthetic sweat as donor solution.

2.6. Blanks

The blank cells were treated the same as the other cells with the exception that no platinum or rhodium was added to the synthetic sweat placed in the donor compartment.

2.7. Skin content evaluation

The skin pieces were removed from the vial and weighed for skin digestion purposes. The vials were rinsed with acetone to remove any residue from the vial. The rinsed solution was placed into a beaker with the skin where the acetone was evaporated using a hot plate. Nitric acid and hydrogen peroxide were added to the skin mixture in separate stages and evaporated to destroy the organic material. Hydrochloric acid was added to the beaker to ensure all the platinum was in a soluble state. The final solution was diluted to 10 ml with hydrochloric acid, which was analysed by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP–OES) (Spectro Arcos) to establish the concentration of the metals remaining in the skin.

2.8. Standardisation experiments

Various standardisation experiments were conducted to establish the appropriate concentration for the donor solution; these experiments included receptor solution removals at one, two and four hours. Analyses of these results predominantly indicated values close to or below the detection limit of analysis. These experiments also indicated that exposure up to six hours did not contribute to the steady state flux through the skin, and therefore removals at these time intervals were excluded from subsequent experiments.

2.9. Analytical measurements

The receptor solutions were analysed with high resolution Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) (Thermo Scientific Element XR) to establish the concentration of platinum and rhodium in the solutions. An internal standard of 10 μg/L rhenium was added to all the solutions to compensate for volume inaccuracies. For platinum the analysis was done using the Pt195 isotope in low resolution mode, whereas the Rh103 isotope was used in high resolution mode for rhodium. The instrument was calibrated with Pt or Rh calibration standards that were matrix matched with a range of 0, 5, 10, 20, 50, and 100 ng/L solutions containing platinum, rhodium and the internal standard rhenium to provide the initial calibration curves. If necessary, the calibration was extended to 1, 2, 5, 10, 20 or even 50 μg/L if the sample concentrations required it. The lower limit of detection for platinum was 30 ng/L and 20 ng/L for rhodium.

The concentration of platinum and rhodium in the donor solutions and in the solutions resulting from the skin digestion were analysed by ICP-OES (Spectro Arcos). An internal standard of 4 mg/L yttrium was added to the solutions to compensate for volume inaccuracies. The wavelengths used for determination was 265.945 nm and 214.423 nm for platinum, and 343.489 nm for rhodium. The instrument was calibrated with a standard solution of 0, 1, 2, 3, 4, 5, 10 and 20 mg/L to provide the calibration curves.

2.10. Transmission electron microscopy (TEM)

Solutions of K_2PtCl_4 and $RhCl_3$ dissolved in synthetic sweat (donor solutions) were characterised with a FEI Tecnai G^2 high resolution transmission electron microscope operated at 200 kV. Micrographs were captured and particles measured using Digital-Micrograph™ (Gatan Inc.) computer software.

2.11. Data analysis

The concentration of platinum and rhodium (ng/cm^3) in the receptor solution was converted to the total mass that permeated through the exposed skin area (ng/cm^2) and then plotted against time as the cumulative mass of metal that permeated per area of skin.

Flux permeation was calculated from the steady-state region of the graph after curve-fitting. Eq. (1) was developed by Díez-Sales et al. (1991) to describe the mass of substance crossing a membrane at a given time:

$$Q(t) = AKhC_v\left[D\frac{t}{h} - \frac{1}{6} - \frac{2}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}\exp\left(\frac{-Dn^2\pi^2t}{h^2}\right)\right] \quad (1)$$

where $Q(t)$ is the mass that passed through the membrane at a given time t, A is the actual surface diffusion area, K is the partition coefficient between the skin and donor solution, h is the membrane thickness, C_v is the concentration in the donor solution and D is the diffusion coefficient of the permeant in the skin. As t approaches infinity the exponential term becomes negligible and the equation can be simplified to Eq. (2):

$$Q(t) = AKhC_v\left[D\frac{t}{h} - \frac{1}{6}\right] \quad (2)$$

Since K, D and h are unknown, the products Kh and D/h^2 were replaced with α and β which were calculated by fitting Eq. (2) to the data by using Easyplot Version 4.0.5 (Spiral Software, Aerious Ltd.). The permeability coefficient (k_p) and flux (J) were calculated using Eqs. (3) and (4) respectively:

$$k_p = \frac{KD}{h}(=\alpha\beta) \quad (3)$$

$$J = k_pC_v \quad (4)$$

Lag time was calculated as the intercept of the steady-state region with the x-axis. Average blank concentrations were subtracted from experimental data.

Data analysis was performed using Statistica Version 11 (Statsoft Inc.). Data was not normally distributed, and therefore Box–Cox transformed (Box and Cox, 1964). Data is reported as mean ± standard error of means (SEM). The difference between independent Box–Cox transformed data was assessed by means of the t-test with separate variance. A p value of $\leqslant 0.05$ was considered as statistically significant.

3. Results

Platinum and rhodium were able to permeate through intact Caucasian skin, and permeation increased cumulatively over time. After 8 h of skin exposure, permeation of platinum into the receptor solution was 0.53 ± 0.18 ng/cm^2 and rhodium permeation was 0.2 ± 0.05 ng/cm^2; whereas after 12 h of skin exposure permeation was 0.97 ± 0.3 ng/cm^2 and 0.47 ± 0.1 ng/cm^2 respectively. After 24 h the permeation increased more than two fold to 2.57 ± 0.57 ng/cm^2 and 1.11 ± 0.29 ng/cm^2 respectively. The flux of platinum across the skin of 0.12 ± 0.02 ng/cm^2/h was significantly higher ($p = 0.015$) than the flux of rhodium of 0.05 ± 0.01 ng/cm^2/h. After 24 h of skin exposure to these metals, 1459.47 ± 99.42 ng/cm^2 of platinum was retained inside the skin, which was significantly higher ($p < 0.001$) than that of rhodium (757.04 ± 70.43 ng/cm^2).

TEM investigation estimated the size of platinum and rhodium particles to be similar with an average diameter of 9.7 ± 0.7 nm ($n = 8$) and 9.6 ± 0.9 nm ($n = 6$) respectively, considering the difficulty in identifying one particle as multiple particles agglomerated together. The diameter of the agglomerates were calculated with an average diameter of 42.9 ± 7.6 nm for platinum ($n = 12$) and 52.5 ± 8.3 nm ($n = 9$) for rhodium. However when observing the agglomerates the platinum agglomerates with a diameter of

30–40 nm were more abundant, whereas the rhodium agglomerates with a diameter of >50 nm were more abundant.

4. Discussion

In this study the skin permeation of a platinum and rhodium salts using intact full thickness human skin was investigated. The results indicated that both platinum and rhodium were able to permeate through intact Caucasian skin and a cumulative increase in permeation over time. Results indicated that the cumulative mass of platinum that permeated through the skin after eight, 12 and 24 h was consistently higher when compared to rhodium permeation. In occupational settings usual work shifts last for eight hours, however some shift work requires workers to work for 12 h. Results indicate that the extended working hours from eight to 12 h will increase permeation by 83% for platinum and 135% for rhodium with continued skin contamination after the additional four hours of exposure. In the event of poor personal hygiene, where skin contamination is not carefully removed by the workers, the permeation of these metals may continue even after working hours, which is considered as the 'take-home' effect. Results indicated an increase in skin permeation of 385% and 455% for platinum and rhodium exposure respectively between eight and 24 h of exposure (See Fig. 1). If the skin is not decontaminated (washed) these metals remain on the skin, and will be able to permeate until it is properly removed. As a worst-case scenario workers may return to work with already contaminated skin and further skin contamination may occur, increasing dermal exposure, and skin permeation of the metal salts. These results emphasise the importance of good personal hygiene after the work shift, as well as providing appropriate facilities for decontamination in the workplace.

The percentage of metal that permeated through the skin after 24 h of exposure was calculated taking the applied concentration into account. These results indicated low overall permeation with platinum permeation of 2.3×10^{-4}% and rhodium permeation of 1×10^{-4}% when compared to the initial concentration of 0.3 mg/ml applied to the skin. Although overall permeation was low, it is important to note that permeation through the skin occurred, and that these metals were also retained inside the skin. The mass of metals retained in the skin was calculated using the full thickness skin, without distinguishing between the epidermis and stratum corneum. After 24 h the mass of platinum retained inside the skin was almost double the mass of rhodium, expressed in percentage of 2.24% and 1.16% for platinum and rhodium respectively. There is a 10,000 fold increased percentage retained inside the skin than the percentage that permeated through the skin (See Table 1). These metals are retained inside the skin, and may continue to act as haptens and form antigens in the skin, contributing to dermal effects even after skin contamination was removed. Skin was obtained from four Caucasian donors, and the authors acknowledge the possibility of variability between donors as a limitation of the study and results should be interpreted accordingly.

Flux describes the rate of diffusion of the metal through the skin over time (McDougal and Boeniger, 2002). Flux provides information that is useful for risk assessment purposes since it gives an indication of the mass that will permeate through the skin per hour of exposure. Flux can therefore be compared for different substances. With the *in vitro* method, an infinite dose was applied to the skin to simulate the highest flux possible if the skin was loaded with high concentrations of the substance, therefore representing the worst-case permeation when dermal exposure is in excess (McDougal and Boeniger, 2002). From these results it is evident that platinum flux across the skin is more than double the flux of rhodium, indicating faster permeation of platinum through the skin.

The molecular size of substances may influence permeation through the skin as absorption is inversely related to molecular weight, however this influence is negligible for molecules with a molecular weight of less than 500 g/mol (Dalton) (Barry, 2007). Absorption of substances through intact human skin declines rapidly for molecules with a molecular weight over 500 g/mol (Dalton) (Bos and Meinardi, 2000). Taking the molecular weight of potassium tetrachloroplatinate (415.09 g/mol) and rhodium (III) chloride (209.26 g/mol) into account, the size of the entire molecule is not likely to be a contributing factor to the rate of permeation. However during TEM analysis smaller agglomerates of platinum were more frequently observed when compared to the agglomerates of rhodium, suggesting that the smaller agglomerates of platinum contributed to the faster permeation reported for Pt (See Fig. 2). The follicular route may provide a significant permeation pathway for these metals, as suggested by Knorr et al. (2009).

In contrast to molecular size, the charge of a molecule will influence permeation through the skin. Skin permeation of charged molecules is normally very poor because of their high polarity (Hadgraft et al., 1989). With substances that are ionisable, the quantity of charged and uncharged species is dependent on pH, and the transport of these charged species through the skin is less rapid than uncharged species (Smith, 1990). If these metal salts dissociate completely, K_2PtCl_4 would lead to Pt^{2+}, whereas $RhCl_3$ would result in Rh^{3+}. The difference in charge could be a contributing factor to the faster permeation of platinum through the skin. However dissociation of these metals in synthetic sweat at a pH of 6.5 is unknown and during TEM analysis agglomerates with suspected chloride attachments as well as agglomerates without the salts were observed. Further research is suggested to gain insight into the dissociation of these metal salts in sweat.

The dermal route was previously suggested as an alternative route leading to sensitisation in refinery workers where skin contamination occurred, however both eight hour and short term respiratory exposure levels measured were below the OEL (Maynard et al., 1997). The *in vitro* skin permeation results confirmed that these metal salts were able to permeate through the skin and thereby could contribute to sensitisation. Literature on platinum and rhodium sensitisation in the workplace list symptoms associated with the skin, such as dermatitis, eczema, urticaria and contact dermatitis; however these studies report these symptoms as a consequence of respiratory sensitisation (Bedello et al., 1987; De La Cuadra and Grau-Massanées, 1991; Calverley et al., 1995; Merget et al., 2000; Cristaudo et al., 2005). The probability of dermal exposure contributing to these symptoms was not observed. Considering results revealing that platinum and rhodium are able to permeate through the skin, and notable percentages thereof were retained inside the skin, the probability exists that

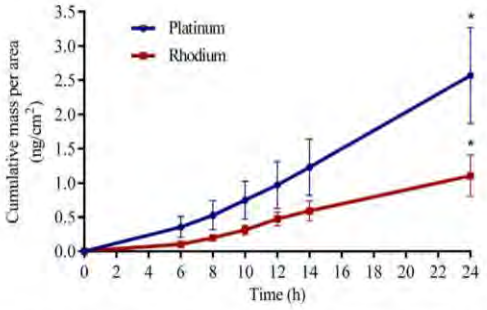

Fig. 1. Cumulative mass of platinum ($n = 15$, Human skin from 2 donors) and rhodium ($n = 11$, Human skin from 2 donors) permeated per area (mean and SEM).

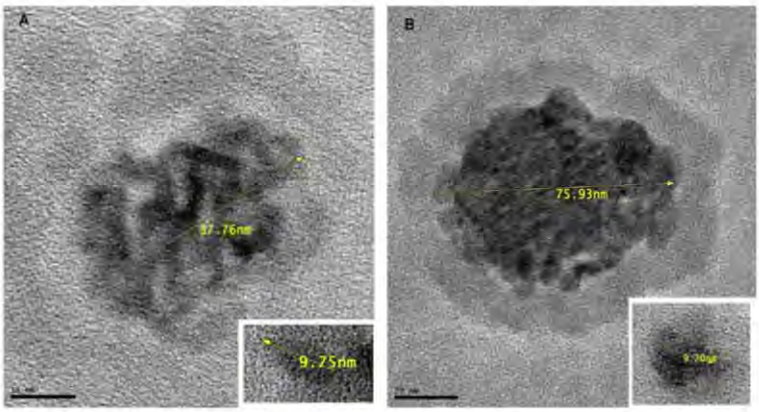

Fig. 2. Platinum agglomerate (A) bar 10 nm; and rhodium agglomerate (B) bar 20 nm visualised by TEM in the donor solution. Estimated individual particles as inserts.

Table 1
Platinum and rhodium concentrations in the receptor solution, retained inside the skin and permeation (mean ± SEM).

	Receptor solution		Skin		Permeation	
	Cumulative mass permeated in 24 h (ng/cm^2)	Percentage diffused after 24 h of exposure (%)	Mass retained inside the skin (ng/cm^2)	Percentage in the skin after 24 h (%)	Flux (ng/cm^2)	Lag time (h)
Pt	2.57 ± 0.57	$2.3 \times 10^{-4} \pm 0.5 \times 10^{-4}$	1459.47 ± 99.42	2.24 ± 0.15	0.12 ± 0.02	3.53 ± 0.40
Rh	1.11 ± 0.29	$1.0 \times 10^{-4} \pm 0.3 \times 10^{-4}$	757.04 ± 70.43	1.16 ± 0.11	0.05 ± 0.01	4.41 ± 0.48
p	**0.016***	**0.016**	**<0.001**	**<0.001**	**0.015**	0.147

Values in bold indicate statistical significant differences.

dermal exposure could have contributed to the dermal symptoms reported. Therefore, a combination of respiratory and dermal exposure could contribute to workers' sensitisation and subsequent symptoms.

Lag time represents the time it takes for the permeation to reach the steady state (McDougal and Boeniger, 2002). There was no significant difference between the lag time of platinum and rhodium, where an average exposure of 4.4 h is needed before rhodium permeation will reach the steady state compared to 3.5 h for platinum. The lag times were consistent with the flux results indicating platinum permeation to be faster than permeation of rhodium. However Kierstan et al. (2001) reported that lag time as an indicator of permeation should be used with caution as it is unreliable. Lag time is often calculated by using the membrane thickness (h) and the diffusion coefficient (D), or the intercept of the steady state curve with the x-axis. When using the steady state plot to calculate lag time, a large number of data points are needed to obtain reliable results (Guy and Hadgraft, 1991; Kao, 1991). Membrane thickness may vary significantly between skin samples, and this intrinsic variability in biological membranes leads to difficulty in obtaining reproducible values of lag time (Kierstan et al., 2001). The authors acknowledge the intrinsic variation in these skin samples even though all full thickness skin samples used in these experiments were measured using a Vernier caliper, ensuring a thickness of <1 mm (see Table 1).

For a substance to penetrate through the skin, it has to penetrate the outer barrier, the stratum corneum which is lipophilic. Once the substance is able to penetrate this barrier, the viable epidermis and dermis is more aqueous and the substance should be able to partition into this phase (Hadgraft and Wolff, 1993). The partition coefficient is the measure that describes the substance's solubility in the different phases of the skin and its surroundings, whereas the diffusion coefficient is the measure of the rate of diffusion by means of different diffusional pathways through of the skin (Guy and Hadgraft, 1989; Hadgraft and Wolff, 1993). When calculating flux the diffusion- and partition coefficients were calculated. For platinum the calculated partition coefficient was 0.9×10^{-5} and the diffusion coefficient was 0.048 cm/h, whereas for rhodium it was 0.4×10^{-5} and 0.23 cm/h respectively. Therefore platinum's partitioning (solubility) through the skin was greater, whereas rhodium had less resistance to diffusion. The permeability coefficient was calculated by multiplying the partition coefficient and the diffusion coefficient resulting in a permeability coefficient of 0.4×10^{-6} for platinum and 0.2×10^{-6} for rhodium. Taking the significantly higher ($p = 0.01$) partition coefficient and the significantly higher ($p = 0.01$) permeability coefficient of platinum into consideration, it is speculated that the partition coefficient contributed significantly to the overall permeation across the skin. These results correspond with the reported higher flux and cumulative mass of platinum that permeated after 24 h of exposure.

Recent studies characterising the skin condition of base metal refinery workers reported low barrier function even before the start of the shift, and further deterioration thereof during the shift as indicated by an increase in trans epidermal water loss (Du Plessis et al., 2010, 2013). Mechanical and chemical skin damage or skin lesions are likely to occur in refinery workers performing manual tasks in an acidic environment of a refinery. Larese Filon et al. (2009) confirmed increased skin permeation of metals in mechanically damaged skin during *in vitro* experiments. Therefore, refinery workers are likely at an increased risk for dermal permeation as a decreased barrier function as a result of chemically or mechanically damaged skin could result in increased permeation of substances through the skin.

In conclusion, this study has reported for the first time that platinum and rhodium are able to permeate through intact Caucasian skin in an *in vitro* diffusion system. Dermal exposure and subsequent skin permeation could contribute to sensitisation even when respiratory exposure is controlled below the respiratory OEL or when respiratory exposure is absent. Therefore, dermal

exposure to these metal salts is recognised as a risk for occupational exposure resulting in sensitisation. The retention of these metals in the skin after exposure and potential continued permeation through the skin after the work shift advocate the need for good personal hygiene together with preventative measures for dermal exposure of workers producing these metals.

Recommended measures for refinery workers: (i) appropriate washing facilities throughout the work environment and change house should be supplied to ensure thorough decontamination of exposed skin during and after the work shift to prevent the 'take-home' effect; (ii) secondary skin contamination should be prevented by preventing surface contamination; (iii) workers should be trained and supervised to ensure frequent hand washing during the day; (iv) workers should be trained properly in donning and doffing of protective clothing to avoid skin contamination; (v) workers should be supplied with appropriate protective clothing such as gloves to provide mechanical and chemical protection and disposable overalls to prevent skin contamination.

Disclaimer

Any opinion, findings and conclusions or recommendations expressed in this material are those of the author(s) and therefore the NRF do not accept any liability in regard thereto.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Transparency Document

The Transparency document associated with this article can be found in the online version.

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Chapter 5: Article III

Franken, A., Eloff, F.C., Du Plessis, J., Badenhorst, C.J., Du Plessis, J.L., *In vitro* permeation of platinum through African and Caucasian skin. Submitted to Toxicology Letters for consideration of publication.

5.1 Background

The Franz diffusion method has been used to report skin permeation of various metals. However, these experiments only included Caucasian skin. Literature on racial differences in skin permeability is limited to one *in vitro* experiment, and *in vivo* experiments reporting concentrations in blood or urine, or alternatively inducing vasodilatory responses. Literature on anatomic and functional differences between races suggests African skin to be more resistant with an enhanced barrier. However, no *in vitro* studies are available to confirm this. The experiment included in Chapter 5 is novel by including African skin to investigate the racial differences in the permeation of platinum through human skin. This is especially important for African countries such as South Africa where the majority of the workforce are Africans. This article was submitted to *Toxicology Letters*, the official journal of EUROTOX, for consideration for publication.

5.2 Instructions to authors (excerpt)

Toxicology Letters is an international journal for the rapid publication of novel reports on a range of aspects of toxicology, especially mechanisms of toxicity.

Toxicology Letters serves as a multidisciplinary forum for research in toxicology. The prime aim is the rapid publication of research studies that are both novel and advance our understanding of a particular area.

Conflict of interest: The journal follows ICMJE recommendations regarding conflict of interest disclosures. All authors are required to report the following information with each submission: All third-party financial support for the work in the submitted manuscript. All financial relationships with any entities that could be viewed as relevant to the general area of the submitted manuscript. All sources of revenue with relevance to the submitted work who made payments to you, or to your institution on your behalf, in the 36 months prior to submission. Any other interactions with the sponsor of outside of the submitted work should also be reported. Any relevant patents or copyrights (planned, pending, or issued). Any other relationships or affiliations that may be perceived by readers to have influenced, or give the appearance of potentially influencing, what you wrote in the submitted work.

Language: Text should be written in good English, American or British usage is accepted. For this thesis British spelling was used throughout.

Article structure: Manuscripts should be typewritten 1.5 spaced and divided into numbered sections including Introduction, Material and methods, Results, Discussion and Conclusions. The short conclusions section may stand alone or form part of the Discussion. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. The manuscript should include three to five bullet point highlights that convey the core findings of the article. A maximum of six keywords should be included in the manuscript.

Tables: Tables should be numbered with Arabic numbers and bear a short descriptive title. Number tables consecutively in accordance with their appearance in the text. Place footnotes to tables below the table body and indicate them with superscript lowercase letters. Avoid vertical rules. Be sparing in the use of tables and ensure that the data presented in tables do not duplicate results described elsewhere in the article. For the purpose of this thesis the Figures and Tables are included in the text for readability.

References: References should be consistent using the following style:

Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2010. The art of writing a scientific article. J. Sci. Commun. 163, 51–59.

Strunk Jr., W., White, E.B., 2000. The Elements of Style, fourth ed. Longman, New York, pp. 60-61.

Mettam, G.R., Adams, L.B., 2009. How to prepare an electronic version of your article, in: Jones, B.S., Smith , R.Z. (Eds.), Introduction to the Electronic Age. E-Publishing Inc., New York, pp. 281–304.

5.3 *In vitro* permeation of platinum through African and Caucasian skin.

Abstract

The majority of the South African workforce are Africans, therefore potential racial differences should be considered in risk and exposure assessments in the workplace. Literature suggests African skin to be a superior barrier against permeation and irritants. Previous *in vitro* studies on metals only included skin from Caucasian donors, whereas this study compared the permeation of platinum through African and Caucasian skin. A donor solution of 0.3 mg/ml of potassium tetrachloroplatinate (K_2PtCl_4) dissolved in synthetic sweat was applied to the vertical Franz diffusion cells with full thickness abdominal skin. Skin from three female African and three female Caucasian donors were included. The receptor solution was removed at various intervals during the 24 hour experiment, and analysed with high resolution Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). Skin was digested and analysed by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES). Significantly higher permeation of platinum through intact African skin, as well as a significantly higher mass of platinum retention in African skin in comparison with Caucasian skin occurred. Significant inter-donor variation was found in both racial groups. Results indicate that African workers have increased risk of dermal permeation and possible sensitisation caused by dermal exposure to platinum salts. These results are contradictory to limited literature suggesting a superior barrier in African skin and further investigation is necessary to explain the higher permeation through African skin.

Keywords

Platinum Group Metals (PGMs), Franz diffusion cells, Skin penetration, Racial differences, Potassium tetrachloroplatinate

Highlights

- Platinum permeated through intact African skin.
- Permeation of platinum through African skin is significantly higher than through Caucasian skin.
- Retention of platinum in the skin is significantly higher in African skin.
- Retention of platinum in the skin could contribute to dermal symptoms.
- Increased permeation through African skin is contradictory to limited literature regarding racial differences.

Introduction

In South Africa more than 40% of the employed population are African males, and more than 34% are African females, which is in stark contrast to the 6.4% Caucasian males and 4.9% Caucasian females (Department of Labour, 2013). The mining industry has employed approximately 419 000 workers at the end of 2013 of which 260 000 were estimated to be African men (Chamber of Mines, 2013; Statistics South Africa, 2014). One of the largest contributors to the mining sector in South Africa is the mining and refining of platinum group metals (PGMs) contributing on average 2.2% of the gross domestic product of South Africa (Chamber of Mines, 2013). During the refining process as well as secondary uses such as catalyst productions, workers are potentially exposed to various forms of these precious metals. This includes possible exposure to the potential sensitising halogenated compounds often containing chloride, via the respiratory tract or the skin (Cleare *et al.,* 1976; Cristaudo *et al*., 2005; Ngo and Maibach, 2010). Maynard *et al.* (1997) suggested the dermal route as an additional exposure route contributing to sensitisation. They found workers were being sensitised to platinum even though respiratory exposure was below the occupational exposure limit (OEL). *In vitro* research confirmed dermal permeation of metals such as nickel, cobalt and chromium through intact Caucasian skin, and more recently for platinum and rhodium (Larese *et al.,* 2007; Larese Filon *et al.,* 2009; Franken *et al.,* 2014). However, the available research on metal permeation was conducted using Caucasian skin, and no information is available for African skin. Considering the potential structural and functional differences between skin of different races and the potentially exposed South Africa workforce, it is important to include African skin in research on PGM permeability.

For the purpose of this article the term *race* will be used to define a specific population based on genetic similarities where racial divisions are based on differences in skin colour and physical features (Anand, 1999). The majority of literature regarding racial differences in skin anatomy, barrier function and permeability proved to be contradictory and dates back prior to 1994. Berardesca and Maibach (2003) concluded that African and Caucasian skin are equal in thickness, however African skin has an increased number of cell layers with greater intercellular cohesion. African skin has increased lipid content, a decreased amount of ceramides, and also increased resistance to stripping and increased recovery after stripping. However these differences reviewed by Berardesca and Maibach (2003) were based on literature published between 1955 and 1994, with some studies including only one African and three Caucasian participants (Thomson, 1955; Reinertson and Wheatley, 1959; Freeman *et al.,* 1962; Weigand *et al.,* 1974; Reed *et al.,* 1994).

The limited literature regarding the difference in permeation between African and Caucasian skin suggests less permeation of drugs through African skin with variation in permeation dependent on the molecule (Wedig and Maibach, 1981; Berardesca and Maibach, 1990; Williams *et al.,* 1991; Kompaore

et al., 1993). These studies were conducted *in vivo* with topically applied drugs sampling urinary excretion, blood concentration or vasodilatory effects. Lotte *et al.* (1993) and Leopold and Maibach (1996) reported non-significant lower permeation through African skin with the last mentioned suggesting that an increase in population size would provide significant differences in permeation between skin from different races. The number of participants included in the above mentioned *in vivo* studies ranged from four to 12 per race, limiting the findings of these studies.

The dermal exposure route has been suggested as an additional exposure route that may lead to sensitisation; however information on skin permeability of metals is limited. A number of metals have been proven to permeate through the skin; however these *in vitro* studies were conducted using only Caucasian skin. Although literature is conflicting, the overall consensus suggests African skin to be less permeable to topically applied drugs and more resistant to irritants, therefore suggesting that the African workforce should be less susceptible to sensitisation and irritation in the workplace. Considering the majority of workers in the mining sector of South Africa are Africans, it is important to include this racial group in research. Therefore this study aimed to determine the permeation of platinum in the salt form through intact African and Caucasian skin, and to determine if there is a difference in permeation through the skin of these two racial groups.

Materials and methods

Chemicals

All chemicals were 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 disodium hydrogen phosphate were purchased from Sigma Aldrich, South Africa. Nitric acid (65%) and hydrochloric acid (33%) were purchased from De Bruyn Spectroscopic Solutions, South Africa. Hydrogen peroxide (50%) and acetone were purchased from Associated Chemical Enterprises, South Africa. Potassium tetrachloroplatinate were obtained from Johnson Matthey and sponsored by Anglo American Technical Solutions, South Africa. Type one water used for the solutions was produced with a Millipore purification pack system (milliQ water).

A 0.9% sodium chloride solution was used to test the integrity of the skin. The physiological solution (receptor solution) was prepared by dissolving 4.76 g Na_2HPO_4, 0.28 g KH_2PO_4 and 18 g NaCl into two litre of milliQ water, the final pH was adjusted to 7.35. The synthetic sweat solution used as the donor solution was prepared with 0.5% NaCl, 0.1% lactic acid and 0.1% urea in milliQ water. Synthetic sweat was used as donor solution to replicate actual workplace conditions as a realistic surrogate to reproduce what workers may be exposed to. After dissolving 0.03233 g potassium tetrachloroplatinate (K_2PtCl_4) in

50 ml synthetic sweat the pH was adjusted to 6.5 with ammonia. A final concentration of 0.3 mg of platinum per millilitre was used as the donor solution. The donor solution was pre-heated to 32 °C prior to the onset of the diffusion study.

Preparation of the skin

Human abdominal full thickness skin was obtained after surgical abdominoplasty procedures with the approval of the Human Research Ethics Committee in the Faculty of Health Sciences of the North-West University (NWU-00114-11-A5). Informed consent was given by the donors. The skin was stored in a freezer at -18 °C for a maximum period of four months. Harrison *et al.* (1984) reported that freezing the skin did not affect the absorption of water by comparing frozen and fresh skin segments. On the day of the experiment, the skin was allowed to thaw, and all subcutaneous fat was removed. The skin was cut into circles with a diameter of 2.4 cm and clamped between the two parts of the Franz cell. Skin integrity was tested before and after each experiment using a Tinsley LCR Data bridge (Model 6401). The measurement was taken at one kHz in the parallel equivalent circuit using two stainless steel electrodes (Fasano and Hinderliter, 2004). Cells with an electrical resistance below 10 kΩ were rejected, and cells with similar resistance values within one skin donor were selected for diffusion studies. Skin from three female African (ages between 28 and 31 years) and three female Caucasian donors (ages between 37 and 52 years) were used.

In vitro diffusion system

Vertical Franz diffusion cells with a two millilitre receptor compartment were used (Franz, 1975). The temperature of the receptor compartment was maintained at 37 °C in a water bath to simulate the normal temperature below the skin. The physiological solution used as receptor solution was continuously stirred using a magnetic stirrer bar. Each piece of skin was clamped between the two compartments of the Franz cell with a stainless steel clamp. The mean exposed skin area was 1.066 cm^2, and the thickness of the skin was measured with a vernier caliper as less than one mm.

Experiment I - African skin

The receptor compartment was filled with two millilitre of physiological (receptor) solution, and the donor compartment was filled with one millilitre of the K_2PtCl_4 synthetic sweat solution. Between six and nine cells were used as experimental cells, and between six and ten cells as blanks for each experiment. The receptor solution was removed at 6, 8, 10, 12, 14 and 24 hours and placed into clearly marked vials. During each removal the receptor compartment was also rinsed with an additional two millilitre of physiological solution, and this rinsing solution was added to the original sample removed. The receptor compartment was rinsed to remove all the platinum in this compartment at the specific

time interval. After each experiment the donor solution was removed and placed into a vial, and the compartment was rinsed four times using one millilitre of synthetic sweat each time to remove all remaining platinum from the skin. The donor rinsing solution was added to the original donor solution for analysis. After testing the skin integrity the cell was dismantled and the piece of skin was placed into a vial for analysis. This experiment was repeated three times by using skin from three different African donors.

Experiment II - Caucasian skin

The above mentioned experiment was repeated three times by using skin from three different Caucasian donors.

Blanks

The blank cells were treated the same as the other cells with the exception that no platinum was added to the synthetic sweat placed in the donor compartment.

Analysis – platinum in donor and receptor solutions

Receptor solutions were analysed with high resolution Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) (Thermo Scientific Element XR) to establish the concentration of platinum in the solutions. An internal standard of 10 µg/L rhenium was added to all the solutions to compensate for volume inaccuracies. The analysis was done using the Pt195 isotope in low resolution mode. The instrument was calibrated with Pt calibration standards that were matrix matched with a range of 0 - 100 ng/L solutions containing platinum and the internal standard rhenium to provide the initial calibration curves. If necessary, the calibration was extended to 1 - 50 µg/L if the sample concentrations required it. The lower limit of detection of platinum was 30 ng/L.

The concentration of platinum in the donor solutions were analysed by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) (Spectro Arcos). An internal standard of 4 mg/L yttrium was added to the solutions to compensate for volume inaccuracies. The wavelengths used for platinum determination was 265.945 nm and 214.423 nm. The instrument was calibrated with a series standard solutions (0 - 20 mg/L) to provide the calibration curves.

Analysis - platinum in the skin

The skin pieces were removed from the vial and weighed for skin digestion purposes. The vials were rinsed with acetone to remove any residue from the vial. The rinse solution was placed into a beaker with the skin where the acetone was evaporated using a hot plate. Nitric acid and hydrogen peroxide were added to the skin mixture in separate stages and evaporated to destroy all organic material.

Hydrochloric acid was added to ensure all the platinum was in a soluble state. The final solution was diluted to 10 ml with hydrochloric acid, which was analysed by ICP-OES as already described, to establish the concentration of platinum remaining in the skin.

Standardisation experiments

Various standardisation experiments were conducted to establish the appropriate concentration for the donor solution; these experiments included receptor solution removals at one, two and four hours. Analyses of these results predominantly indicated values close to or below the detection limit of analysis. These experiments also indicated that exposure up to six hours did not contribute to the steady state flux through the skin, and therefore removals at these time intervals were excluded from subsequent experiments.

Transdermal data analysis

The concentration of platinum (ng/cm^3) in the receptor solution was converted to the total mass that permeated through the exposed skin area (ng/cm^2) and then plotted against time as the cumulative mass of platinum that permeated per area of skin.

Flux permeation was calculated from the steady-state region of the graph after curve-fitting. Equation 1 (Eq 1) was developed by Díez-Sales *et al.* (1991) to describe the mass of substance crossing a membrane at a given time:

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

Where $Q(t)$ is the mass that passed through the membrane at a given time t, A is the actual surface diffusion area, K is the partition coefficient between the skin and donor solution, h is the membrane thickness, C_v is the concentration in the donor solution and D is the diffusion coefficient of the permeant in the skin. As t approaches infinity the exponential term becomes negligible and the equation can be simplified to Eq 2:

$$Q(t) = AKhC_v \left[D\,\frac{t}{h} - \frac{1}{6}\right] \quad (2)$$

Since K, D and h are unknown, the products Kh and D/h^2 were replaced with α and β which were calculated by fitting equation 2 to the data by using Easyplot Version 4.0.5 (Spiral Software, Aerious Ltd.). The permeability coefficient (k_p) and flux (J) were calculated using Eq 3 and Eq 4 respectively:

$$k_p = \frac{KD}{h} (= \alpha\beta) \quad (3)$$

$$J = k_p C_v \quad (4)$$

The curve fit model was used to calculate the partition and diffusion coefficients. Lag time was calculated as the intercept of the steady-state region with the x-axis. Mean blank concentrations were subtracted from experimental data.

Statistical data analysis

Data analysis was performed using Statistica Version 12 (Statsoft Inc.). Data was not normally distributed, and therefore Box-Cox transformed under supervision of a bio-statistician since this method is maximally effective in transforming a variable toward normality (Box and Cox, 1964). Data is reported as mean ± standard error of means (SEM). The difference between independent Box-Cox transformed data was established with a t-test with separate variance. Differences between the three donors within each racial group were established by using an one-way analysis of covariance (ANCOVA) with adjustment for age using the Bonferroni post-hoc test. Throughout a p value of ≤ 0.05 was considered as statistically significant.

Results

Results indicated that platinum (Pt) permeated through intact African and Caucasian skin, and that permeation through African skin was significantly higher than through Caucasian skin (Figure 1). After eight hours of exposure the mean cumulative mass per area platinum that permeated through African skin was 3.19 ± 0.71 ng/cm^2 (range 0.03 – 10.2 ng/cm^2), which was significantly higher ($p = 0.004$) than the mean permeation of 0.46 ± 0.13 ng/cm^2 (range 0.02 – 2.5 ng/cm^2) through Caucasian skin. The mean permeation after 12 hours was also significantly higher ($p = 0.002$) through African skin (8.78 ± 1.79 ng/cm^2) than through Caucasian skin (1.51 ± 0.44 ng/cm^2). After 24 hours the permeation increased more than fourfold to 37.52 ± 10.61 ng/cm^2 (range 0.32 – 158.8 ng/cm^2) for African skin, and more than threefold to 5.05 ± 1.53 ng/cm^2 (range 0.37 – 27.1 ng/cm^2) for Caucasian skin ($p = 0.04$) in comparison with 12 hours. After 24 hours of skin exposure 3 064.13 ± 642.31 ng/cm^2 of platinum was retained inside African skin, which was significantly higher ($p < 0.002$) than 1 486.32 ± 75.27 ng/cm^2 which was retained in Caucasian skin. As indicated in Table 1, both the flux of platinum and the permeability coefficient across African skin was significantly higher ($p = 0.040$ and $p = 0.041$ respectively) than Caucasian skin. No significant differences in lag time were found between the two racial groups. Significant inter-donor variation was found for African skin, with less variation for Caucasian skin.

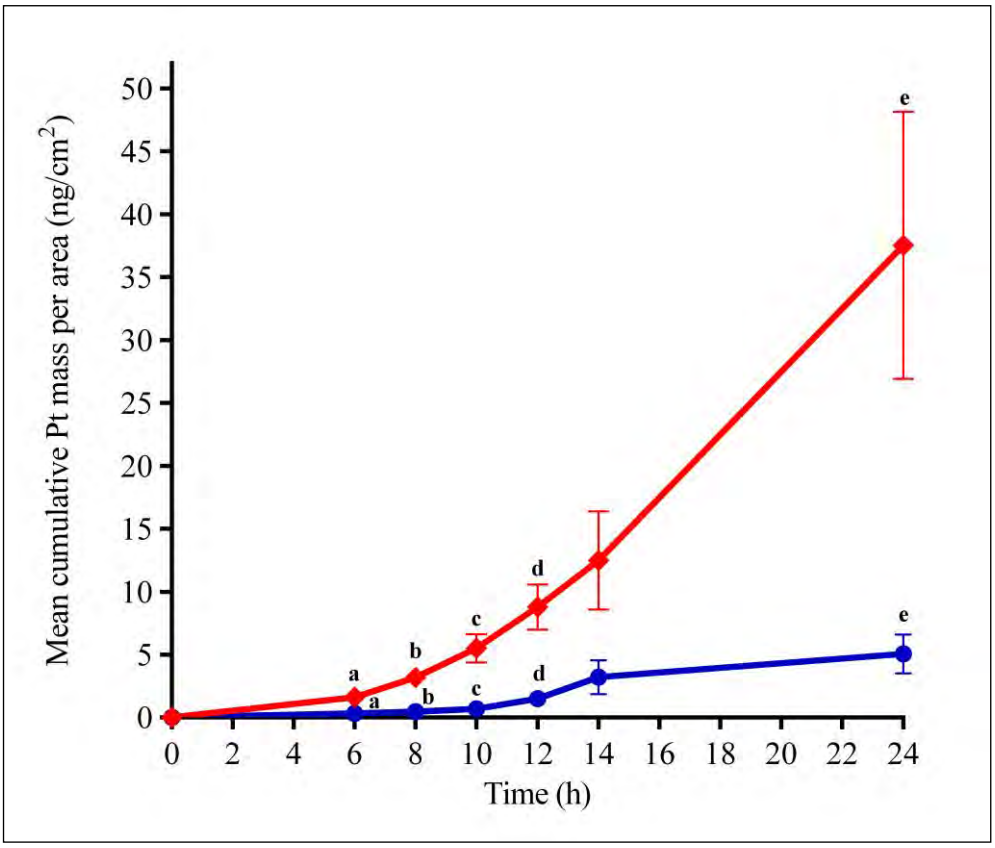

Figure 1: Mean cumulative mass of platinum permeated per area through African skin (n = 21, Human skin from 3 donors) and Caucasian skin (n = 21, Human skin from 3 donors) (mean ± SEM). a-e indicates significant differences between African and Caucasian skin ($p \leq 0.05$).

Table 1: Platinum in the receptor solution, retained inside the skin and permeation (mean ± SEM).

	Receptor solution		Skin		Permeation		
	Mean cumulative mass permeated in 24 hours	Percentage diffused after 24 hours of exposure	Mass retained inside the skin	Percentage in the skin after 24 hours	Permeability coefficient	Mean flux	Lag time
	ng/cm^2	%	ng/cm^2	%	cm/h	$ng/cm^2/h$	h
African skin	37.52 ± 10.61	3.43×10^{-3} ± 1.0×10^{-3}	3 064.13 ± 642.31	4.78 ± 1.00	6.6×10^{-6} ± 1.9×10^{-6}	1.93 ± 0.55	4.91 ± 0.48
Caucasian skin	5.05 ± 1.54	0.47×10^{-3} ± 0.1×10^{-3}	1 486.32 ± 75.27	2.31 ± 0.11	9.3×10^{-7} ± 3.2×10^{-7}	0.27 ± 0.09	4.51 ± 0.48
p	**0.044**	**0.045**	**0.002**	**0.001**	**0.041**	**0.040**	0.695

Values in bold indicate statistical significant differences.

Variation between donors of each racial group determined by ANCOVA indicated significant differences between the three African donors for permeability coefficient, mean flux, mean cumulative mass permeated in 24 hours, and the percentage diffused ($p < 0.001$). Significant differences for the above mentioned parameters were found between two of the three Caucasian donors ($p < 0.02$).

Discussion

In this study the skin permeation of platinum using full thickness African and Caucasian skin was investigated. For the first time abdominal African skin was used in the *in vitro* diffusion system to determine the permeation of a metal, specifically platinum. The inclusion of African skin in *in vitro* permeation studies is especially important for African countries such as South Africa where the majority of the workforce are Africans (Department of Labour, 2013). The results indicated that platinum permeated through intact African skin, and that permeation was significantly higher through African skin in comparison with Caucasian skin. Figure 1 indicates significantly higher mean cumulative mass permeation through African skin for all the time intervals, except 14 hours, when compared with Caucasian skin. After eight hours of exposure the mean cumulative mass per area platinum that permeated through African skin was approximately seven times higher than the mean permeation through Caucasian skin, and approximately six times higher after 12 hours of exposure. Workers are often required to work shifts of 12 hours instead of the usual eight hours. In these additional four hours permeation increased by 175% in African skin, and 228% in Caucasian skin in the *in vitro* diffusion system. If the platinum was to remain on the skin after the 12 hours, permeation will increase by 327% in African skin, and 234% in Caucasian skin in the following 12 hours of exposure. These results indicate the importance of good personal hygiene throughout the work shift to prevent skin loading and permeation of platinum through the skin. It is also important to effectively remove any contaminants from the entire body after a work shift to prevent further permeation from extended contamination. However, care should be taken when skin is decontaminated as normal washing methods may not be effective in removing all the contaminants and certain washing procedures may even increase skin uptake (Larese Filon *et al.,* 2006; Julander *et al.,* 2010).

Moody *et al.* (2009) calculated possible systemic loading of nickel after exposure to nickel contaminated soil *in vitro*. In accordance estimated systemic loading of platinum was calculated by using the flux values obtained from the *in vitro* experiments. The flux through African skin reported as 1.93 ng/cm^2/h x 840 cm^2 (area of adult male hand) x 8 h (work day) yields an estimated total systemic exposure of 12.96 µg per day. The flux through Caucasian skin (0.27 ng/cm^2/h) yields an estimated systemic loading of 1.81 µg per day. The systemic loading calculated as an estimate for worst-case exposure would be 7.2 times higher in African workers than Caucasian workers under similar exposure

conditions. These calculations are only an estimate of systemic exposure under the present experimental conditions and will in all likelihood be overestimated by the high concentration applied to the skin. It is important to consider the skin area of exposure since these estimates were calculated based only on exposure to the hands; however in the workplace the arms and other areas may also be contaminated. In addition potential anatomical differences in skin permeation should be considered since these estimates were calculated form results obtained with abdominal skin. Therefore Moody *et al.* (2009) cautioned that these estimates may be affected by exposure conditions and individual differences in skin permeability.

The percentage platinum permeation through the skin was calculated to indicate permeation after exposure to 0.3 mg Pt/ml. A significantly higher percentage of platinum permeated through African skin than Caucasian skin. The mass of metals retained in the skin was calculated using the full thickness skin, without distinguishing between the epidermis and the stratum corneum (SC). After 24 hours of exposure, more than double the mass of platinum was retained inside African skin in comparison with Caucasian skin. For African skin there was a ratio of 1:1 300 between the percentage that permeated through the skin and the percentage retained inside the skin, in comparison with the ratio of 1:4 900 for Caucasian skin. Caucasian skin yielded a greater difference between the percentages permeated and the percentage retained, indicating a higher retention level for Caucasian skin when compared with the mass permeated. However, larger variations were found in African skin where the retention of platinum in the skin ranged between 1 257.9 and 11 343.8 ng/cm^2 in comparison with retention in Caucasian skin ranging between 706.7 and 2 048.3 ng/cm^2. As a result of the platinum retention in the skin of both racial groups, platinum could act as haptens leading to the formation of antigens in the skin and thereby contribute to dermal effects or sensitisation even after the contaminant is removed from the skin (Cleare *et al.,* 1976).

In biological membranes such as the skin there is inherent variability, including inter-donor variation in *in vitro* permeability studies (Kierstan *et al.,* 2001; Green, 2005). This was confirmed by a robustness study of *in vitro* methodology as part of the Evaluations and Predictions of Dermal Absorption of Toxic Chemicals (EDETOX) team where they reported variability between absorption properties of skin from different donors (Van der Sandt, 2004). The variation was determined by ANCOVA with the adjustment for age since the age of the skin donor could influence dermal absorption with diminishing blood supply, diminishing surface lipid content or decrease in skin thickness in aging skin (Hostýnek, 2003). The inter-donor variability is also evident in our results with higher variability found in African skin. Significant differences were found between the three African donors for permeability coefficient, mean flux, mean cumulative mass permeated in 24 hours and the percentage diffused. Skin from Caucasian donors showed less variability between donors with significant differences between two of the three

donors for the four parameters mentioned. No significant differences between the donors of either racial group for mass retained in the skin or percentage in the skin after 24 hours were found. The variability between donors is intrinsic, which highlights the importance of including multiple donors in *in vitro* studies. The variation found between donors of the same race should be taken into consideration when these results are interpreted and conclusions on racial differences are made.

The flux through African skin, ranging between 0.019 and 8.44 ng/cm^2/h, was significantly higher ($p = 0.04$) than the flux through Caucasian skin ranging between 0.016 and 1.62 ng/cm^2/h. Flux is a measure of the rate of diffusion of the metal through the skin over time (McDougal and Boeniger, 2002). The permeability coefficient, describing the movement of the metal over time, through African skin was one order of magnitude greater than Caucasian skin. These results indicate higher permeation of platinum through African skin in comparison with Caucasian skin, which is in contrast to a study by Shehata-Karam *et al.* (1988) reporting no difference between the permeation of pesticides through African or Caucasian skin, however this experiment used foreskin from human newborns. No similar *in vitro* studies using abdominal skin comparing African and Caucasian skin are available. In contrast to our findings, selected *in vivo* studies have reported significantly lower permeation of drugs through African skin (Wedig and Maibach, 1981; Berardesca and Maibach, 1990; Williams *et al.*, 1991; Kompaore *et al.,* 1993). Wedig and Maibach (1981) compared urinary excretion of dipyrithione from four African and four Caucasian participants. Besides the small number of participants, the authors mentioned possible experimental errors of the study such as the completeness of excretion and differences in excretions, distribution or metabolism between the two groups. Other studies reported lower permeation through African skin indirectly by evaluating the vasodilatory effects of certain drugs (Berardesca and Maibach, 1990; Kompaore *et al.,* 1993). Berardesca and Maibach (1990) concluded decreased susceptibility of African skin to nicotinates by determining the nicotinate-induced vasodilation with laser Doppler velocimetry including nine to ten participants of each race. However, the results indicated significant differences between races only for area under the curve, whereas the initial response and peak response were non-significant. Kompaore *et al.* (1993) and Kompaore and Tsurata (1993) also reported absorption of methyl nicotinate by determination of the lag time before vasodilation with laser Doppler velocimetry including seven to eight participants per race. The authors concluded African skin to be less permeable after results indicated a longer lag time before vasodilatation was induced. In contrast Sinha *et al.* (1978) found no difference in skin permeation of diflorasone diacetate between African and Caucasian skin by determining urine, faeces and blood concentrations in three participants of each race. In accordance Guy *et al.* (1985) found no significant differences in the vasodilatory response to methyl nicotinate in six participants per race. The differences in permeation and vasodilatory effects reported between African and Caucasian skin were obtained by different techniques and methodologies, and therefore cannot be compared directly to the results of platinum *in vitro*

permeation obtained with abdominal skin. No *in vitro* studies using abdominal African skin are available, with the scarceness of African skin as a definite reason for it.

Anatomical differences stated in the literature also suggest African skin to have an enhanced barrier against permeation and also to be more resistant to irritants. These include more cell layers in the SC of African skin with greater cellular cohesion reported by Weigand *et al.* (1974). Therefore African skin is suggested to be more compact than Caucasian skin, since African and Caucasian skin are equal in thickness (Weigand *et al*., 1974). However these anatomical data is 40 years old and no recent publications are available to confirm these findings as all the literature stating equal thickness of the SC acknowledges this lone publication. Anatomical differences in skin appendages, such as number of pores and glands, between African and Caucasian skin could be a contributing factor leading to increased permeation through the shunt route of African skin, thereby contributing to the difference in permeation reported in this study (Rawlings, 2006; Hillebrand *et al.,* 2007).

Permeation of metals through the skin is dependent on the chemical species and the valence or oxidation state (Hostýnek, 2003). To our knowledge no information is available on the behaviour of platinum in sweat or in the skin. In this study platinum was applied to the skin as K_2PtCl_4 in synthetic sweat, and if the salt dissolved completely it would have led to the formation of Pt^{2+}. Platinum is most commonly found in the oxidation states of 0, $Pt^{2+,}$ Pt^{4+} or Pt^{6+}; and less commonly found in the Pt^{3+} state (Lykissa and Maharaj, 2006; Wilson and Lippard, 2012). The oxidation state of the platinum could influence skin permeation as proven for chromium, where chromium(III) was found to be less permeable than chromium(VI). Chromium(III) had a stronger affinity for the skin contributing to the lower permeation (Van Lierde *et al.,* 2006). In addition studies with nickel proved that counter ions play an important part in permeation as nickel chloride compounds were reported to be more permeable than nickel sulphate compounds (Fullerton *et al.,* 1986). During the refining process platinum is mostly found in the salt form attached to chloride, therefore the counter ions of chloride may contribute to higher skin permeation of platinum after dermal exposure in the workplace. Further studies are needed to determine the valence state of platinum in the donor solution as well as in the skin.

In conclusion, this study has reported for the first time that platinum permeated through intact African skin in an *in vitro* diffusion system, and is also retained inside the skin. The comparison of permeation between races indicated significantly higher permeation through African skin, as well as significantly higher retention inside African skin. The findings of these *in vitro* experiments contradict limited *in vivo* literature reporting African skin to be less permeable to drugs when compared with Caucasian skin. However the permeation of platinum as a metal cannot be compared to the permeation of topically applied drugs specifically manufactured for optimal permeation. This study suggests African skin to have an inferior barrier to Caucasian skin when considering *in vitro* platinum permeation results;

however considering the inter-donor variations reported, the results should be interpreted accordingly. The results, based on seven times higher flux values for African skin, indicate an increased risk to these workers when platinum dermal exposure occurs in a refinery setting. In view of the diverse workforce present in refineries it is important to consider these racial differences when future health risk assessments are conducted. Results indicating platinum permeation through intact human skin highlight the possibility that platinum could contribute to sensitisation following dermal exposure; therefore dermal exposure to platinum should be controlled adequately. A general shortage of recent literature regarding racial differences in skin anatomy, barrier function and permeability is evident. Racial differences have only been reported for drug permeation *in vivo*, and no flux values have been reported by using *in vitro* methods. Studies should also be conducted to determine racial differences in structural and functional skin anatomy, such as SC thickness, number of cell layers and lipid content by including a larger number of participants in these studies. Future *in vitro* studies should also include African skin in permeation studies to evaluate racial differences in permeability of substances.

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Disclaimer

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Chapter 6: Concluding chapter

This chapter offers conclusions with an emphasis on the aims, objectives and hypotheses postulated for this thesis. Recommendations are made for preventing and reducing skin contact to PGMs to prevent skin permeation. In addition, recommendations are made to improve future *in vitro* experiments and finally the limitations of the *in vitro* skin permeation experiments and possible future studies will be discussed.

6.1 Conclusions

A critical review of published literature on *in vitro* skin permeation of metals specifically utilising human skin, is provided in Chapter 3 of this thesis. Various studies using the *in vitro* method have found skin permeation of chromium, cobalt, gold, lead, mercury, nickel and silver, with only limited comparisons between studies. After a thorough exploration of the experimental design used in different studies, a number of discrepancies in the apparatus used, as well as skin related experimental aspects were identified and thereby the objective to give a critical review of the *in vitro* diffusion method used to determine metal permeability through human skin was achieved. The majority of the studies utilised a Franz type diffusion cell. However, the studies used various donor and receptor solutions, different temperatures at which the receptor solution was kept and different surface areas of skin exposed. Disparity in the anatomical area of skin, the gender and the age of the donors, as well as the thickness of the skin utilised was observed. The concentration of metals, as well as the units in which results were reported differed significantly. The most significant shortcoming, however, was the lack of detail provided regarding the methodology of published studies, for example only a few studies included the number of skin donors, the gender or the age of donors. The disparity in the experimental design can influence the findings and deductions made and impede the comparison of results from different studies. It is important to standardise the *in vitro* methodology as well as the format of data reporting for studies to be conducted in future. Guidelines for standardisation are included in Chapter 3 and are elaborated on in Section 6.2.1 of this chapter.

Skin permeation of nickel, chromium and cobalt, amongst others, were shown with the Franz diffusion system, and consequently this method was utilised to investigate the permeability of platinum and rhodium in the salt form through human abdominal skin. For the purpose of this thesis potassium tetrachloroplatinate (K_2PtCl_4) and rhodium chloride ($RhCl_3$) were utilised in the *in vitro* skin permeation experiments as representatives of platinum and rhodium salts. The methodology used for *in vitro* experiments in this thesis was based on work published by leading researchers in the field of occupational toxicology. The results that were published in *Toxicology in Vitro* (Chapter 4 of this thesis)

indicate the permeation of both platinum and rhodium through intact human skin. Therefore the objective of investigating the permeability of the two PGM salts was achieved. The *first hypothesis* stated: *Published in vitro skin permeation studies reported the permeation of nickel, cobalt and chromium through intact skin. Based on the permeability of these metals the permeation of platinum and rhodium through intact human skin is hypothesised.* This hypothesis is accepted. Furthermore, platinum permeation was found to be significantly higher than rhodium permeation, and the objective to evaluate the difference in permeability of platinum and rhodium was achieved. Consequently, the *second hypothesis;* which stated: *Published in vitro skin permeation studies with nickel, chromium and cobalt reported significant differences in the permeability of these metals. Based on the difference in charge it is hypothesised that platinum and rhodium permeation differ statistically significantly from each other;* is accepted. It is important to consider that these findings are based on one platinum and one rhodium salt. However, no other *in vitro* studies are available for comparison. The findings on the permeability of platinum and rhodium should be viewed in this context, and are dependent on future studies utilising other platinum and rhodium salts.

In the past, platinum sensitisation was mainly attributed to respiratory exposure, with only recent inquiry into the contribution of dermal exposure (Maynard *et al.,* 1997; Kimber and Dearman, 2002). Platinum is currently listed only as a respiratory sensitiser, and rhodium is not listed as a sensitiser at all. However, the skin symptoms described in workers sensitised to platinum and rhodium, such as erythematous or urticarial skin lesions, are consistent with symptoms described during a type IV hypersensitivity reaction (Hunter *et al.,* 1945; Cristaudo *et al.,* 2005). Therefore these symptoms could possibly be a result of dermal exposure and sensitisation. There is no information available regarding dermal exposure to platinum leading to respiratory sensitisation in humans, but it was reported in experiments with mice (Dearman *et al.,* 1998). The results included in Chapter 4 of this thesis found that platinum and rhodium permeated through intact skin, and thereby supports the possibility that dermal exposure could contribute to sensitisation and subsequent development of skin symptoms. It is furthermore suggested that a combination of dermal and respiratory exposure may possibly cause sensitisation in workers, leading to development of respiratory as well as skin related symptoms. In addition to the immunological reaction with Langerhans cells in the skin leading to sensitisation and allergic contact dermatitis, a non-immune reaction is possible. For allergic contact dermatitis the 'danger model' is proposed where the hapten causes release from non-immune skin cells such as the keratinocytes. The release of this danger signal produces sensitisation by means of the immunological reaction to the antigen. Therefore a hapten can deliver both antigenic and irritant signals, however the possibility of platinum and rhodium causing the danger signal is still unknown (Smith *et al.,* 2002). Based on limited literature the sensitisation potential of rhodium is lower than that of platinum, and this should be considered when assessing the potential risks involved with respiratory and dermal exposure.

The investigation of platinum permeation through intact African and Caucasian skin was submitted for publication in *Toxicology Letters* (Chapter 5 of this thesis). It was found that platinum permeated through intact skin of both racial groups, with statistically significantly higher permeation through African skin. After 24 hours of exposure the permeation through African skin was more than seven times higher than that of Caucasian skin. The final objective was achieved by showing a significant difference in permeability between African and Caucasian skin. The *third hypothesis* stated: *Published literature report significant differences in skin structure, barrier function and permeation of drugs between African and Caucasian skin, which suggests that African skin is less permeable. It is therefore hypothesised that platinum has a statistically significantly lower permeation through African skin.* This hypothesis is rejected because results indicate statistically significantly higher permeation of platinum through African skin. Literature regarding racial differences in skin structure is from past decades and in some instances based on a very limited number of subjects. Differences in permeation are based on concentrations of the substance detected in the blood or urine, or on the vasodilatory effect (*in vivo*), and often by testing pharmaceutical compounds (drugs) manufactured for optimal permeation. Results obtained from the *in vitro* diffusion method included in Chapter 5 of this thesis cannot be directly compared to *in vivo* obtained results. The permeation of metals has only been conducted with Caucasian skin, and only one *in vitro* study utilised African and Caucasian skin and reported no difference in pesticide permeation between different racial groups. However, it should be noted that this study utilised human foreskin from newborns, and no other *in vitro* studies utilising abdominal skin is available (Shehata-Karam *et al.,* 1988). The results included in this thesis are novel in indicating permeation of platinum and rhodium through human skin. Additionally, by including African skin in the experiments, the difference in *in vitro* platinum permeation between skin from different racial groups was shown. These findings are important in the South African context where PGM mining and refining is a noteworthy contributor to the economy and the majority of the workforce are Africans. Even though it was a challenge to obtain African skin from donors, it is an important field of research to pursue in future.

Apart from exposure to metals refinery workers, while wearing gloves, may also be co-exposed to irritants, such as acids and other hazardous chemicals. These irritants may damage the skin barrier and thereby increase permeability of the skin, and furthermore the damaged barrier will facilitate absorption of irritants leading to further deterioration of the barrier (Kezic and Nielsen, 2009). African skin is suggested to be less susceptible to chemical irritants with various studies reporting lower irritation for African skin (Weigand and Mershon, 1970; Weigand and Gaylor, 1974; Frosch and Kligman, 1977). Therefore irritants in the workplace will affect the skin of African workers to a lesser extent when compared to the skin of Caucasian workers and may also be responsible for the lower incidence of sensitisation in African workers.

Digestion of the skin after 24 hours of exposure indicated significant retention of both platinum (average retention between 2.31% and 4.78% of the applied dose), and rhodium (1.16%) in the skin. The retention in the skin indicates a possible reservoir formation in the skin where large quantities could penetrate into the skin as a result of exposure. This reservoir can cause prolonged permeation through the skin, even after the contaminant has been removed from the skin surface. The retention of these metals in the skin increases the time available for the metals to form antigens capable of eliciting immune reactions (Lacy *et al.,* 1996). Therefore, significant skin retention increases the potential to contribute to sensitisation. All skin donors were females with no occupational exposure to PGMs, yet background platinum levels ranging between 0.03 and 7.55 ng and rhodium ranging between 0.098 and 2.35 ng in non-exposed skin samples (blanks) were detected, indicating possible environmental exposure.

During pilot studies conducted, permeation through the skin was observed as early as two hours of exposure, and subsequent experiments indicated significant permeation after six hours of exposure. PGM salts easily dissolved in synthetic sweat, which suggests that the salt could dissolve in sweat on the skin after dermal contact in workplace conditions, and thereby start to permeate into and through the skin as early as two hours after the start of exposure. For the mining and other industries utilising these PGMs, these results should place emphasis on the importance of including the dermal route when assessing occupational exposure. Dermal exposure to these metals should be prevented since these metals are potential sensitisers. The results obtained with the *in vitro* skin permeation experiments provided valuable information on the permeability of selected PGMs salts through intact human skin, which could be useful to determine potential health risks following dermal exposure, thereby realising the general aim of this thesis. More specific recommendations for refineries or other industries utilising these metals are given in Section 6.2.2 of this chapter.

6.2 Recommendations

Recommendations based on the findings reported in Chapter 3, 4 and 5 will be discussed.

6.2.1 Recommendations for *in vitro* experiments in general

- Continued use of human skin instead of animal skin, such as pig skin, since animal skin is generally more permeable than human skin, thereby overestimating the permeation of substances (OECD, 2004). Furthermore, human skin is the 'gold' standard and the best predictor for skin permeation of metals in real life conditions (Sartorelli *et al.,* 2000).

- It is important to include skin from other races as anatomical differences between skin form different racial groups suggest a difference in skin structure and skin barrier and therefore permeability. To date only Caucasian skin has been used to determine *in vitro* permeation of metals through human skin. Future studies should include African skin to investigate the permeability of other metals.

- The use of fresh skin is not always practicable; therefore skin may be frozen at -20 °C for up to four months without loss of barrier function, provided that skin integrity is tested before the skin is used. The length of time frozen and the temperature at which it was stored should be reported (Sartorelli *et al.,* 2000; OECD, 2004).

- Include skin integrity testing by measuring electrical resistance before and after the experiment. Measurement before the start of the experiment enables the exclusion of damaged skin from the experiment (OECD, 2004). Pieces of skin with electrical resistance measurement values within a narrow range should be selected for the experiment. If the effect of skin damage on permeation is investigated, skin integrity should be tested again after the skin has been damaged to obtain an indication of the damage caused. After conclusion of the experiment, skin integrity testing should be included to ensure no damage or experimental fault occurred. The skin integrity testing measurement methods should be described in publications.

- Use of abdominal skin as it generally has a larger surface area than skin obtained from other regions (such as breast). By using skin only from the abdominal area, variability between different anatomical areas as a result of differences in skin thickness or follicle density is minimised (Sartorelli *et al.,* 2000). The use of abdominal skin is further recommended because of the availability thereof as a result of surgery.

- Include skin from a sufficient number of different donors in experiments to compensate for inter-donor variability (Sartorelli *et al.,* 2000). Biological membranes such as skin are variable, and therefore variation in results from different donors is inherent. By including skin from multiple donors, this possible variability will be reflected in the data reported. The age of the skin donor should also be considered to prevent age related variability in the results. Aged skin thins and undergoes changes in vasculature and collagen fibres that support the skin, thereby compromising skin integrity (Ngo and Maibach, 2010; Ngo *et al.,* 2010). Therefore it is important to report the origin of the skin, including the age and gender of the donor and the anatomic site where it was obtained from, as well as methods of skin handling.

- Use of full thickness skin to simulate actual conditions in the workplace and to limit disturbance of the barrier function by cutting or separation methods. However, excessive thickness (> 1mm) should be avoided and therefore the thickness of skin used should be established and reported in publications (Sartorelli *et al.,* 2000; OECD, 2004). However, measurement of skin thickness by making use of an accurate electronic vernier caliper should be standardised.

- To represent occupational conditions it is recommended to use synthetic sweat as donor solution with a pH between 4.5 and 6.5 as suggested by Larese *et al.* (2007) and Larese Filon *et al.* (2009a). For occupational toxicological studies this solution is a realistic surrogate to reproduce what workers may be exposed to in workplace conditions (Sartorelli *et al.,* 2000). In addition, the physicochemical properties, such as solubility of the metal tested, should be regarded when deciding on a pH of the donor solution.

- The metal should be applied as an infinite dose able to reach steady state conditions to enable the calculation of a permeation profile. The concentration applied to the skin should simulate probable dermal exposure in the workplace based on available literature. If no literature on dermal exposure is available, the lowest concentration possible for accurate analysis should be established through pilot studies, and used subsequently.

- To simulate the physiology of the human body it is recommended to use a physiological solution with a salt concentration similar to that of blood as a receptor solution with a pH of 7.35 (Larese Filon *et al.,* 2013).

- To simulate normal temperature underneath the skin the receptor solution should be maintained at a constant temperature of 37 °C, instead of the 32 °C used by some studies which simulates the temperature on the skin surface (Franz, 1975).

- The duration of 24 hours is suggested to allow for steady state conditions and enable flux calculations. However, removal of the receptor solution at eight and 12 hours is recommended for occupational toxicology studies to allow reporting of permeation based on work shifts. Overall, at least five to six interval removals should be included to determine a permeation profile (OECD, 2004). In addition, it is recommended to collect receptor solution after short term exposures, such as 30 minutes, to simulate realistic workplace exposures. However, detection limits of analysis methods may prevent quantification of these removals (McDougal and Boeniger, 2002).

- Include tape stripping before digestion of the skin to determine the localisation of the metal within the skin. To remove the SC, 15 to 25 strips should be used (OECD, 2004). The tape strips and the skin should be digested separately and analysed to report metal content in the different layers of the skin.

- Recovery of the metal should be calculated by including total cumulative concentration permeated though the skin, the concentration retained inside the skin, as well as the concentration in the donor solution after the experiment. This will enable the calculation of a mass balance to ensure proper experimental setup (Sartorelli *et al.,* 2000).

- Results should preferably be given as flux and permeability coefficient to enable comparison with other studies. In addition, the concentration in the receptor solution and in the skin, or alternatively the percentage, should be reported.

6.2.2 Recommendations for industries working with PGMs

Recommendations are made to employers of companies where workers are potentially exposed to PGMs in order to protect the workers' health. These companies include refineries, catalyst manufacturers as well as other industries utilising PGMs during production.

- Skin exposure should be monitored in conjunction with respiratory exposure monitoring to identify work areas or work practices with skin exposure to PGMs. The removal method using GhostWipes^{TM} as recommended by the ISO standard (ISO/TR 14294:2011) and also previously utilised for dermal sampling of metals such as nickel and cobalt, could be used to determine skin exposure to PGMs (Du Plessis *et al.,* 2010). These wipes should be analysed using ICP-MS according to the methods for the determination of hazardous substances (MDHS) method 46/2 (HSE, 1996) by an accredited laboratory. The dermal exposure should be calculated by dividing the total mass of contaminant (µg) by the area wiped (cm^2) to yield exposure in $\mu g/cm^2$.

- Surface sampling should be included on a regular basis to determine if surfaces are contaminated. These surfaces should include control rooms, door handles, stair railings, lockers in the change house, canteen surfaces and other areas frequently touched with bare hands. If contaminated, these surfaces should be cleaned at regular intervals to prevent skin contact. The cleaning method should be evaluated by wipe testing the surface with the colorimetric technique explained below. If there is a reaction on the wipe test, the surface should be cleaned again.

- A colorimetric technique could be used to determine surface contamination immediately without having to wait for laboratory analysis results. Sodium borohydride ($NaBH_4$) reacts with platinum species and converts it into metallic platinum, which is insoluble and non-toxic. This reaction turns the metallic platinum black, often forming small grains. A 1% sodium borohydride solution can be sprayed onto surfaces and wiped with tissue paper. If the sodium borohydride solution wipe test turns black, it provides a qualitative indication that platinum species are present on the surface.

- Provide appropriate personal protective equipment (PPE), such as hardhats and long sleeved acid resistant coveralls without pockets or pocket openings to prevent skin contact. Respirators (FFP3) such as 3M Premium series for metal fume and dust, or in extreme cases supplied air respirators, should be provided. Mechanical protective gloves such as PVC or rubber gloves of elbow length should be provided to ensure adequate protection of the hands and wrists. Gloves should not contain any knitted material as dust particles could penetrate this material. In addition, workers should be encouraged to wear nitrile disposable gloves underneath mechanical protective gloves to further prevent skin contamination. These disposable gloves will also prevent skin contact of the hands where workers have to remove the large gloves to perform fine motor skills with the fingers.

- Employers should provide appropriate training on the correct procedure for donning and doffing of PPE. Specific instructions should be provided on how to remove contaminated PPE such as gloves to prevent skin contact. It is important to caution workers not to touch contaminated areas of PPE such as the palm or finger areas of gloves after removal.

- Workers should be encouraged to shower after the work shift to decontaminate their skin, and thereby preventing the 'take-home' effect as skin contamination that continues after the work shift could cause prolonged skin permeation. Workers should not be allowed to take any clothing or PPE home with them.

- Potential racial differences in skin barrier and susceptibility to skin conditions should be considered during risk assessments. Employers should not assume the skin of African workers to be more resistant with an enhanced barrier function.

6.3 Limitations of the *in vitro* skin permeation experiments

- The results obtained during these *in vitro* skin permeation studies are limited to one platinum and one rhodium salt, and the permeability findings of these metals should be viewed in this context.

- During the *in vitro* experiments the skin was exposed to solutions for the duration of the experiment, which was 24 hours, thereby over-hydrating the skin. Excessive hydration of the skin could potentially increase permeation. However, literature regarding the effect of hydration and occlusion on permeability is contradictory. Increasing hydration of the skin increases the penetration rate of certain substances, such as nicotinates, whereas the permeation of other substances, such as caffeine, is not affected (Treffel *et al.,* 1992; Zhai *et al.,* 2002; Benson, 2005). In actual workplace conditions the skin would rarely be exposed to solutions constantly, and certainly not for 24 consecutive hours. As a result of over-hydration, results obtained in these *in vitro* studies could possibly overestimate actual skin permeation, but this would be dependent on the substance applied to the skin. However, significant permeation already occurred after eight hours of exposure *in vitro*, and therefore these results could be useful to indicate possible permeation as a result of workplace exposure. The possibility of overestimation should be considered when results are reported and interpreted. However, the duration of 24 hours is necessary to obtain a permeation profile and calculate a flux or permeability coefficient.

- The excised skin used during *in vitro* experiments is dead without active transport processes and metabolism and therefore lack the normal active processes in living cells and tissue. In addition the sink conditions of the peripheral blood flow may not be fully reproduced *in vitro* (OECD, 2004; Larese Filon *et al.,* 2011). Therefore *in vitro* experiments using excised skin do not consider the influence of living processes such as metabolism (Environmental Protection Agency, 1992; Larese Filon *et al.,* 2011). This could potentially influence permeability and lead to discrepancies in permeation between *in vitro* experiments and *in vivo* conditions. In the pharmaceutical industry the permeation of topical medications are often tested by comparing *in vitro* and *in vivo* results. However, this is not possible for PGM permeability since PGMs are potential sensitisers and therefore cannot be topically administered *in vivo*. In addition, the manipulation of the skin to insert it into diffusion cells could possibly damage the skin, which would also lead to discrepancies in permeation results (Barry, 2007). Therefore the skin condition should be considered when extrapolating *in vitro* results to workplace conditions.

- The majority of *in vitro* studies utilise normal skin with a presumed intact barrier. Therefore results obtained with intact skin would underestimate the possible permeation occurring in the workplace, since workers are likely to have mechanical damage to their skin, which would increase the permeation through the skin.

- The *in vitro* experiments utilised in this thesis extended over 24 hours, with the first removal only after six hours. Therefore, these experiments did not investigate the skin permeability of possible

short term exposures, such as splashes of high concentration PGM solutions directly onto the skin. This is discussed further in Section 6.4 of this chapter as a recommendation for future studies.

- In general *in vitro* experiments determine permeation over 24 hours of exposure. However, in the workplace workers will be exposed for eight hours, or in some cases a maximum of 12 hours. Therefore results could overestimate the risk involved with dermal exposure to the metal. In addition, workers are likely to wash their hands during the day; therefore exposure should not be continuous. However, Julander *et al.* (2010) reported detectable levels of metals (Ni, Co, Cr) on the skin using an acid wipe sampling technique after workers cleaned their skin with soap and water. Washing the skin was not effective in removing all the contaminants, and the possibility of continuous exposure exists.

- Franz cells are static, whereas in occupational settings the skin will be flexed at certain areas, as at the wrist and finger digits, which would provide kinetic energy moving particles into the skin. The flexing motion has been proven to result in permeation of particles into the dermis, where no permeation was possible without flexing (Tinkle *et al.,* 2003; Rouse *et al.,* 2007). In the workplace the flexing motion could enhance permeation of metals that are already permeable. Therefore the *in vitro* results obtained without flexing may underestimate the permeability of metals in workplace conditions.

6.4 Future studies

- Future studies should investigate the skin permeation and skin retention of PGMs after short term exposure to simulate workplace conditions such as splashes or spillages. For these studies higher concentrations should be used to compensate for detection limits of analysis methods. It is recommended to use three times the current concentration, therefore a concentration of 0.9 mg PGM/ml, to attempt to obtain results quantifiable by the analysis method. The PGM solution should be applied for 30 minutes, whereafter the skin should be cleaned with synthetic sweat and cotton swabs. Synthetic sweat without PGMs should be placed on the skin after cleaning to simulate continuous sweating in workplace conditions and the receptor solution removals should continue for 24 hours. This will indicate the skin permeability after short term exposure to high concentrations and the possible continued permeation after decontamination of the skin.

- The influence of damaged skin on permeation of PGMs should be investigated by comparing the permeation and retention of damaged skin with that of intact skin. The skin should be abraded by using a 19-gauge hypodermic needle and drawing across the surface two times in one direction, and

two times perpendicular as suggested by Bronaugh and Stewart (1985). This method was also utilised by Larese Filon *et al.* (2009a; 2009b), although the skin was abraded with forty marks in total in their experiments.

- The contribution of chloride atoms to the permeability of platinum could be investigated by comparing the permeability of different platinum salts since the presence of a counter ion such as chloride could influence permeation. Therefore, future studies should compare the permeation of a hexachloroplatinate compound, such as ammonium hexachloroplatinate, with a tetrachloroplatinate compound, such as potassium tetrachloroplatinate. This will also indicate if permeability of the salts could contribute to the allergenicity of the salts, since hexachloroplatinate is considered to be a more powerful sensitiser (Cristaudo *et al.,* 2005).

- Future studies should continue to include skin from different races to determine differences in *in vitro* permeability of metals. For the South African context the use of Indian skin could also be included, but this will be dependent on the availability of skin donors. The potential difference in skin permeation of rhodium and other PGMs between African and Caucasian should be investigated in future.

- The influence of long term hydration on permeability could be investigated by comparing different volumes applied to the skin. In one experiment the skin could be exposed to 1 ml of the metal dissolved in synthetic sweat for 24 hours as reported in Chapter 4 and 5. Another experiment could include the same concentration of PGM salt in 0.1 or 0.2 ml of synthetic sweat, allowing the sweat to evaporate, but continuing the experiment for 24 hours. The second experiment will simulate workplace conditions where splashes onto the skin is possible, but where the solution will be able to evaporate and thereby leave the PGM in a powder form on the skin.

- Flexing of the skin could be included in future studies to determine if flexing, as seen at the wrist and finger digits, will influence permeation of platinum and rhodium.

- The concentrations of metal salts used in these *in vitro* studies could be compared to the concentrations used *in vivo* in patch testing for platinum and rhodium sensitivity.

6.5 References

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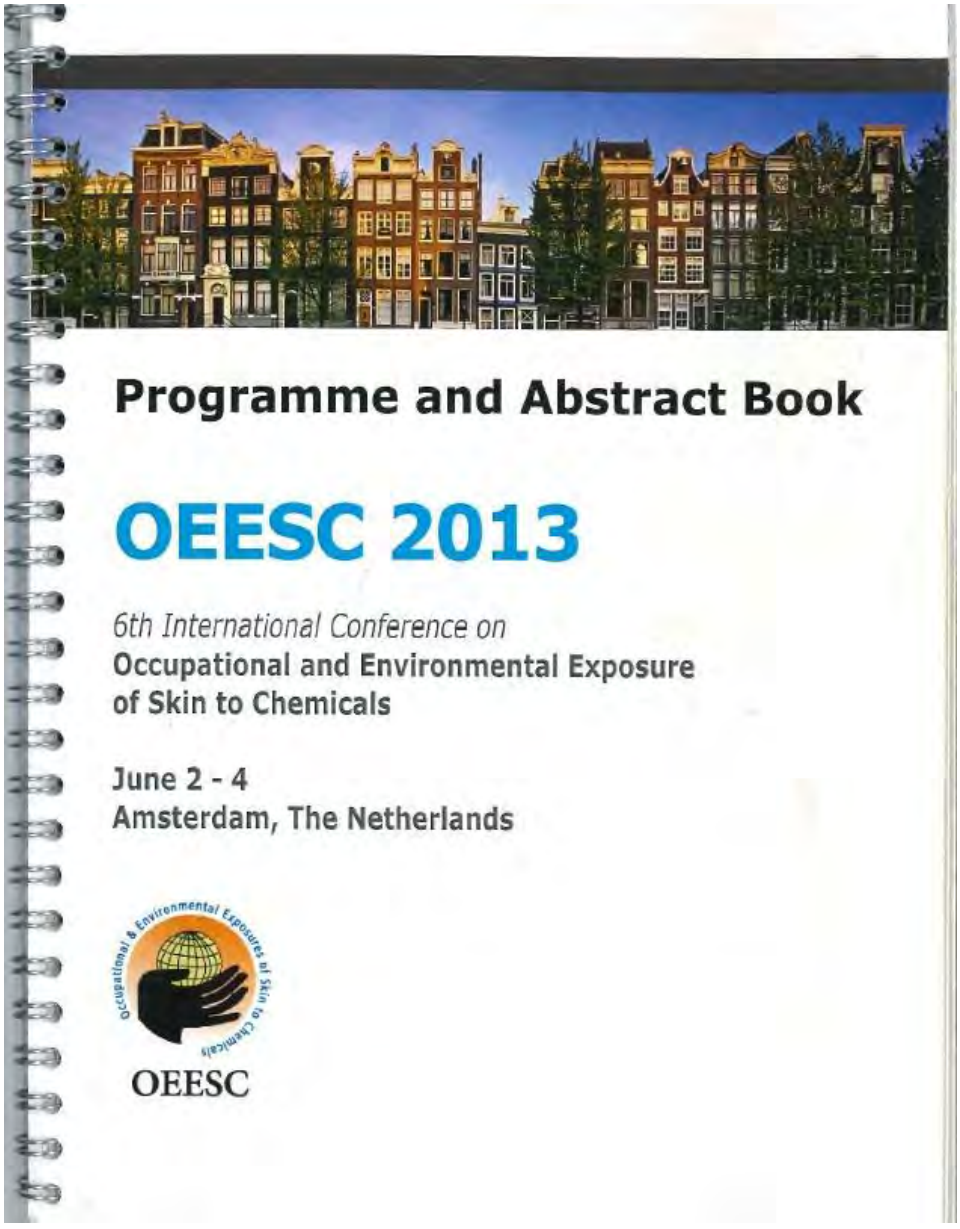
Appendix A: Contributions at conferences

A.1. International Conference – oral presentation

Occupational and Environmental Exposure to Skin Chemicals (OEESC). 6th International Conference. 2-4 June 2013. Amsterdam, The Netherlands. Oral presentation: *In vitro* percutaneous absorption of a platinum salt through African and Caucasian skin. Franken, A., Du Plessis, J.L., Eloff, F.C., Du Plessis, J., Badenhorst, C.J., Van der Merwe, C.J., Laubscher, P.J.



Abstract submitted:

83 (Oral)

In vitro percutaneous absorption of a platinum salt through African and Caucasian skin.

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Introduction: The possibility of dermal absorption of platinum group metals has come into question as an alternative route of exposure leading to sensitisation. Dermal absorption of nickel, cobalt, chromium and gold, to name just a few, have been proven through Caucasian skin. Studies have shown conflicting evidence of differences in skin permeation between Caucasian and African skin, nonetheless no in vitro studies have been done to prove or contradict this. The aim of this study is to determine if platinum is permeable through intact human skin, and if there is a difference in skin permeation between intact Caucasian and African skin.

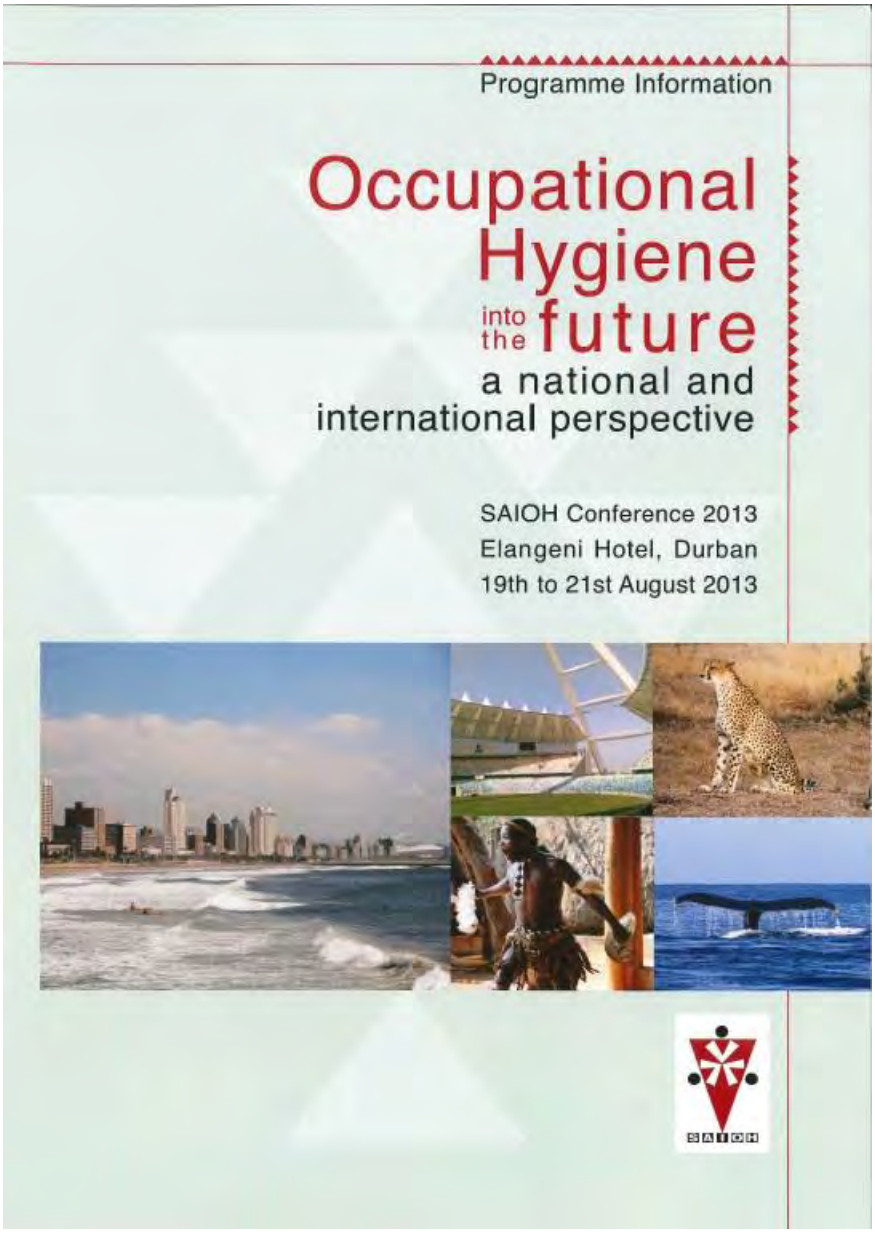
Material & methods: Female human abdominal skin obtained after cosmetic procedures from two Caucasian (44 and 48 years) and two African (38 and 41 years) donors were used. A suspension of 32.33 mg K2PtCl4 in 50 ml of synthetic sweat (pH 6.5) was prepared and applied to the outer surface of the skin in a vertical Franz diffusion cell. The concentration of the metals in the physiological solution (pH 7.35) placed inside the receptor phase, was determined by high resolution ICP-MS after extraction at various intervals during the 24 hours of the study. After completion of the study the skin was digested by using hydrogen peroxide, nitric acid and hydrochloric acid during different steps, so that the concentration of the metals remaining in the skin could be determined by ICP-OES.

Results: Preliminary results indicated that platinum started to permeate through the skin at 1 hour after the start of exposure, and reached a peak at approximately 6 hours. There was a 41.8 % increase in permeation from 1 to 6 hours for Caucasian skin, and an increase of 67.4 % for African skin. These results indicate a possible difference in permeation between Caucasian and African skin, and further data analysis is in progress.

Conclusion: This study confirmed that dermal exposure to platinum salts is of concern as it is able to penetrate through intact Caucasian and African skin and is also detained inside the skin in an in vitro diffusion cell system.

A.2. National Conference – oral presentation

Southern African Institute for Occupational Hygiene (SAIOH) Annual Conference. 19-21 August 2013. Durban, South Africa. Oral presentation: *In vitro* permeation of a platinum salt through intact Caucasian skin: preliminary results. Franken, A., Du Plessis, J.L., Eloff, F.C., Du Plessis, J., Badenhorst, C.J., Van der Merwe, C.J., Laubscher, P.J., Van der Merwe, A., Ramotsehoa, C.



Abstract submitted:

Title: *In vitro* percutaneous absorption of a platinum salt through intact Caucasian skin: preliminary results.

Anja Franken, Johan L du Plessis, Fritz C Eloff, Jeanetta du Plessis, Cas J Badenhorst, Cornelius J Van der Merwe, Petrus J Laubscher, Alicia Van der Merwe, Cynthia Ramotsehoa

Purpose: The possibility of dermal absorption of platinum group metals has come into question as an alternative route of exposure leading to sensitisation. Dermal absorption of nickel, cobalt, chromium and gold, to name just a few, has been proven through intact Caucasian skin. The aim of this study is to determine if platinum is permeable through intact Caucasian skin.

Methods: Abdominal skin obtained after cosmetic procedures from two female Caucasian donors were used. A suspension of 19.4 mg K_2PtCl_4 in 30 ml of synthetic sweat (pH 6.5) was prepared and applied to the outer surface of the skin in a vertical Franz diffusion cell. The concentration of the metals in the physiological solution (pH 7.35) placed inside the receptor phase, was determined by high resolution ICP-MS after extraction at various intervals during the 24 hours of the study. After completion of the study, the skin was rinsed four times using 1 ml of synthetic sweat each time to remove any platinum remaining on the skin surface. The skin was removed from the Franz cell and sent to an analytical laboratory where it was digested using H_2O_2, HNO_3 and HCl during different steps so that the concentration of the metals remaining in the skin could be determined by ICP-OES.

Results and Discussion: Preliminary results indicate that platinum already permeates through the skin at 2 hours after the onset of exposure which indicates that short exposure to platinum can lead to absorption through the skin. The concentration that permeates through the skin increases after 6 hours of exposure and platinum is also retained inside the skin after 24 hours of exposure.

Conclusions: This study confirmed that dermal exposure to platinum salts is of concern as it is able to penetrate through intact Caucasian skin and platinum is also retained inside the skin in an *in vitro* diffusion cell system.

A.3. International Conference – poster presentation

Perspectives in Percutaneous Penetration 14th International Conference. 23-25 April 2014. La Grande Motte, France. Poster presentation: *In vitro* permeation of platinum and rhodium through Caucasian skin. Franken, A., Eloff, F.C., Du Plessis, J., Badenhorst, C.J., Van der Merwe, C.J., Ramotsehoa, C., Van der Merwe, A., Du Plessis, J.L.

IN VITRO PERMEATION OF PLATINUM AND RHODIUM THROUGH INTACT CAUCASIAN SKIN

A. Franken[1], F.C. Eloff[1], J. Du Plessis[2], C.J. Badenhorst[1], C.J. Van der Merwe[1], C. Ramotsehoa[1], A. Van der Merwe[1], J.L. Du Plessis[1].

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Background

The possibility of dermal permeation of platinum group metals has come into question as an alternative route of exposure leading to sensitisation in the workplace[1]. Maynard *et al.* (1997) suggested the dermal route since refinery workers developed sensitisation, despite respiratory exposure found to be below the OEL. Dermal permeation of nickel, cobalt, and chromium[2-3], has been proven through intact Caucasian skin. The aim of this study was to investigate platinum and rhodium permeation through intact Caucasian skin.

Methods

- Abdominal skin was obtained from four female Caucasian donors after cosmetic procedures .
- The study was approved by the NWU Ethics Committee (NWU-00114-11-A5).
- Vertical Franz diffusion cells with an exposed skin area of 1.066 cm^2 were used, with full thickness skin (< 1 mm).
- Skin integrity was tested with a Tinsley LCR Data bridge Model 6401.
- The donor solutions of 32.46 mg K_2PtCl_4 in 50 ml of synthetic sweat (pH 6.5), and 43.15 mg $RhCl_3$ in 50 ml of synthetic sweat (pH 6.5) were prepared with a final concentration of 0.3 mg/ml.
- Physiological solution (pH 7.35) was placed inside the receptor phase and removed at specific time intervals.
- After 24 hour exposure the skin was rinsed four times to remove platinum or rhodium remaining on the skin surface.
- Solutions were analysed by ICP-MS and digested skin solutions were analysed by ICP-OES.

Results

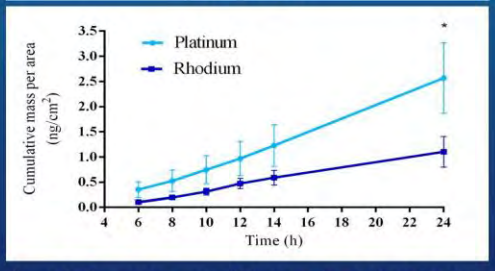

Figure 1: Cumulative mass of platinum (n = 15) and rhodium (n = 11) that permeated per area (mean ± SEM) (* p = 0.016).

Table 1: Platinum and rhodium concentrations in the receptor solution, retained inside the skin and permeation (Mean ± SEM).

	Receptor solution	Skin		Permeation
	Percentage diffused (%)	Mass retained inside skin (ng/cm^2)	Percentage in the skin (%)	Flux (ng/cm^2/h)
Pt	$2.3 \times 10^{-4} \pm 0.5 \times 10^{-4}$	1459.47 ± 99.42	2.24 ± 0.15	0.12 ± 0.02
Rh	$1.0 \times 10^{-4} \pm 0.3 \times 10^{-4}$	757.04 ± 70.43	1.16 ± 0.11	0.05 ± 0.01
p	**0.016**	**< 0.001**	**< 0.001**	**0.015**

Discussion & Conclusion

- Platinum and rhodium permeation through intact Caucasian skin in an *in vitro* diffusion system occurred.
- Within 3.5 and 4.4 hours respectively platinum and rhodium permeated through the skin (lag time).
- Platinum permeation and retention in the skin was significantly higher than that of rhodium.
- Permeation increased cumulatively with prolonged exposure, therefore thorough cleaning of the skin is imperative to prevent continued exposure and further permeation.
- The permeated and skin retained platinum and rhodium may therefore contribute to sensitisation and indicates a health risk associated with dermal exposure in the workplace.

References

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