In vitro permeation of potassium hexachloroplatinate through full thickness human abdominal skin

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Dissertation accepted in fulfilment of the requirements for the degree Master of Health Sciences in Occupational Hygiene at the North-West University

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Graduation:	December 2021
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ACKNOWLEDGEMENTS

The author of this research study would like to express her gratitude to all who contributed to the execution of this study especially;

***Prof A Franken**,* for her remarkable guidance and support throughout the research process.

Mr CJ van der Merwe, for his well-timed questions that helped me search for more understanding in the field.

Prof JL du Plessis, for his patience and willingness to make sure everyone stays on track.

The Occupational Hygiene and Health Research Initiative (OHHRI), for all the encouragement and support throughout the years.

All my friends and family, for their incredible support and love.

All the surgeons, their staff, and the donors, without whose cooperation and donations this project would not have been possible.

The International Platinum Group Metals Association, with special thanks to Mr Mark Hosford, for their patience and funding of this project.

Johnson Matthey Technology Centre (Sonning Common, UK), with special thanks to Dr Peter Ash and Mrs Raquel Garcia Sanchez, for their professionalism, patience and for analyzing the samples produced. Furthermore, for the organization, and considerable effort put into the symposium discussing the analysis essay, various difficulties in experimentation and additional considerations for current and future studies.

PREFACE

This mini-dissertation is written in article format, with referencing throughout following the rules and language set out by the *Toxicology Letters* journal for uniformity. This particular journal was chosen as a possible publication journal for the manuscript/article elaborated in this mini-dissertation. The journal does not have specific reference guidelines for new submissions and thus any reference format may be used. Consistency is, however, emphasized throughout. Author(s) name(s), journal title/ book title, chapter title/ article title, year of publication, volume number/ book chapter and the article number or pagination should be added where applicable and the use of Digital Object Identifier (DOI) system and American (English) spelling are encouraged.

The detailed author guidelines for the *Toxicology Letters* journal can be found in Chapter 3 (p. **51-53**).

The language of choice for the mini-dissertation is based on American spelling throughout except for direct quotations, or names in references originally written using British spelling.

The outline of the mini-dissertation is as follows:

Chapter 1 – For introductory purposes, this chapter contains the problem statement, aims and objectives as well as the hypothesis of the study.

Chapter 2 – A literature review composed of detailed information on the relevant topics of the study.

Chapter 3 – A manuscript/article to be submitted to *Toxicology Letters* for possible publication entitled '*In vitro* permeation of potassium hexachloroplatinate through full thickness human abdominal skin'.

Chapter 4 – A concluding chapter including the recommendations and limitations of the study.

Chapter 5 – Annexures containing certificates regarding language editing Turnitin (similarity/plagiarism) report and ethical approval.

In order to prevent confusion, the following definitions are presented:

Exogenous compound: A chemical originating from outside the living organism.

Absorption: The mass of exogenous compound that moved through the full thickness of the skin membrane, human or otherwise, and detected in the blood, synthetic or otherwise.

Penetration: The mass of exogenous compound found retained in the skin barrier without diffusing into the blood, synthetic or otherwise.

Permeation: The movement of a compound through different layers of the skin.

Platinum salts: Otherwise known as soluble platinum compounds, are chemical platinum compounds of a soluble nature. Examples included K_2PtCl_6, K_2PtCl_4, Na_2PtCl_6, Na_2PtCl_4, H_2PtCl_6 and $(NH_4)_2[PtCl_6]$.

AUTHORS' CONTRIBUTIONS

The research study was planned and executed by a research team. The individual team members' contributions are listed in Table 1.

Table 1. Contributions by each listed author.

Author	Contribution
Ms BS Bosch	• Planning the study. • Compiling the proposal submitted for ethical and scientific approval. • Completing paperwork necessary for ethical approval. • Setting up and maintaining budget. • Conducting literature research. • Conducting laboratory experiments as per *in vitro* methodology training. • Managing research laboratory. • Statistical analysis of the data. • Interpretation of data results. • Formulating recommendations. • Writing the mini-dissertation, including the manuscript. • Preparing for and attending Johnson Matthey symposium. • Writing of International PGM Association report.
Prof A Franken	• Supervisor. • Assisting with the planning and design of the study, provided laboratory training, developed and approved the study protocol, selection of statistical analysis methods, and review of the results and mini-dissertation. • Preparing for and attending Johnson Matthey symposium. • Co-author of International PGM Association report.
Prof JL du Plessis	• Co-supervisor. • Assisting with the planning of the study, executed administrative duties and review of the mini-dissertation. • Assisting with the review of the International PGM Association report.
Mr CJ van der Merwe	• Assistant supervisor. • Moral and technical support.

The following is a statement from the co-authors that confirms each individual's role in the study:

I declare that I have approved the manuscript 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 BS Bosch's MHSc (Occupational Hygiene) mini-dissertation.

Prof A Franken	Prof JL du Plessis	Mr CJ van der Merwe
(Supervisor)	(Co-supervisor)	(Assistant supervisor)

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LIST OF SYMBOLS AND ABBREVIATIONS

ACGIH	American Conference of Governmental Industrial Hygienists
ANOVA	Analysis of variance
Cl^-	Chloride
DEL	Department of Employment and Labor
DECOS	Dutch Expert Committee on Occupational Standards
DLS	Dynamic Light Scattering
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic Acid
DOI	Digital Object Identifier
DoL	Department of Labor
STATS SA	Department of Statistics South Africa
FFP3	Filtering face piece 3
GM	Geometric mean
HCl	Hydrochloric acid

HEPA	High Efficiency Particulate Air
HNO_3	Nitric acid
HSE	Health and Safety Executive
H_2O_2	Hydrogen peroxide
H_2PtCl_6	Chloroplatinic acid
ICMM	International Council on Mining and Metals
ICP-MS	Inductively Coupled Plasma-Mass Spectrometry
ICP-OES	Inductively Coupled Plasma-Optical Emission Spectrometry
IgE	Immunoglobulin E
IPA	International Platinum Group Metals Association
IPCS	International Program on Chemical Safety
Ir	Iridium
ISBN	International Standard Book Number
ISO	International Organization for Standardization
JM	Johnson Matthey
KH_2PO_4	Potassium dihydrogen phosphate
K_2PtCl_6	Potassium hexachloroplatinate
K_2PtCl_4	Potassium tetrachloroplatinate
LEV	Local exhaust ventilation
MDHS	Methods for the Determination of Hazardous Substances
MHS	Mine Health and Safety
MHSA	Mine Health and Safety Act
MDI	Diphenylmethane-4,4-diisocyanate
MW	Molecular Weight
Na	Sodium
$NaBH_4$	Sodium borohydride
NaCl	Sodium chloride
Na_2HPO_4	Disodium hydrogen phosphate

Na_2PtCl_6	Disodium hexachloroplatinate
$(NH_4)_2[PtCl_6]$	Ammonium hexachloroplatinate
NIOSH	National Institute for Occupational Safety and Health
NP	Nanoparticles
NWU	North-West University
OECD	Organization for Economic Co-operation and Development
OEL	Occupational Exposure Limit
OHS	Occupational Health and Safety
Os	Osmium
OsO_4	Osmium tetroxide
PbO	Inorganic Lead
Pd	Palladium
PGM	Platinum Group Metals
PPE	Personal protective equipment
Pt	Platinum
$PtCl_4$	Tetrachloroplatinate
$PtCl_6$	Hexachloroplatinate
Rh	Rhodium
$RhCl_3$	Rhodium chloride
Ru	Ruthenium
SEM	Standard Error of Means
Sen	Sensitization notation
SK	Skin notation
TEM	Transmission Electron Microscopy
UK	United Kingdom
UV	Ultraviolet
WHO	World Health Organization

STANDARD UNITS

pKa	Acid dissolution constant
˚C	Celsius
cm	Centimeter
cm/h	Centimeter per hour
Da	Dalton
g	Gram
g/mol	Gram per mole
>	Greater than
≥	Greater than or equal to
h	Hours
kHz	Kilo-Hertz
kΩ	Kilo-ohm
<	Less than
≤	Less than or equal to
L	Liter
µg/cm^2	Microgram per square centimeter
µg/m^3	Microgram per cubic meter
µg/g	Microgram per gram
mg	Miligram
mg/L	Milligram per liter
mg/mL	Miligram per milileter
mg/m^3	Miligram per cubic meter
mL	Milliliter
mm	Millimeter
min	Minutes
M	Mole

ng/cm^2	Nano-gram per square centimeter
$ng/cm^2/h$	Nano-gram per square centimeter per hour
ng/L	Nano-gram per liter
%	Percentage
K_p	Permeability Coefficient
±	Plus-minus
pH	Potential of hydrogen

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SUMMARY

Title: *In vitro* permeation of potassium hexachloroplatinate through full thickness human abdominal skin.

Background: During the processing and refining of platinum (Pt), workers are exposed to respiratory sensitizing platinum salts. The most toxic of these platinum salts are tetra- and hexachloroplatinate, especially when attached to potassium. Although multiple researches have associated the respiratory exposure route with the development of respiratory sensitization to platinum salts, the notion of an additional dermal route of exposure was suggested. This notion was suggested due to active dermal exposure to these platinum salts in many instances and the high sensitization occurrence during low levels of respiratory exposure. Dermal exposure has previously been associated with the development of respiratory sensitization in animal studies Subsequent *in vitro* studies investigated the permeation of potassium tetrachloroplatinate through intact abdominal skin and substantiated the plausibility of the dermal route of exposure contributing to platinum absorption. However, the more toxic sensitizer, potassium hexachloroplatinate has not been investigated regarding its permeation profile.

Aims and objections: *In vitro* laboratory experiments were conducted to investigate the permeation profile of potassium hexachloroplatinate (K_2PtCl_6) through full thickness human abdominal skin. The research objectives were to quantify the permeation of potassium hexachloroplatinate by utilizing the static Franz diffusion cell method, to calculate the percentage of platinum retained in the skin, and lastly, to evaluate the effect of exposure duration on the permeation of platinum at 8-, 12- and 24-hour intervals.

Methods: Intact abdominal skin from Caucasian female donors, aged between 41 and 48, were obtained after abdominoplasty procedures following ethics approval and informed consent. Utilizing the Franz diffusion compartment methodology, the permeation of 0.3 mg/mL K_2PtCl_6 was quantified at time intervals of 2-, 4-, 6-, 8-, 10-, 12-, 14-, and 24-hours after the onset of the experiment. After the 24-hour extraction, the skin was digested and analyzed by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), quantifying the penetrated platinum mass inside the skin. Whereas the permeated platinum mass in the receptor solution was quantified by the means of Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Blank cells not containing the platinum salt, were used for quality control purposes.

Results: Platinum permeation, increasing with the continued duration of exposure, occurred through the intact full thickness female Caucasian abdominal skin. The influence of time on permeation was significantly indicated by the 68 % increase in dermal absorption between 8

and 12 hours, and 62 % increase between 12 and 24 hours. The dose percentage of platinum mass contained in the skin after 24-hours was calculated as 3.11 ± 0.25 % (8848.03 ± 699.02 ng). Flux and lag time were calculated as 0.32 ± 0.05 ng/cm^2/h and 2.26 ± 0.31 hours, respectively.

Conclusion: With the results all aims and objectives were achieved. Results indicating a considerable platinum reservoir forming in the skin together with the short lag time implicated that continuous real-world permeation may occur adding to the body burden of platinum even after removal from the source of exposure. The presence of detectable levels of platinum mass in the receptor fluid at the 2-hour interval further suggested that even short periods of exposure to potassium hexachloroplatinate presents a risk of permeation into the body. Significant increase in mean permeation between 8 and 12 hours was substantial as extended work shifts are commonly implemented. Furthermore, exposure circumstances such as the so called 'take-home' effect and the layering of new deposition onto previous deposition on the skin, emphasize the importance of proper decontamination protocols. Indications were made towards a shorter lag time and heightened cumulative mass parameter when utilizing potassium hexachloroplatinate compared to other PGM research. Recommendations were made based on the real-world implications of the results in occupational settings to be applied by future PGM permeation investigations.

Keywords: Franz diffusion cells; occupational health; platinum group metals; platinum salts; skin retention.

CHAPTER 1: GENERAL INTRODUCTION

1.1 Problem statement

Platinum is a precious metal that is part of the Platinum Group Metals (PGMs), based on similarities between these metals. The other PGMs are palladium, rhodium, ruthenium, osmium and iridium (Ravindra *et al*., 2004; Wiseman and Zereini, 2009). The use of platinum has extended over numerous years and includes the manufacturing of jewelry, dentistry instruments and catalysts, to name a few (Hunter *et al*., 1945; Kielhorn *et al*., 2002). According to the most recent PGM reports, a consistent average of 73 % of the platinum used by the above-mentioned industries originates from South Africa, where it is mined and refined (JM, 2018, 2019, 2020). According to the South African Minerals Council, there were 163 538 workers directly employed in the South African platinum mining industry in 2020 (Minerals Council South Africa, 2021). During the processing and recycling of platinum refinery, workers are exposed to platinum salts (Hunter *et al.*, 1945; Kielhorn *et al.*, 2002; Ravindra *et al*., 2004). The most well-known and toxic of these platinum salts are tetrachloroplatinate and hexachloroplatinate, especially when attached to potassium (Ravindra *et al*., 2004; Bencs *et al*., 2011). Adverse health effects associated with these platinum salts include respiratory sensitization presenting as asthma and rhinitis, and dermal manifestations of urticaria, itching, and dermatitis (Hunter *et al*., 1945; Calverley *et al*., 1995; Merget *et al*., 2000).

Multiple studies have shown the association between inhalation exposure of platinum salts and respiratory sensitization (Maynard *et al.*, 1997; Ravindra *et al*., 2004; Cristaudo *et al*., 2007; Colombo *et al*., 2008; Wiseman and Zereini, 2009; Heederik *et al*., 2016). A study found that the magnitude of the sensitization reactions was, however, not justified by the level of inhalation exposure alone, which was below the legal respiratory occupational exposure limit (OEL) (Maynard *et al*., 1997). This led to the notion that an additional exposure route is supplementing the respiratory sensitization to these platinum salts (Maynard *et al*., 1997; Heederik *et al*., 2016; NIOSH, 2016). This was substantiated by *in vivo* studies, conducted on mice and guinea pigs, which proved respiratory sensitization by exclusively dermal exposure, thus concluding that respiratory sensitization was not a manifestation of inhalation exposure alone (Schuppe *et al*., 1997; Kimber and Dearman, 2002). During these experiments, Schuppe *et al.* (1997) also indicated that dermal exposure to disodium hexachloroplatinate (Na_2PtCl_6) exacerbated respiratory sensitization effects in mice. Thus, the dermal route became the focus of interest for the suspected additional route of worker exposure (Maynard *et al*., 1997; Heederik *et al*., 2016; NIOSH, 2016).

Resulting from the *in vivo* animal data, *in vitro* utilizing human skin studies were conducted to evaluate the possible dermal permeation of these platinum salts and the factors that may influence the permeation. These studies reported that absorption by means of a dermal route is plausible as permeation occurred through intact human abdominal skin. The duration of dermal exposure was evaluated by these studies as significantly influential to the total permeated platinum mass. This was determined on the basis that the cumulative mass increased with time, and the statistically significant increase in mass permeated between eight and 12 hours (Franken *et al*., 2014, 2015; Van Nieuwenhuizen, 2016). The positive association between exposure time and mass permeation has been indicated by other experiments as well (Swarbrick *et al*., 1982; Larese Filon *et al*., 2006, 2011; Barbero and Frasch, 2016), and warrants consideration in further platinum permeation studies. Further observations deducted from the *in vitro* results were the considerable amount of platinum mass retained inside of the skin (Franken *et al*., 2014, 2015; Van Nieuwenhuizen, 2016). However, the research on platinum permeation previously mentioned, solely focused on potassium tetrachloroplatinate leaving the more reactive potassium hexachloroplatinate's permeation profile in question. Hexachloroplatinate is more reactive and thus more toxic than tetrachloroplatinate based on the number of halide bonds (i.e. Cl^-) (Cleare *et al*., 1976). Speciation has been proven to influence dermal permeation, such as during experimentation with chromium salts (Gammelgaard *et al*., 1992; Zhang *et al*., 2012) and thus warrants experimentation on both compounds (Linnett and Hughes, 1999; Van Briesen *et al*., 2010). The proposed study, therefore, aimed at providing more information on the *in vitro* permeation of potassium hexachloroplatinate through full thickness human abdominal skin by utilizing Franz diffusion cells. Static Franz diffusion cells utilized with intact human abdominal skin have been applied in multiple experiments to evaluate permeation of a variety of compounds (Franz, 1975; Larese Filon *et al*., 2007; Ng *et al*., 2010; Mauro *et al*., 2015; Oh *et al.*, 2020).

1.2 Research aim and Objectives

The aim of the study was to investigate the *in vitro* permeation of potassium hexachloroplatinate (K_2PtCl_6) through full thickness human abdominal skin.

The research objectives of this study were:

1. To utilize the static Franz diffusion cell method to quantify the *in vitro* permeation of potassium hexachloroplatinate (K_2PtCl_6) through intact human abdominal skin.
2. To calculate the percentage of platinum retained in the skin after 24 hours of exposure.
3. To investigate the influence of exposure time at 8-, 12- and 24-hour intervals on dermal permeation of potassium hexachloroplatinate.

1.3 Hypothesis

Previous research on the sensitization effects of platinum salts indicated the possibility of a dermal exposure route (Maynard *et al*., 1997; Franken *et al*., 2014; Heederik *et al*., 2016; NIOSH, 2016). This was based on observations of platinum salt sensitization with low inhalation exposure (Maynard *et al*., 1997), as well as animal studies proving respiratory sensitization (Schuppe *et al*., 1997) and exacerbation of respiratory sensitization following dermal exposure (Schuppe *et al*., 1997). Developing from these results, subsequent *in vitro* dermal permeation studies were based on potassium tetrachloroplatinate (Franken *et al.*, 2014, 2015; Van Nieuwenhuizen, 2016) and nano-sized sodium hexachloroplatinate (Mauro *et al*., 2015) but no data is available on the more reactive potassium hexachloroplatinate. Previous *in vitro* literature based on potassium tetrachloroplatinate (K_2PtCl_4), proved that platinum permeates through full thickness human abdominal skin (Franken *et al.*, 2014, 2015; Van Nieuwenhuizen, 2016). It is, therefore, hypothesized that potassium hexachloroplatinate (K_2PtCl_6) permeates through intact human abdominal skin at a quantifiable level when utilizing *in vitro* methods. For this purpose, a quantifiable level is defined as a value above the detection limit of the analytical method for platinum (> 1 ng/L).

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CHAPTER 2: LITERATURE STUDY

2.1 Introduction

This chapter is aimed at providing an understanding of platinum permeation through human skin during occupational exposure. The chemical and physical properties of platinum are discussed as well as its industrial use to provide insight into the occurrence of occupational exposure. South African legislation governing the occupational exposure of platinum compounds is considered and the adverse health effects of certain platinum salts experienced by exposed workers are emphasized. In order to assess the exposure, emphasis is placed on the toxicokinetics of platinum salts since existing information is inadequate in clarifying the probable dermal exposure route. Then the dermal route of exposure is discussed in terms of dermal anatomy and physiology. Focus then shifts towards factors influencing dermal permeation and previous Platinum Group Metal (PGM) permeation research. Lastly, the methodology used to evaluate the dermal permeation is discussed.

2.2 Platinum and Platinum salts

2.2.1 Chemical and Physical properties

Platinum was first scientifically described in the eighteenth century as a contaminant of gold. After many experiments, such as the production of platinum into sheets by transforming the metal into potassium chloroplatinate, the scientific community realized its potential as a precious metal (Anderson, 2015). Today, platinum is categorized as a noble metal that is part of the Platinum Group Metals (PGMs) (Mauro *et al*., 2015). Although the focus of this discussion is on platinum and its associated salts, a brief description of the aforementioned metal grouping is appropriate. The PGMs are platinum (Pt), palladium (Pd), rhodium (Rh), ruthenium (Ru), osmium (Os) and iridium (Ir). This grouping is based on specific similarities between the metals (Hunt, 1982; Ravindra *et al*., 2004; Wiseman and Zereini, 2009; Iavicoli *et al*., 2012). These similarities include a silvery white appearance, high melting point, having similar ductile and malleable characteristics, being resistant to corrosion and oxidation, their catalytic activity and having the ability to conduct electricity (WHO, 1991; Cotton *et al*., 1999; Hoppstock and Sures, 2004; Xiao and Laplante, 2004).

Platinum is naturally found in several oxidation states namely Pt^{0}, Pt^{2+}, Pt^{4+} and Pt^{6+} of which Pt^{2+} is the most common (Lindell, 1997; Cotton *et al*., 1999). Due to its multiple oxidative states, it easily forms complexes and platinum salts by attaching to ions or neutral molecules. However, metallic platinum's thermodynamic stability is of such a nature that the reaction conditions necessary to convert the platinum to a higher oxidative state, such as in platinum salt compounds, require extreme oxidative and acidic conditions (Cotton *et al.*, 1999; Brook, 2006).

Platinum refineries often have a more acidic environment and thus platinum salts are commonly formed there. As a metal or attached to oxygen, platinum is insoluble (hydrophobic) but if attached to form a salt, it is water soluble (hydrophilic). Examples of these platinum salts are chloroplatinates, displaying various degrees of water solubility. This variance in water solubility is evident when comparing potassium tetrachloroplatinate (K_2PtCl_4) and its less water-soluble hexa- counterpart (K_2PtCl_6) (Lindell, 1997). Furthermore, platinum containing compounds are commonly recognized by three components over and above the oxidative state namely the ligands stabilizing the center of platinum and the number thereof (referred to as the coordination number), and lastly the degree of aggregates (Brook, 2006). For example, Pt^0 and Pt^{2+} have, under normal circumstances, a coordination number of four, such as potassium tetrachloroplatinate (Cotton *et al*., 1999; Brook, 2006). Potassium hexachloroplatinate (coordination number of six), possesses an oxidation state of Pt^{4+} (Brook, 2006). Decreasing the number of stabilizing ligands leads to aggregation of the particles, this is especially true when Pt^0 is present. Thus, a reasonable prediction could be made that K_2PtCl_4 aggregate to a greater degree than K_2PtCl_6. The collective platinum characteristics make it a valuable metal to a wide variety of industries (Xiao and Laplante, 2004).

2.2.2 Industrial use

Platinum's usage ranges from jewelry making to catalyzing exhaust fumes to ensure emission of less harmful gases into the environment. Platinum jewelry is widely popular due to its silvery white appearance and rarity. Although characterized as one of the precious metals together with gold and silver, platinum's other physical characteristics such as its resistance to molten glass' abrasion is utilized in the medical industry for producing optical glasses. Further medical usage includes the production of cisplatin and carboplatin, compounds used to treat malignant tumors (Hunt, 1982; Xiao and Laplante, 2004) and the production of dental instruments (Hunter *et al*., 1945; IPA, 2017). Other industries, such as the chemical industry, also use platinum's characteristics advantageously in equipment and to increase the octane rating of petroleum/gasoline or hydrogenate alkenes (Hoppstock and Sures, 2004; Xiao and Laplante, 2004; Brook, 2006). Further examples include the use of the catalyzation activities of platinum in a hydrosilylation reaction to crosslink silicone or converting toxic gases (hydrocarbons and carbon monoxide) into less harmful automotive emissions such as carbon dioxide and water (Hoppstock and Sures, 2004; Xiao and Laplante, 2004; Brook, 2006; Rauch and Peucker-Ehrenbrink, 2015). Catalysts in a chemical reaction are recovered in their original form at the end of the reaction (Brook, 2006). Chloroplatinic acid (H_2PtCl_6) was utilized to catalyze the automotive emissions' reaction until recently when an organosoluble Pt^0 was implemented. Due to the extensive application of platinum, the occurrence of the risk towards occupational exposure becomes apparent.

2.2.3 Occupational exposure

During mining, refining, processing, recycling and use of platinum, workers are exposed to a variety of platinum compounds including platinum salts (Linnett and Hughes, 1999; Brook, 2006). In refineries the removal of ammonium hexachloroplatinate salts from the filter in the calcination furnaces requires physical handling of the platinum salts by the workers. This removal is thus a useful example of worker exposure to platinum salts. Further examples are found during recycling phases where materials are placed in aqua regia (HCl and HNO_3 in the ratio 3:1) to draw out the PGMs' chloro-compounds. Thereafter, the chloro-complexes could be converted to metallic forms (Seymour and O'Farrelly, 2012). The proses during the refining of platinum initiates the formation of various platinum compounds/salts during various steps of the proses (Santucci *et al*., 2000), although the specifics of these processes and formations are beyond the scope of this study.

Until recently, the main focus of exposure studies to platinum salts were solely aimed at inhalation exposure. These studies have found that worker inhalation exposure to platinum salts often exceed the legal exposure limits (Calverley *et al.*, 1995; Linnett and Hughes, 1999; Linde *et al*., 2017, 2018a). Furthermore, higher exposure levels were associated with increased probability to develop adverse health effects of these platinum salts (Calverley *et al.*, 1995). However, in areas indicating low levels of inhalation exposure the magnitude of the adverse health effects, i.e. respiratory sensitization and subsequent reactions, were still evident (Maynard *et al*., 1997). This gave rise to theories concerned with an additional route of exposure, such as the skin (Maynard *et al*., 1997; Heederik *et al*., 2016; NIOSH, 2016; Linde *et al*., 2018a). The proposal of dermal involvement in the platinum salt respiratory sensitization was further supported by *in vivo* animal studies that resulted in respiratory sensitization after exclusive dermal exposure towards diphenylmethane-4,4-diisocyanate (MDI) (Rattray *et al*., 1994). This concluded that platinum inhalation exposure is not solely responsible for immune responses towards these sensitizers (Kimber and Dearman, 2002). Schuppe *et al.* (1997) postulated that dermal exposure could not merely cause respiratory sensitization but additionally exacerbate the respiratory sensitization in mice.

In an occupational setting the possible dermal involvement in the development of adverse health effects caused by platinum salts, was further implicated after observations of dermal contact with the platinum salts in the workplace (Maynard *et al*., 1997). In addition, Linde *et al.* (2018a) reported a positive association between dermal and inhalation exposure, and urinary excretion of platinum salts while assessing exposure in two South African precious metals refineries. Furthermore, Linde *et al.* (2018a) indicated that an increase in airborne platinum salts could lead to an increase in the deposition of platinum salts on the skin, and thus

dermal protection is advisable (Rice and Mauro, 2013). Schneider *et al.* (1999) describes a model depicting interactions of several compartments leading to the contamination of the skin, i.e. dermal exposure to exogenous compounds (a chemical originating from outside the living organism). Among these compartments the source of the contaminant, the air, surfaces upon which the contaminant could have deposited (via the air or contact transfer), the outside and inside of the worker's clothing and the skin itself are described. Furthermore, contamination could spread from one anatomical area to another. Continuous contamination, and subsequent exposure prevails unless decontamination (the removal of the contaminant from the system/combined compartments) is initiated (Schneider *et al.,* 1999). The 'take-home' effect is a prime example of the continuous exposure on the skin. The 'take-home' effect is the term often used for the phenomenon found with poor personal hygiene where dermal exposure continues after shift hours due to lack of proper decontamination of the skin area (Franken *et al*., 2014). Moreover, contamination takes place between the skin and the inner and outer clothing compartments, when decontamination is not sufficiently implemented. This was explained in an *in vitro* platinum and rhodium experimental study that argued that poor hygiene and the ineffective removal or cleaning of contaminated clothing and skin, could cause continuous exposure, after work shifts (Franken *et al*., 2014). Although, the model described by Schneider *et al.* (1999) is relevant when applied to potassium hexachloroplatinate exposure, current knowledge is lacking in describing the involvement of dermal exposure to associated dermal permeation, as depicted as the last step in the model (Ravindra *et al*., 2004; Cristaudo *et al*., 2007; Bullock, 2010; Sartorelli *et al*., 2012; Linde *et al.*, 2018a).

2.2.4 Legislation

In order to protect exposed workers from the adverse health effects of these platinum salts, the industry relies on appropriate legislation and occupational hygiene measures. According to Johnson Matthey (JM), South-Africa possesses 80 % of the world's platinum reserves and provided 73 % of the world's platinum in 2017 and 2019 (JM, 2018, 2019, 2020). The South-African Minerals Council claimed that an estimated 163 538 workers were directly employed in the South-African platinum mine industry in 2020 (Minerals Council South Africa, 2021). Thus, emphasis will be given to the South African legislation, namely the Occupational Health and Safety Act (OHS Act, Act 85 of 1993) and Mine Health and Safety Act (MHSA, Act 29 of 1996) and the relevant regulations associated with them. Preceding regulation changes to the OHS Act Regulations for Hazardous Chemical Agents published in March 2021, the 1995 regulation indicated a respiratory Occupational Exposure Limit (OEL) for platinum (metal) of 5 mg/m^3 (DoL, 1993; DMR, 1996) and platinum mine dust (respirable fraction i.e. 0.001-1000 µm) of 3 mg/m^3 (DMR, 1996; Schröder, 2014). However, for inhalation exposure to platinum salts (soluble), as platinum, an OEL eight hour time weighted average (TWA) was set at 0.002 mg/m^3 with only a respiratory sensitization notation (Sen). The sensitization notation indicated the

capability of developing respiratory sensitization towards the compound, whereas a skin notation (SK) was used to warn against likely dermal absorption of the compound (DoL, 1993; DMR, 1996). Thus, there was no reference to any dermal involvement (no skin notation) or regulatory guidelines for biological monitoring of platinum and its many complex forms (DoL, 1993; DMR, 1996; HSE, 2011). The revised regulations published in March 2021 (the Regulations for Hazardous Chemical Agents), depict a stricter OEL of 1 mg/m^3 although the OEL for soluble platinum salts (0.002 mg/m^3) remains the same. Both OEL's are set under the Restricted Limit (RL) categorization (renamed from the former Recommended Limit) indicating that control measures are legally adequate provided that the set limit is not exceeded. Furthermore, the addition to both respiratory- (RSEN) and dermal sensitization (DSEN) notations were assigned to platinum salts (DEL, 2021). Although the addition of a new dermal sensitization notation would require employers to initiate dermal protective measures to ensure protection from the dermal effects of these platinum salts, multiple published studies have indicated that inhalation exposure to platinum salts lower than the current OEL-RL (0.002 mg/m^3) still proved sensitizing (Calverley *et al*., 1995; Maynard *et al.*, 1997; Linnett and Hughes, 1999; WHO, 2000; Bullock, 2010; Vos, 2012; Heederik *et al*., 2016). The lowering of the OEL to a safe level could be so low that it is questionable due to the subsequent economic consequences for the businesses (Rijnkels *et al.,* 2008). However, after sensitization has occurred even very low exposure levels may lead to intense reactions that could prove life threatening (ACGIH, 2007) as was observed after sensitized workers were exposed to 0.05 µg/m^3 (40 times lower) (Di Gioacchino *et al*., 2004). Furthermore, provided that the MHS Act governs the mining and refinery processes (DMR, 1996) and not the OHS Act, during which the greater part of occupational exposure to platinum salts occurs (Linnett and Hughes, 1999; Brook, 2006), the lack of OEL revision and no current indication of dermal involvement during either absorption or development of sensitization are cause for concern.

In addition to the above mentioned, the lack of speciation differentiation in the setting of the OEL is troublesome. Chemical speciation refers to the specific form that the elements is presented in regarding isotopic composition, oxidative state and/or molecular structure (Templeton *et al.*, 2000). It is used to predict the element's behavior in a system and thus describes the chemical toxicity of the element (Van Briesen *et al*., 2010). Due to the close association between speciation and toxicity, it is crucial to consider speciation when setting OEL's. Categorizing all forms of platinum salts under one OEL speciation is not advisable. Disregarding speciation subsequently excludes major differences in toxicity even between compounds that may have remarkably similar chemical formulas. Toxicity of a compound is related to the reactivity of the compound, which is important when considering possible health effects of the compound (Linnett and Hughes, 1999). An example specifically relevant to the

research presented here, is the toxicity and health effect differences between chloroplatinate salts, as not all chloride containing platinum salts are sensitizing.

The most common and potent sensitizers are hexachloroplatinate ($PtCl_6$) and tetrachloroplatinate ($PtCl_4$). Hexachloroplatinate is the more one, and both tetra- and hexachloroplatinate are more sensitizing when attached to potassium or ammonium (Linnett and Hughes, 1999; Ravindra *et al*., 2004; Bencs *et al*., 2011). This is further shown in experimentation with ammonium platinum salts where ammonium hexachloroplatinate, $(NH_4)_2[PtCl_6]$, was indicated as a more potent sensitizer than its tetra-counterpart $(NH_4)_2[PtCl_4]$) (Cleare *et al*., 1976; Boscolo *et al*., 2004; Di Gioacchino *et al*., 2004). Furthermore, the sensitizing reactions of $PtCl_4$ and $PtCl_2$ were extremely low compared to the reactions caused by the ammonium counterparts (Di Gioacchino *et al*., 2004). Speciation is only addressed in a small guideline paragraph (paragraph 108 -109) of the newly published regulations, stating that a platinum ion attached to a halide compound (i.e. chloride, bromide, fluoride and iodide) is classified as a halogeno-platinum compound causing sensitization (DEL, 2021).

Occupational hygiene measures are used to comply with legislation and protect workers from potential health effects. Sufficient and current information on exposure routes is crucial for implementing effective protective measures (Schröder and Stanton, 2014). Furthermore, the understanding of all possible routes of exposure is vital for evaluating total worker exposure (Angerer *et al*., 2007). With insufficient understanding, subsequent sensitization to the platinum salts may result in the compulsory removal of the worker from the exposed working environment. This often leads to detrimental socioeconomic consequences for the worker and the employer (Bullock, 2010; Merget *et al*., 2017). However, the removal from the exposed area does not necessarily prevent further adverse health effects from occurring (Brooks *et al*., 1990; Merget *et al*., 2017).

Further consequences of insufficient knowledge could be observed in the structuring of legislation. This is demonstrated when assessing the difference in exposure limits between the 1963 ACGIH threshold limit and 2008 Dutch Expert Committee on Occupational Standard. The ACGIH limit of 2 $\mu g/m^3$ for all platinum salts was based upon qualitative assessments only whereas, when considering further speciation and health assessments, a 400 times lower limit of 0.005 $\mu g/m^3$ for chloroplatinate salts was set by the Dutch Expert Committee on Occupational Standard (Heederik *et al*., 2016). Thus, evaluating the possible dermal involvement, as an exposure route, may lead to more knowledge for more informed legislation and protective measures against the potential adverse health effects.

2.2.5 Adverse health effects

Adverse health effects due to platinum salt exposure have been investigated since 1945 (Hunter *et al*., 1945). Since then, many studies proved that inhalation exposure to these platinum salts cause severe respiratory sensitization, even though metallic platinum itself is inert (WHO, 1991; Calverley *et al*., 1995; Niezborala and Garnier, 1996; Merget *et al*., 2000; Ravindra *et al*., 2004; Brook, 2006; Heederik *et al*., 2016). The most sensitizing of these platinum salts are tetra- and hexachloroplatinate, especially when attached to potassium (Ravindra *et al*., 2004; Bencs *et al*., 2011). After reviewing studies on occupational inhalation exposure to PGMs Linde *et al*. (2017) summarized that a variety of respiratory and dermal reactions have been associated with these platinum salts after sensitization. These reactions include bronchial asthma, allergic rhinitis, dermal itching, urticaria and dermatitis (Hunter *et al*., 1945; Lown *et al*., 1980; Calverley *et al*., 1995; Merget *et al*., 2000; Brook, 2006).

The immunological mechanism of the platinum salts' sensitizing effects is theoretically described as a type I reaction mediated by immunoglobulin E (IgE) (Heederik *et al*., 2016). Type I hypersensitivity reactions, also known as immediate or IgE-mediated hypersensitivity, is the toxic reaction of the immune system to a previously exposed compound or a structurally similar one (Eaton and Gilbert, 2013; Rice and Mauro, 2013). This reaction, true for most hypersensitivity reactions, are compartmentalized into two phases i.e., sensitization and elicitation. The initial exposure to the compound initiates the sensitization phase where the immune system identifies and reacts to the presence of the chemical by forming antibodies. The elicitation phase involves the subsequent exposure and hypersensitivity reactions where previously formed antibodies react towards the compound (Gregus, 2013). Smaller molecules, such as platinum salts, are usually not large enough to be identified by the immune system during the initial sensitization phase. These molecules are known as haptens and form part of an antigen only when attached to an endogenous protein (Moore *et al*., 1975b; Eaton and Gilbert, 2013). This bond may lead to changes in the rate or level of the specific proteins' synthesis. The influence of platinum attaching to endogenous proteins has been postulated to relate to other metals such as cadmium and lead, whereas the protein synthesis in the body is induced by the bond (Lown *et al*., 1980). The larger antigen is identified by the immune systems' T-helper cells and due to the T-helper cell association to B-cells, the B-cells react by means of differentiating and secreting antibodies (Eaton and Gilbert, 2013). These antibodies are referred to as immunoglobulin (Ig), although when defined by a specific antigen the Ig has an identifiable pre-fix (e.g. anti-sRBC IgM) (Kaplan *et al*., 2013). A normal reaction would cause secretory antibodies (IgA), complement fixation with efficient agglutination (IgM) or with placenta permeation capabilities (IgG) to be released and respond to the antigen, leading the antigen to be cleared from the body without any sensitization reaction (Kaplan *et al*., 2013;

Rice and Mauro, 2013). However, with the initiation of a sensitization reaction the B-cells secrete IgE which causes mast-cell degranulation sensitization response (Kaplan *et al*., 2013). Thus, in atopic or hypersensitive cases IgE antibodies form, and subsequent exposure causes attaching of the IgE to local mast cells. IgE travels by means of the circulatory system and attaches to mast cells in other tissues as well, such as the lungs or skin (Kaplan *et al*., 2013). This bond causes degranulation with the release of mediators and cytokines that activate other immune response cells and provoke the sensitization reaction manifestations. Therefore, the level of IgE in the circulatory system is often used to evaluate the magnitude of sensitization reactions after platinum salt exposure (Murdoch *et al*., 1986; Merget *et al*., 1988; Brooks *et al.*, 1990; Bolm-Audorff *et al.*, 1992; Ban *et al.*, 2010; Merget *et al*., 2017). However, due to persistence of the immune responses and the time passed between the last exposure and the analysis of the IgE levels, the detection of this indicator is not always successful. However, whether detection is successful or not the secretion of IgE is necessary for the development of sensitization reactions towards platinum salt exposure (Kimber and Dearman, 2002). The specifics of why this reaction appears are unknown although research indicates that genetic, environmental and a trigger event or combination thereof may be involved (Kaplan *et al*., 2013). In some cases, such as in platinum salt exposure sensitization reactions, both the dermal (urticaria, itching and dermatitis) and respiratory systems (asthma and rhinitis) are involved (Eaton and Gilbert, 2013).

Urticaria, more commonly known as hives, is an outbreak of reddish wheals that raise above the dermal surface and cause itchiness in the area. Hives are formed in the skin after a type I immune reactions Type I immune reactions, i.e. degranulation of cutaneous mast cells after reacting to liganded IgE, leads to the release of histamine, a known vasoactive substance. Vasoconstriction of the blood vessels in the skin lead to fluid build-up manifesting as hives (Rice and Mauro, 2013). The release of mast cell mediators after this reaction, causes constriction of the airway smooth muscle (asthma) (Holgate, 2013) and rhinitis. Rhinitis is characterized by sneezing and nasal discharge (Bousquet *et al*., 2008). The release of histamine and other mediators promote the production of adhesion molecules leading to the infiltration of inflammatory cells into the nasal tissue causing the symptoms associated with rhinitis (Hayden, 2004). Furthermore, dermatitis is commonly listed as one of the dermal manifestations of platinum salt exposure, although most literature does not specify the type i.e., irritant, contact, erythematous, or eczematous dermatitis (Hunter *et al*., 1945; Lown *et al*., 1980; Calverley *et al*., 1995; Merget *et al*., 2000). Due to the various mechanisms involved in the different types of dermatitis, such as type IV hypersensitivity in contact allergic dermatitis and type II in eczematous dermatitis (Kaplan *et al*., 2013; Langan *et al*., 2020) and the controversial observations of studies on platinum salt exposure induced dermatitis (Lindell, 1997; WHO, 2000; Linde, 2018), the various mechanisms will not be discussed here.

An influential factor on the development of adverse health effects in exposed workers, include the workers' smoking habits. Smokers are associated with a higher risk in developing respiratory sensitizing reactions towards platinum salts (Calverley *et al*., 1995; Schierl *et al*., 1998; Linnett and Hughes, 1999). Cigarette smoking induces the production of IgE and therefore it is theorized to explain the increased risk of developing respiratory sensitization to various substances associated with type I immune responses (Hendrick, 1989).

2.2.6 Toxicokinetics

Toxicokinetics focuses on the absorption, distribution, metabolism and elimination (excretion) of an exogenous compound in the body after exposure (Shen, 2013). Toxicokinetic data on platinum salts are lacking and is only discussed in the broad group of soluble platinum compounds (IPA, 2017). However, it is known that the toxicokinetics of exogenous compounds are highly dependent on the physicochemical properties (e.g. charge, particle size and solubility) and the route of exposure (Lindell, 1997; IPA, 2017). A simple example of this effect is the conclusion of a rat study reporting that soluble platinum salts are more toxic than their insoluble counterparts and will be illustrated in the following four toxicokinetic subsections (Holbrook *et al*., 1975). Due to the focus of this literature review being on exposure and absorption (i.e. dermal permeation and absorption) of platinum salts, only brief discussions of the other toxicokinetic mechanisms will be provided.

2.2.6.1 Absorption

Absorption of exogenous compound relates to the physical uptake of the chemicals through natural bodily barriers into the systemic circulation (Gregus, 2013). In an occupational setting these exposure routes include the respiratory system (inhalation exposure), gastrointestinal tract (accidental ingestion) or the skin (dermal exposure) (Eaton and Gilbert, 2013).

2.2.6.1.1 Inhalation

Studies done on inhalation exposure, show that platinum salts may dissociate from their compound molecules (for example the potassium and chloride molecules attached to platinum in potassium hexachloroplatinate) and be transported into the alveoli as platinum ions (Lindell, 1997). This fact has been shown in rat studies where urine platinum concentrations indicated absorption via the lungs into the circulatory system, and thus into the excretion through the kidneys, even after initial rapid clearance of platinum in the lungs via theoretical mucociliary action (Moore *et al*., 1975b). A more recent occupational exposure study agrees with respiratory absorption of platinum with a strong positive association found between urine platinum concentrations and inhalation exposure to platinum salts (Linde *et al*., 2018a).

2.2.6.1.2 Ingestion

Accidental ingestion may occur, for example, after eating with contaminated hands or practicing certain habits such as nail biting (Gorman Ng *et al*., 2016; Klasson *et al*., 2017; IPA, 2017). After accidental ingestion dissociated platinum ions may be absorbed through the gastro-intestinal tract. Although, this uptake is theorized to be dependent on the specific particle size (Lindell, 1997). Examples of ingestion studies include animal studies that indicate poor absorption of disodium hexachloroplatinate (Na_2PtCl_6) and tetrachloroplatinate (Na_2PtCl_4) through the gastrointestinal tract, leading to high platinum concentrations eliminated in the faeces compared to low platinum concentration in the circulatory system (Moore *et al*., 1975a, 1975b; Massaro *et al*., 1981). Another study highlights the importance of speciation between halogen-based platinum salts, with varying concentration results absorbed between the species after accidental ingestion by workers (IPA, 2017).

2.2.6.1.3 Dermal

Dermal absorption of an exogenous compound could be defined as the mass of exogenous compound that moved through the full thickness of the skin membrane, human or otherwise, and detected in the blood, synthetic or otherwise. Dermal absorption should not be confused with dermal penetration which is the mass of exogenous compound found retained in the skin barrier without diffusing into the blood or dermal permeation which is the movement of a compound through different layers of the skin (Zsikó *et al*., 2019; Hopf *et al*., 2020). An animal study done in 1984 reported dermal absorption with trace amounts of platinum in most organs (Roshchin *et al*., 1984; Lindell, 1997). Further mice studies substantiated these results by indicating respiratory sensitization after exclusive dermal exposure to disodium hexachloroplatinate ($Na_2[PtCl_6]$) (Schuppe *et al*., 1997). Recent human *in vitro* studies strengthened these findings with traceable amounts of platinum absorbed through human skin after exposure to potassium tetrachloroplatinate (Franken *et al*., 2014, 2015; Van Nieuwenhuizen, 2016). A strong positive association was indicated between dermal exposure monitoring and subsequent urine sampling (Linde *et al*., 2018a). More research should be done to establish a better understanding on the topic of platinum salt exposure and absorption especially considering the effects of differences in physicochemical properties between chemicals.

2.2.6.2 Distribution

After an exogenous compound enters the body (absorption), it is distributed through the circulatory system and enters different tissues due to physicochemical characteristics of the chemical (Gregus, 2013). Examples of these physicochemical characteristics include solubility, particle size and oxidative state (Moore *et al*., 1975b; Lown *et al*., 1980; Massaro *et al*., 1981;

Lindell, 1997). Platinum salts attaches to albumin and transferrin in the blood, (Sykes *et al*., 1985; Trynda and Kudu-Jaworska, 1994) with 65-80 % of absorbed platinum located in erythrocytes (Lindell, 1997). Subsequently, platinum is distributed to all tissues with a low concentration in the brain and a high concentration in the kidneys (Moore *et al*., 1975a, 1975b; Lown *et al*., 1980; Massaro *et al*., 1981). It is theorized that concentration distributions of platinum are influenced by platinum's positive charge and large particle size (Moore *et al*., 1975b; Lown *et al*., 1980; Massaro *et al*., 1981; Lindell, 1997). Charge and particle size are known influential factors in blood-brain barrier penetration (Lehman-McKeeman, 2013). The kidney concentration was thought to cause possible damage to the kidneys themselves leading to less efficient urine elimination of platinum, thereby increasing the body burden (Lown *et al*., 1980; Massaro *et al*., 1981). This kidney damage leading to an increase in body burden, pertains to gastrointestinal tract damage after high levels of ingestion as well (Lown *et al*., 1980). The damage to the kidneys, as indicated by increased serum creatinine levels after ingestion of $PtCl_2$ and $PtCl_4$ in rats (Reichlmayr-Lais *et al*., 1992), was theorized to be representative of the interaction of platinum compounds and sulfhydryl groups in kidney proteins (Roshchin *et al*., 1984).

Distribution of exogenous compounds is often hampered by accumulation (storage), although redeposition into the circulatory system may occur (Gregus, 2013). Pertaining to platinum salt dermal exposure, the skin was indicated as an accumulation site in human *in vitro* studies (dermal penetration), and that platinum was retained in the skin itself (Franken *et al*., 2014, 2015; Mauro *et al*., 2015; Van Nieuwenhuizen, 2016). Furthermore, animal studies indicated possible platinum accumulation in the lungs and gastrointestinal tract (Moore *et al*., 1975b) substantiated by trace amounts of platinum in the blood parallel to tissue concentrations for up to 32 days after an intravenous injection (Lindell, 1997). Human autopsy data suggested accumulation in the subcutaneous fat. However, prior metabolic transformation is necessary to increase lipid-solubility, as most platinum compounds are insoluble in lipids (hydrophilic/lipophobic) and fat cells contain a high lipid content (Duffield *et al*., 1976).

2.2.6.3 Metabolism

Current literature regarding the metabolism of platinum and the toxicity of platinum is incomplete. However, Duffield *et al*. (1976) and Massaro *et al*. (1981) theorizes possible methylation of platinum comparable to the metabolism of mercury, and in comparison, derive the same physicochemical alteration as mercury. Methylated mercury is more toxic and have increased bio-accumulation characteristics (Duffield *et al*., 1976; Massaro *et al*., 1981). Methylation is the biochemical addition of a methyl group (CH_3) to a compound. This theory holds more merit when considering that platinic sulfate and potassium hexachloroplatinate could

be methylated under laboratory conditions by utilizing the same methyl cobalamin as in the reaction of mercury (Duffield *et al*.,1976).

2.2.6.4 Elimination

Studies have indicated the elimination of platinum by means of urine and feces, in rodents (Moore *et al*., 1975a, 1975b; Massaro *et al*., 1981; Lindell, 1997) and humans (Lindell, 1997; Linde *et al*., 2018a, 2018b). The urine excretion indicated absorption through the lungs whereafter kidney filtration took place. The presence of platinum concentration in the feces indicated poor absorption through the gastrointestinal tract (Moore *et al*., 1975b) especially after initial clearance through mucociliary action of the lungs followed by the swallowing of platinum particles (Tokar *et al.*, 2013). The rate of clearance was indicated as an initial rapid clearance that subsided to a much slower phase of 42 %, 24 hours after ingestion (Lindell, 1997). The evaluation of urinary excretion of two volunteers after once-off inhalation exposure to ammonium hexachloroplatinate reported a maximum excretion around ten hours after exposure. A calculated first biological half-life was at ± 50 hours for both volunteers and a second half-life of 24 days for volunteer A (Schierl *et al*., 1998). The biological half-life is often used as a parameter to describe the rate of elimination for 50 % of the exogenous compound from the circulatory system (Eaton and Gilbert, 2013). Workers exposed to high platinum levels have indicated much longer half-lives than the average population (Brook, 2006). A study into the metabolism of platinum half-life amongst an average population has indicated a half-life of < 3 days (Nuttall *et al*., 1994). Schierl *et al*. (1998) and Linde *et al.* (2018a) theorized that platinum salt accumulation in various parts of the body could account for the slow excretion. This was further implicated by the presence of 25 times above control group urinary platinum concentrations even after six years of no exposure to potassium tetrachloroplatinate and platinum nitrate. These results were based on tests done on hypersensitive workers that were removed from the exposed area (Schierl *et al*., 1998). The lungs and skin have been indicated as a possible reservoir (accumulation) location (Duffield *et al*., 1976; Benes *et al*., 2000; Franken *et al*., 2015).

2.3 Dermal permeation

2.3.1 Dermal anatomy and Physiological function

The skin prevents excess water loss, play a role in thermoregulation and is the first line of defense against exogenous compounds (Kolarsick *et al*., 2011). The skin cells, such as other mammalian cells, have lipid bilayer membranes of phospholipids with hydrophilic polar heads and hydrophobic tails (Lehman-McKeeman, 2013). These cells are then organized into three layers, the outer epidermis, the dermis and the subcutaneous adipose tissue. Although the dermis compromises ± 90 % of the skin thickness, the main purpose is solely supportive and will

thus not be discussed in detail here. The average thickness of the epidermis is between 10-20 µm and the dermis between 50-100 µm. Mast cells, surrounding the blood vessels in the subcutaneous fat and dermis, are secretory cells associated with hypersensitive immune responses. Mast cell granules contain histamine and other inflammatory mediators including many IgE receptors on their surface. Capillaries located in the basement ridges of the basement membrane separating the epidermis and dermis supply the viable epidermis and epidermal appendages with blood circulation (Kolarsick *et al*., 2011; Rice and Mauro, 2013; Selzer *et al*., 2013).

Epidermal appendages, such as hair follicles, sebaceous- and eccrine glands, that are rooted deep in the dermis account for a very small area of the total dermal surface (Poet and McDougal, 2002; Selzer *et al*., 2013). Other cells in the epidermis include macrophages (Langerhans cells) playing a crucial part in immune reactions in the skin (Rice and Mauro, 2013) including the metabolism of certain exogenous compounds (WHO, 2006). The epidermis is divided into two parts, the physiologically dead stratum corneum which forms the outermost part of the skin and the viable epidermis underneath (Selzer *et al*., 2013). The stratum corneum is the main barrier to exogenous compounds and thus the rate limiting step during permeation and/or penetration (Rice and Mauro, 2013). Due to the slow rate of saturation of the stratum corneum, a reservoir of the compound forms in the stratum corneum, which could lead to continuous slow penetration of the compound into the deeper layers of the epidermis. Subsequently permeation of the compound into the underlying viable epidermis layers may occur even after efficient decontamination of the skin or removal from the exposure site (Lehman-McKeeman, 2013). The stratum corneum consists of corneocytes that are 80 % keratin and are formed by migrated basal cells that undergo differentiation to become physiologically dead cells (Kolarsick *et al*., 2011; Rice and Mauro, 2013; Raney *et al*., 2015). The outer-most layer of corneocytes is shed from the body in a 14-day cycle (Rice and Mauro, 2013). The stratum corneum is highly hydrophobic due to the high protein content, while the viable layer underneath is hydrophilic (Elias, 2005; Rice and Mauro, 2013; Selzer *et al*., 2013). Hydrophilic compounds diffuse into intracellular water whereas hydrophobic compounds partition/move into the cells, both of which readily diffuse into the capillaries that supply the viable layer of the epidermis (Elias, 2005; Rice and Mauro, 2013). Corneocytes' physical and biochemical properties, vary according to the location in the stratum corneum. This is demonstrated by increased water-binding (hydrophilic) capacity of the stratum corneum cells in the middle compared to the cells closer to the viable layer of the epidermis (more hydrophobic). This phenomenon is associated with high concentration of free amino acids in the middle layer compared to those of the more densely compacted deeper stratum corneum cells (Kolarsick *et al*., 2011). The deeper cells are

connected by tight junctions, desmosomes, giving them their compacted structure (Rice and Mauro, 2013).

Compared to the stratum corneum, the deeper viable epidermal layers are porous, aqueous and is composed of less protein. Thus, resistance to diffusion in these layers are less, for hydrophilic/lipophobic exogenous compounds, although the rate of diffusion into the systemic circulation is dependent on blood flow, movement of the interstitial fluid, and interactions with dermal constituents (Poet and McDougal, 2002; Lehman-McKeeman, 2013).

2.3.2 Routes of dermal permeation

There are three ways in which an exogenous compound may lead to adverse health effects namely damage to the skin itself, dermal permeation, and immune-mediated responses. As discussed earlier, when evaluating adverse health effects caused by platinum salts, the potential of permeation through the skin is emphasized in this section. Permeation through the skin can occur through one (or more) of three routes, i.e. transcellular, intercellular and appendageal. Lipophilic/hydrophobic compounds could diffuse through the lamellar acyl chains of the phospholipid tails of the lipid-bilayer skin cell membranes while hydrophilic compounds diffuse through the polar heads, transcellular. Only hydrophilic compounds diffuse through the intercellular water in between the skin cells with the intercellular route. Lastly, hydrophilic particles of small size could penetrate through the epidermal appendages (Essa *et al.*, 2002; Poet and McDougal, 2002; Konda *et al*., 2012; Lehman-McKeeman, 2013; Rice and Mauro, 2013). The hydrophobic/lipophilic compounds' diffusion through the stratum corneum is directly proportional to lipid solubility but inversely proportional to their molecular weight. However, the dermis is composed of less protein and thus diffusion here is restricted. The reverse is true for hydrophilic/lipophobic compounds (Poet and McDougal, 2002; Lehman-McKeeman, 2013). The epidermal follicular route is commonly referred to as the shunt route (Otberg *et al.*, 2007). The shunt route is often neglected due to the small (< 1 %) dermal surface area that it covers (Lehman-McKeeman, 2013). However, if an exogenous compound is able to permeate by this route it avoids the restriction of the stratum corneum barrier and the other layers of the skin that may inhibit diffusion (Poet and McDougal, 2002; Otberg *et al*., 2007; Desai *et al*., 2010; Liu *et al.*, 2011; Lehman-McKeeman, 2013; Rice and Mauro, 2013). Particle size, especially particles between 300 and 600 nm, have been shown to penetrate via this route easily (Lademann *et al.*, 2011; Lehman-McKeeman, 2013). If an exogenous compound is able to permeate through the skin layers, it could diffuse into the circulatory system through the blood capillaries in the upper levels of the dermis (Selzer *et al*., 2013).

2.3.3 Influencing permeation factors

Permeation of exogenous compounds are extremely chemical specie-specific and influenced by physicochemical factors (Hostýnek, 2003). These factors can be divided into two categories, namely intrinsic and extrinsic factors. Extrinsic factors are related to the exposure conditions such as dose, exposure time, size of exposed dermal surface area, hydration, speciation, solubility, molecular weight, oxidative state and particulate size. Whereas intrinsic factors are related to the specific exposed worker including anatomical area exposed, skin pH, skin metabolism, integrity of the skin and the exposed worker's race, sex and age (Poet and McDougal, 2002; Konda *et al*., 2012; Rice and Mauro, 2013; Lehman-McKeeman, 2013). Some noteworthy factors that will not be discussed in this section are temperature, humidity, occlusion, vehicle, counterion mechanisms and dermal metabolism (Rougier *et al*., 1987; Hostýnek, 2003; Otberg *et al*., 2007; Finnin *et al*., 2012; Lehman-McKeeman, 2013; Rice and Mauro, 2013). These factors are all interrelated and complex causing difficulties in the design of a predictive model in how they interact to influence permeation. Some factors, such as oxidative state of metallic species, elude modeling all together (Hostýnek, 2003). The interplay and complexity of the factors discussed here should be considered during the evaluation of previously conducted research and with the implementation of experimental methodology.

2.3.3.1 Dose

The applied or deposited compound concentration is commonly referred to as the dose (ICMM, 2007). In permeation research the dose could be classified as infinite or finite. When an infinite dose is deposited on the skin, the effects of permeation through the skin or evaporation do not lead to depletion of the contaminant on the skin. Thus, continuous permeation could take place in contrast to a finite dose that could be depleted, ceasing further permeation possibilities (Selzer *et al*., 2013). Furthermore, Selzer *et al.* (2013) theorized that large doses normally permeate through the intercellular spaces of the stratum corneum. This may provide a more direct and quicker permeation route in contrast to the transcellular route. The transcellular route requires the additional movement through corneocytes and the lipid layers that is absent in intercellular permeation (Zsikó *et al*., 2019). The exact manner in which the dose affects permeation is unique to each individual compound. For example, chromate ions (Wahlberg, 1965), displayed an increase in permeation leading up to a threshold that causes a plateau effect even with further increases of concentration (Hostýnek, 2003). Whereas others, such as dichromate, indicate a negative association between dose and mass permeated (Gammelgaard *et al*., 1992). The decrease in mass permeated is theorized to coincide with an accumulation of the compound inside the skin due to interactions with skin proteins. The accumulation forms a barrier through which further permeation becomes improbable and is

often indicated in long lag times (Hostýnek, 2003). Lag time is a calculated construct used as a permeation parameter in *in vitro* research described in more detail under the '*In vitro* methodology' section (Section 2.5, **below**). Alternatively, the accumulation inside the skin could form a reservoir and create a large concentration gradient across the skin increasing the diffusion through the skin into the blood (Zsikó *et al*., 2019). This reservoir could also cause continuous further permeation and absorption into the circulatory system even after proper decontamination methods were preformed removing deposition of the exogenous compound on the dermal surface (Franken *et al*., 2014, 2015).

2.3.3.2 Exposure time

The duration of exposure has an influence on the permeation of various compounds (OECD, 2011) and toxicity. Depending on the compound, the exposure time or dose could become the predominant influential factor. This is due to dermal cells metabolizing certain compounds and the rate of metabolites forming (McDougal and Boeniger, 2002). Positive associations between the exposure time and permeated mass have been identified in multiple studies investigating various compounds (Swarbrick *et al*., 1982; Larese Filon *et al*., 2006, 2011; Franken *et al*., 2014, 2015; Barbero and Frasch, 2016; Van Nieuwenhuizen, 2016).

2.3.3.3 Size of exposed dermal surface

Workers' hands and forearms are often exposed to exogenous compounds, and subsequent transfer of the contaminant (with or without gloves) to the face and neck is commonly found (Schneider *et al.*, 1999; Rice and Mauro, 2013). Observations in a precious metal smelter indicated that workers touched their faces with contaminated hands an average of 6.3 times per hour (Gorman Ng *et al*., 2016). The larger the contaminated dermal surface area the greater the permeation potential (McDougal and Boeniger, 2002). The contaminant could be transferred to areas which allow easier permeation due to structural differences between anatomical areas and increasing the permeation further (Hostýnek, 2003; Finnin *et al*., 2012). The size of exposed dermal surface together with the dose, exposure time, frequency of contaminant transfer (McDougal and Boeniger, 2002) and the easy transfer of contaminant between surfaces should all be taken into account when assessing the risk of exposure (Fenske, 1993; Schneider *et al.*, 1999). The total size of the exposed area is thus included in the calculations utilized in *in vitro* methodology adapted from previous *in vitro* research by Franken *et al.* (2014, 2015). All these factors play a part in the entwined complexity of these influencing factors on permeation. Furthermore, exposed dermal surface area is one of the factors incorporated into quantification of the mass permeated through a membrane (Díez-Sales *et al.*, 1991).

2.3.3.4 Anatomical area

Permeation across anatomical areas of the same individual may differ due to structural differences between these anatomical areas (Hostýnek, 2003; Finnin *et al*., 2012). Epidermal thickness, density of epidermal appendages, and variation in intercellular lipid composition are some of the structural differences (Poet and McDougal, 2002; Knorr *et al*., 2009; Liu *et al.*, 2011; Lehman-McKeeman, 2013). These structural differences are especially influential in the permeation of compounds involved in inflammatory – and contact hypersensitivity reactions (Wahlberg, 1996; Hostýnek, 2003). However, a consistent model of the influence of these structural variations amongst anatomical areas across compounds have not been indicated (Finnin *et al*., 2012). Lastly, comparing *in vitro* and *in vivo* data that utilized different anatomical areas produced incomparable results (Raney *et al*., 2015).

2.3.3.5 Hydration

Homeostatic hydration of the stratum corneum is maintained at 20 % water, i.e. only partially hydrated. Water in the stratum corneum is a necessity to maintain resistance to trauma and the normal function of enzymes that control desmosome (the tight junctions between the corneocytes) degradation (Gonçalo, 2000; Rice and Mauro, 2013). Thus, stratum corneum hydration below 20 % causes dry skin and decreases permeability of hydrophilic compounds (Wepierre and Marty, 1988). Alternatively, hyperhydration causes swelling of the compact structured corneocytes in the stratum corneum, causing distortion between the structure and creating pores assisting increased permeation of exogenous compounds, especially those with hydrophilic properties (Blank *et al*., 1984; Talreja *et al*., 2001; Hostýnek, 2003; Lehman-McKeeman, 2013; Rice and Mauro, 2013). This swelling also closes the shunt openings of the follicular route of permeation on the epidermal surface (Maghraby *et al*., 2001; Hostýnek, 2003). As some exogenous compounds are theorized to penetrate the skin solely by the facilitation of the follicular route (Mauro *et al*., 2015), the closure of these openings may decrease the mass of compound inside the skin and circulatory system.

2.3.3.6 Skin pH

pH quantifies the free hydrogen ion concentration in negative logarithmic form. A pH lower than seven indicates a solution of an acidic nature, equal to seven is neutral and above seven indicates solutions of an alkaline nature (Schmid-Wendtner and Korting, 2006). The functions of the skin pH include the regulatory homeostasis cycle of maintaining the protein cohesion of the stratum corneum and balancing the symbiotic microbes living on the skin (Stefaniak *et al*., 2013). Normal levels of skin pH are between 4 to 6.5 (Yosipovitch *et al*., 1998). *In vitro* permeation studies standardized the utilization of a pH of 6.5, representing normal and healthy skin (Yosipovitch *et al*., 1998; Larese Filon *et al*., 2004, 2007, 2009a;

Franken *et al*., 2014; Van Nieuwenhuizen, 2016). However, some occupational environments lead to a more acidic (lower) pH (Larese Filon *et al*., 2008, 2009a). Measurement of the pH on the surface of base metal refinery worker's skin indicated a skin pH of between 5 and 6 (Du Plessis *et al.,* 2010). This occupational influence on the skin pH caused some *in vitro* studies to use a more acidic (4.5) skin pH during experiments (Larese Filon *et al*., 2008, 2009a, 2009b, 2011, 2013; Mauro *et al*., 2015; Crosera *et al*., 2018). Increasing pH levels, i.e. less acidic, have a reverse effect on the barrier function of the skin (Hostýnek, 2003). The higher pH cause ionization of compounds which may decrease the available amount of lipophilic (unionized) compound (Konda *et al*., 2012). More acidic salts penetrate deeper into the sweat ducts, as one of the shunt routes, and have a longer-lasting effect on sweat inhibition (Lansdown, 1973; Hostýnek, 2003). Studies evaluating the influence of the pH of sweat on the permeation of PGMs indicated a positive association between a more acidic (pH of 4.5) sweat and permeation of rhodium ($RhCl_3$) and platinum (K_2PtCl_4) (Van Nieuwenhuizen, 2016; Jansen van Rensburg *et al*., 2017). Thus, together with regulating the homeostasis function of the stratum corneum, the pH of the skin may facilitate chemical reactions changing the properties of the exogenous compound, for example decreasing the solubility or changing the oxidative state.

2.3.3.7 Speciation

As previously discussed, chemical speciation could be defined as the specific isotopic composition, oxidative state and/or molecular structure that the element is presenting (Templeton *et al.*, 2000). The importance of chemical speciation is emphasized when considering the immense implications of ignoring toxicity and leaving group differences among various compounds of a metal in studies such as Linnett and Hughes (1999). The differences in toxicity were stated to be influenced by the relationship between reactivity of different compounds (Linnett and Hughes, 1999). Furthermore, the importance of speciation is indicated by incomparable results when *in vitro* and *in vivo* studies utilized different chemical formulations (Raney *et al*., 2015). Speciation closely relates to the molecular weight and oxidative state of a compound (Templeton *et al*., 2000).

2.3.3.7.1 Molecular weight and Solubility

The rate of diffusion is indirectly related to the molecular weight (MW) of the compound and thus the influence of compound solubility and the MW of the compound on permeation often interact. Hydrophobic compounds of low molecular weight permeate through the skin much easier than compounds of high molecular weight and hydrophilic properties. In compounds with low molecular weight, hydrophobicity becomes the most dominant influential factor in dermal permeation (Rice and Mauro, 2013). Examples of the influence of MW on permeation is indicated in the higher permeation of palladium, with lower MW compared to nickel

(Crosera *et al*., 2018). The solubility of a compound affects the diffusion capability (permeation) within the skin layers and the amount of compound available for penetration in the sweat/synthetic sweat, and thus the overall permeation of the compound (Williams and Barry, 2004; WHO, 2006). The octanol/water partition ratio is commonly used to describe a compound's hydrophobicity (Anderson and Raykar, 1989) and has been positively associated with permeation calculations (Fasano and McDougal, 2008; OECD, 2011). Furthermore, hydrophilic/lipophobic compounds are commonly ionized, whereas the opposite is true for lipophilic/hydrophobic compounds. Ionization is determined by the pH of the environment and the pKa value of the compound (acid dissociation constant). When pH < pKa a weak acid predominates in unionized form whereas weak bases would predominate in ionized form (Konda *et al*., 2012). Metal ionization has resulted in increased permeation through the skin (Larese Filon *et al.*, 2006, 2007, 2008).

2.3.3.7.2 Oxidative state

Oxidative state is the positive or negative charge related to the number of outer electrons of an element specie (valence number). The oxidative state is associated with the electrophilic nature of the specific specie which in turn is related to protein reactivity (Hostýnek, 2003). Differences in oxidative state may cause drastic variety in permeation profiles such as the difference in permeation between Cr^{6+} and Cr^{3+} (Van Lierde *et al*., 2006).

2.3.3.8 Particulate size

Particulate size has been indicated as inversely proportional to the degree of permeation through the skin, to the extent that compounds > 400 Da are known as poor permeants (Lademann *et al.*, 2011; Lehman-McKeeman, 2013; Selzer *et al*., 2013). Thus, nanoparticles, due to their small size can penetrate the skin more easily. However, it seems their overall systemic absorption is low (Lademann *et al.*, 2011; Lehman-McKeeman, 2013). This is due to the cavity needed for the compound to move into as it travels through the skin. Thus, the skin lipids temporarily create this cavity allowing the movement. The larger the compound, in reference to the lipid size, the larger the cavity should be and the formation of a cavity of large enough size is less likely (Mitragatri *et al*., 1999). However, molecular size is not the only influential factor on dermal permeation/penetration and only plays a part in the interplay between multiple factors. This is demonstrated by the inability of nano-sized titanium dioxide and zinc oxide to penetrate the skin past the stratum corneum, thus making them ideal for dermal protective measures against solar ultraviolet exposure in sunscreens (Newman *et al.*, 2009; Lehman-McKeeman, 2013).

2.3.3.9 Integrity of the skin

The stratum corneum, as the main barrier of the skin, could be damaged chemically, physically, or pathologically leaving the integrity of the skin as a barrier diminished (Finnin *et al*., 2012). Damaging or removal of the stratum corneum increases the permeation of both hydrophilic and lipophilic exogenous compounds of all sizes (Schaefer *et al*., 1977; Poet and McDougal, 2002; Chiang *et al*., 2012; Finnin *et al*., 2012; Lehman-McKeeman, 2013). Chemically decreasing the barrier functionality of the stratum corneum is exemplified by the application of DMSO (dimethyl sulfoxide). DMSO disrupts the skin's protein structure by removing lipids, altering keratin configurations, and increasing hydration (Lehman-McKeeman, 2013). Physical damage per mechanical injury or pathological damage due to illness such as atopic dermatitis, leads to heightened sensitivity to dermal irritants. Genetic predispositions, including but not limited to susceptibility towards irritants, have been positively associated with the development of some illnesses (Rice and Mauro, 2013). Sensitization to nickel occurs more rapidly when the exposed dermal area has already been irritated previously compared to when intact skin is exposed (Allenby and Basketter, 1993; Nielsen *et al.*, 1999).

2.3.3.10 Race

There are many interpretations to the term 'race'. In this literature study the term is used to describe a specific population with genetic similarities where categorization is based on variance in skin color and physical features (Anand, 1999). Dermal structural differences between races have been observed over the years, although the observations are controversial throughout literature (Muizzuddin *et al*., 2010; Franken *et al*., 2015). These differences include the number of corneocyte cell layers, skin protein cohesion, pH and hair structure and density which all may play a part in the complexities of the skin's barrier function (Weigand *et al*., 1974; Berardesca *et al*., 1998; Mangelsdorf *et al*., 2006; Girardeau *et al.*, 2009; Muizzuddin *et al*., 2010). To illustrate the controversial observations between race comparisons, the contradictions in published research between African and Caucasian skin barrier functions are discussed here. In the past, structural differences between these two races have led researchers to believe that African skin have a superior barrier function towards exogenous compound permeation (Reinertson and Wheatley, 1959; Berardesca and Maibach, 1990; Kompaore *et al.*, 1993; Rawlings, 2006; Fluhr *et al.,* 2008; Gunathilake *et al.,* 2009; Muizzuddin *et al*., 2010). However, more recent *in vitro* platinum dermal permeation research does not support this (Franken *et al*., 2015). Franken *et al.* (2015) indicated that African skin is more permeable, with significantly higher platinum mass absorbed into the receptor solution, while, Caucasian skin's retainment ability to platinum was four times higher (Franken *et al*., 2015). The exclusive use of a particular race in permeation experimentation is standard practice to avoid the ambiguous nature of race as an influencing factor on permeation.

2.3.3.11 Sex

Differences in anatomy, physiology, epidemiology, and disease manifestations have been indicated between men and women. However, the exact mechanism how these differences affect the permeation of various exogenous compounds is contradictory in published literature (Chen *et al*., 2010; Rahrovan *et al*., 2018). To demonstrate this the difference in hydration of the stratum corneum has been measured in various studies with a non-significant difference between the two sexes (Wilhelm *et al*., 1991; Jacobi *et al*., 2005; Firooz *et al*., 2012), whereas others statistically concluded a significant difference between the two sexes (Mac-Mary *et al*., 2006; Liu *et al*., 2012). Common practice during permeation research dictates the use of a single sex to eliminate the possible influence thereof.

2.3.3.12 Age

The exact effect of aging skin on the permeation of exogenous compounds is still controversial in literature. Structural changes due to normal aging of the skin have led to examinations into the specific effect this might have (Konda *et al*., 2012). Degeneration of capillaries supplying the skin with blood and the change in lipid content of the stratum corneum are well established examples of this (Finnin *et al*., 2012; Konda *et al*., 2012). The decrease in blood supply to the skin influences the diffusion gradient across the skin and decreases systemic delivery. Whereas a decrease in lipid content of the skin layers decreases resistance to hydrophilic compound permeation through the skin (Roskos *et al*., 1989; Finnin *et al*., 2012; Hostýnek, 2003). The change in lipid content does not have a significant impact on highly lipophilic compounds (Roskos *et al*., 1989; Finnin *et al*., 2012). Further structural changes are decreases in sweat production, a degeneration in whole skin thickness and higher pH values (Finnin *et al*., 2012; Konda *et al*., 2012). Animal studies indicated inverse proportional associations between permeability and age (quantifiably represented by decreased permeability coefficients), especially apparent for lipophilic compounds (Ngawhirunpat *et al*., 2001). However, a study found the influence of age on water and mannitol permeation insignificant (Dick and Scott, 1992). Further research focusing on the effects of aging related changes such as total skin thickness and pH, delivered contradicting conclusions on the influence on permeation (Wilhelm *et al*., 1991; Fluhr *et al*., 2000; Waller and Maibach, 2005, 2006; Konda *et al*., 2012).

2.4 PGM permeation studies

Although, the focus of this chapter is on dermal permeation studies with platinum salts, related research of dermal exposure to metals, especially PGMs is relevant to this study. The following paragraphs describe the related PGM dermal permeation studies on nano-sized Pd (Larese Filon *et al*., 2016) and Pd powder (Crosera *et al*., 2018), platinum (K_2PtCl_4) and rhodium

salt ($RhCl_3$) (Franken *et al*., 2014), nano-sized platinum (Na_2PtCl_6) and rhodium salt ($RhCl_3 \cdot 3H_2O$) (Mauro *et al*., 2015). These studies and an osmium tetroxide (OsO_4) dermal exposure case study (Friedova *et al*., 2020) illustrate the interplay of influential factors through the results obtained.

Larese Filon *et al*. (2016) investigated the *in vitro* permeation of nanoparticle size Pd. PdNPs utilized were 10.7 nm in size with low inclination to agglomerate. Nanoparticles with a low inclination to agglomerate have a high likelihood of permeation (Faurschou *et al.,* 2011; Muris *et al*., 2014, 2015). The results proved time dependent penetration of PdNPs via the epidermal appendages with concentrations ranging from lowest to highest as receptor solution < dermis < epidermis (Larese Filon *et al.*, 2016). Larese Filon *et al.* (2016) associated the high epidermal concentrations with possible accumulation (storage) of PdNPs in hair follicles, due to their small size. This theory corresponds well with the known delayed mechanism of allergic contact dermatitis caused by Pd exposure (Faurschou *et al.,* 2011; Muris *et al*., 2014, 2015). The accumulation could lead to long term permeation and adverse health effects. Lastly, permeation was significantly higher through damaged skin (11 times higher) than through intact skin with a slightly shorter lag time in damaged skin (Larese Filon *et al*., 2016).

Following the research on PdNPs, Crosera *et al.* (2018) utilized the Franz diffusion cell methodology to evaluate the permeation of fine Pd powder through intact and damaged human skin. The penetration into the skin compared well with the PdNP study (Larese Filon *et al*., 2016), with a concentration profile of receptor solution < dermis < epidermis and high amount of the total mass contained in the skin residing in the epidermis. The results concluded that damaged skin showed six times higher permeation than intact skin and that sufficient decontamination procedures led to no detectable penetration of the skin barrier or absorption through the skin into the receptor solution (Crosera *et al*., 2018). Comparing their results to Larese Filon *et al*. (2016) indicated that smaller amounts of PdNP delivers similar permeation results as when fine Pd powder was used (Crosera *et al*., 2018). Furthermore, compared to K_2PtCl_4 (platinum salt) and $RhCl_3$ (rhodium salt) permeation concentrations, a two times higher concentration of Pd powder permeated, although caution should be taken in this comparison due to the higher pH of the Pt and Rh study and the use of salts (Franken *et al*., 2014; Crosera *et al*., 2018).

The study on platinum salt (K_2PtCl_4) and rhodium salt ($RhCl_3$) permeation was conducted by Franken et *al.* (2014) and indicated that the salts permeate through full thickness abdominal Caucasian skin with a cumulative increase in absorption over time. Comparing the two salts' permeability showed that platinum permeated the skin consistently higher and faster and was

retained twice as much inside the skin when compared to rhodium. The differences in permeation of these two PGMs were attributed to the smaller agglomerates that platinum formed, observed 30-40 nm compared to rhodium > 50 nm, and the more negative charge of the platinum dissolute (Pt^{2+} compared to Rh^{3+}). The penetration into the skin, forming a reservoir, was significantly more than the mass that permeated through the skin for both Pt and Rh. Furthermore, the depiction of possible continuous permeation at the end of a work-shift, namely the 'take-home' effect, was illustrated. The effect was related to the often-detected poor personal hygiene in refineries and consequently no decontamination of exposed dermal areas. This 'take-home' effect is problematic as a significant increase in permeation for Pt and Rh was found between 8 hours (normal shift length) and 24 hours (Franken *et al*., 2014).

Following the *in vitro* research of Franken *et al*. (2014) on Pt and Rh, the permeation of more hazardous nanoparticles' was evaluated through intact and damaged skin, by utilizing Na_2PtCl_6 and $RhCl_3 \cdot 3H_2O$ (Mauro *et al*., 2015). PtNPs were not absorbed through intact or damaged skin, implicating theorized interactions with skin proteins. This was further substantiated by high PtNPs and RhNPs concentrations in the skin. Although the only detectable permeated concentration was a low concentration of RhNPs through damaged skin, the skin content proved that Pt penetrated (but not permeated through) intact skin more than Rh and with the opposite being true for damaged skin. The dermal concentration of both Pt and Rh decreased significantly from the epidermis towards the dermis. The concentration gradient between the epidermis and dermis was associated with the storage of the NP compounds in the epidermal hair follicles (Rancan *et al.*, 2012; Mauro *et al*., 2015). Furthermore, the penetration concentrations were two times and 17 times higher in damaged skin for Pt and Rh respectively. The amount of dermal retention indicated by the two compounds and the higher concentrations of PtNPs in the skin in relation to RhNPs were comparable to the dermal retention found by Franken *et al.* (2014) (Mauro *et al*., 2015).

Exposure to osmium is very scarce and thus the case study of an accidentally exposed laboratory researcher provided valuable information on the permeation of osmium tetroxide. The presence of osmium in the blood and urine after dermal and corneal exposure allowed the extrapolation of absorption through the skin and corneas. However, the exposure conditions should not be excluded as the conclusions were based upon an accidental exposure and the influential factors normally controlled in a laboratory study were not applied (Friedova *et al*., 2020). Further *in vitro* permeation studies with a larger sample size and a regulated laboratory environment could be beneficial to the understanding of osmium's toxicokinetics.

2.5 *In vitro* dermal permeation methodology

The Franz diffusion methodology has been adopted by many studies to evaluate the permeation of a variety of substances (Levintova *et al*., 2011). Examples of these adapted methodology studies include research into cobalt metal, caffeine, platinum, rhodium, palladium and benzene (Larese Filon *et al*., 2004; Liu *et al*., 2011; Franken *et al*., 2014; Crosera *et al*., 2018; Frasch and Barbero, 2018). The method is based on the advantageous use of *in vitro* experiments by utilizing human skin characteristics and simulating the environment where workers are exposed (Raney *et al*., 2015). *In vitro* studies are less time-consuming, less expensive, and reduce overall complexity when compared to *in vivo* studies yet deliver comparably relevant results to when *in vivo* methods are utilized (Wagner *et al.*, 2002; Lehman *et al*., 2011; Selzer *et al*., 2013; Pantelić *et al*., 2018). The results relate to two major points in particular; rate and extent of active compound fraction permeated through human skin and both *in vitro* and *in vivo* reaching the same conclusion (Raney *et al*., 2015).

The Franz diffusion cells are based on two compartments, namely the donor and receptor compartments (Franz, 1975; Ng *et al.*, 2010). In between the two compartments a sampling membrane is clamped. This membrane may be of an animal, human or synthetic origin (Zhang *et al.*, 2017). Although synthetic membranes have been used to provide some information on time dependent drug release (Ng *et al*., 2010; Zhang *et al*., 2017), the results do not include enough influencing factors, such as the effect of a possible dermal reservoir forming, to be relevant when compared to similar human *in vivo* experiments. The factors that affect permeation may include interactions between human skin and the exogenous compound (Pantelić *et al.*, 2018) and structural differences (Jacobi *et al*., 2007; Zhang *et al*., 2017). Consequently, human skin is seen as the best fit or 'golden standard' when evaluating *in vitro* methods (Barbero and Frasch, 2009). Unfortunately, the availability of *ex vivo* skin from enough donors is minimal (Raney *et al*., 2015; Zhang *et al*., 2017). The use of cadaver skin is useful when evaluating poorly soluble compounds, although the biotransformation function of the skin is not utilized in cadaver skin experiments and comparison to live human skin is often needed (Rice and Mauro, 2013). Furthermore, as in this research, the skin thickness is often used as the full epidermis (stratum corneum included) and part of the dermis (Raney *et al*., 2015). With the inclusion of the complete stratum corneum, the primary barrier function of the skin is accounted for in the results obtained (Rice and Mauro, 2013). The thickness of the skin used may influence permeation parameter calculations and is thus considered and measured prior to experimentation.

The methodology requires the use of liquids representing the donor and receptor compartment solutions (Levintova *et al*., 2011). The donor solution is often referred to as the donor fluid

whereas donor solution without the investigated compound (i.e. blank solution) is often called the synthetic sweat. The receptor solution is often referred to as receiver fluid, physiological solution, or synthetic blood plasma. These solutions cause hyperhydration of the stratum corneum, which is well known as the rate limiting layer for most compounds, leading to definite changes in the permeability of the contaminant (Van Hal *et al*., 1996; Bellantone *et al*., 2002; Levintova *et al*., 2011; Rice and Mauro, 2013). Thus, the hyperhydration of the stratum corneum during *in vitro* experimentation should be considered when evaluating the permeation parameters. Pools of water develop in the intercellular lamellar region of the corneocytes during hyperhydration which lead to less resistant permeability pathways for hydrophilic substances (Van Hal *et al*., 1996; Levintova *et al.,* 2011). This phenomenon is represented in partition coefficients of hydrophilic substances, as discussed below (Van Hal *et al*., 1996). Donor compartments are often occluded, sealing the top preventing evaporation and thus reinforcing the hyperhydration of the skin. By leaving the cap off may cause other factors to cause undesirable variations in permeation. These factors include the loss of compound to the environment and the skin might dry out completely. Furthermore, hyperhydration during *in vitro* experimentation cause swelling and consequently the closure of the follicular route openings (Selzer *et al*., 2013).

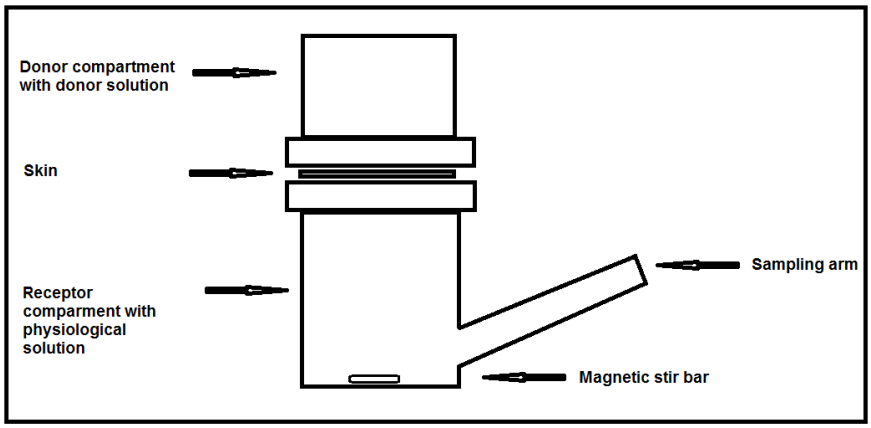

Fig. 1: Static Franz diffusion cell system (adopted from Franz, 1975)

The composition of the compartment solutions should facilitate the solubility of the compound in the receptor solution in order to create optimal sink conditions (Raney *et al*., 2015) and the pH of the donor solution is carefully selected. The pH of this solution, as discussed under permeation influencing factors (Section 2.3.3, **above**), is considered with caution as it greatly influences the permeation of substances (Hostýnek, 2003). The sampling of the receptor solution is done frequently to provide enough data representing the effect of time on permeation and removing enough of the compound to mimic microcirculation in the dermis layer of the skin (Raney *et al*., 2015). Together with frequent sampling of the receptor solution, some guidelines are set regarding the total experimental timeframe. Due to the influence of time on absorption of

a compound, the laboratory experimentation should allow for the accurate evaluation of the permeability profile of a compound. However, to provide for the degradation of the physical integrity of the skin during experimentation, a single experiment should not exceed 72 hours when utilizing infinite dose conditions. After 72 hours the skin is not classified as intact anymore. An experimentation timeframe of 24 or 48 hours in total is common practice (Van de Sandt *et al*., 2004; Elewski, 2007; Selzer *et al*., 2013). Although, written guidelines suggest a 24-hour period (OECD, 2004), published literature suggests that the duration should exceed 2.7 times the lag time period to ensure that steady state conditions are contained in the results. Compounds with extremely low flux rates require strict limits of detection during analysis methods and should have longer experimentation time to allow detectable levels of permeation (Selzer *et al*., 2013).

The results collected during this methodological approach could then be used to calculate common permeation parameters to quantify the permeation profile presented by the compound. These calculations include the diffusion-, partition- and permeability coefficient, lag times, flux, the mass retained in the skin and the percentage compound mass recovered after experimentation.

2.5.1 Permeability coefficient

The permeability coefficient is composed of the partition- and diffusion coefficients. The partition coefficient is defined as an unitless indication of lipophilicity or affinity across the different mediums, i.e. donor solution and skin (WHO, 2006). The skin-vehicle partition coefficient, as described here, could only be calculated from permeation experimentation (McDougal and Boeniger, 2002). Reasonably high partition coefficients indicate an increase in dermal retention of the compound, due to its high affinity to the skin (Zsikó *et al*., 2019). Whereas the diffusion coefficient quantifies the rate of diffusion by the compound through the various routes. Factors that influence this parameter significantly are the particle size of the exogenous compound and the thickness of membrane (skin) through which the compound should diffuse (Singh and Singh, 2003). Thus, a lower diffusion coefficient indicates greater diffusional path length (membrane thickness) and slower diffusion (Watkinson and Brain, 2002). Therefore, the combined effects of partition and diffusion coefficients would describe the movement of the compound over time, the permeability coefficient (Díez-Sales *et al.,* 1991; McDougal and Boeniger, 2002). Permeability coefficients for non-ionized compounds are much higher than those of their ionized counterparts (WHO, 2002). A higher permeability coefficient is associated to a faster lag time (Singh and Singh, 2003).

2.5.2 Lag time

Lag time is the time it takes before the permeation rate becomes constant. This should not be misinterpreted as the time before the compound permeates through the skin into the receptor solution (McDougal and Boeniger, 2002). Lag time could be as fast as a few minutes or as slow as a couple of days and is significant in assessing the risk of exposure when compared to the exposure time (Schneider *et al*., 1999). Even though lag time is a commonly used parameter calculated and utilized to add to a permeation profile of a compound, it is not reproducible. This is mainly due to its extreme sensibility to membrane thickness and thus is impractical to replicate and confirm (Kierstan *et al*., 2001).

2.5.3 Flux

Flux is a permeation parameter describing the compound movement through a membrane (skin or synthetic membrane) per dermal area over time. This is calculated with steady state conditions prevailing (McDougal and Boeniger, 2002). Steady state is the moment in time where the compound exits the stratum corneum towards the viable layer of the epidermis at the same rate as it enters from the outside (Rice and Mauro, 2013). Steady-state conditions are normally following the lag time (McDougal and Boeniger, 2002).

2.5.4 Retention in skin

Penetration is the mass of metal measured inside the skin and thus absorption is the mass of metal that passed through the thickness of the skin into the receptor solution (Hopf *et al*., 2020). The mass of penetrated exogenous compound in reference to the mass retained in the donor solution or permeated into the receptor solution, is associated with the affinity of the compound to the skin (partition coefficient) and may indicate chemical association with proteins in the skin. A reservoir could form in the skin leading to continuous permeation and absorption into the circulatory system even after exposure has ceased in real-world workplace environments (Franken *et al*., 2014, 2015).

2.5.5 Compound recovery calculation

In order to account for the total dose applied and what occurred during experimentation the total mass detected in the receptor- and donor solutions and the mass retained in the skin are collectively used to calculate the percentage of the dose accounted for (OECD, 2004; Selzer *et al*., 2013). Guidelines indicate that this percentage should be between 100 ± 10 % (OECD, 2004) or 100 ± 15 % to enable the reporting of adequate dose recovery.

2.6 Conclusion

Platinum is used in multiple industries due to its chemical and physical characteristics. As the natural occurrence of platinum is in a variety of oxidative states, the formation of complex

platinum salts such as hexachloroplatinate, is common. During mining and refining of platinum for multiple uses, workers may be occupationally exposed to the various forms of platinum. Until recently, studies of occupational exposure to these platinum salts were, mainly concentrated on the respiratory route of exposure although the dermal route has become more prominent in literature. Occupational exposure to these platinum salts causes respiratory sensitization with various dermal manifestations. While the toxicokinetics governing these health effects are not well-known, emphasis could be placed on the uncertainties related to dermal absorption. Thus, further investigation into the uncertainties of dermal absorption and the permeation profile of potassium hexachloroplatinate would be beneficial. The utilization of the static Franz diffusion methodology to quantify the permeation profile of potassium hexachloroplatinate would offer valuable insights into possible dermal permeation.

2.7 References

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CHAPTER 3: ARTICLE

Toxicology Letters is an international journal dedicated to rapid publishing of reports based on all toxicology aspects. *Toxicology Letters* provides different guidelines for new submissions compared to those of revised submissions. The following guidelines are stipulated for new submissions and are thus of relevance to this chapter. This manuscript is written in American English.

3.1 General

Submission manuscripts should be written in English (American spelling) with 1.5 spacing. A title page should be provided with submission containing a concise and informative title of the manuscript. Given the title is used in systems used for information-retrieval, it is advised not to use abbreviations or formulae where possible. The authors' names and affiliations should be stated, and the corresponding author highlighted with his/her relevant contact information. The manuscript should be clearly divided into numbered sections, excluding the abstract. Figures and tables should be numbered with Arabic numbers and have a short descriptive caption. Placement should be next to the relevant text and a detailed caption should be directly below the figure or table.

3.2 Structure

Essential headings include abstract, keywords, introduction, materials and methods, results, conclusions, funding, conflict of interest, acknowledgements and there should be clear sections under each heading.

Abstract

The abstract should describe the purpose, principle results and main conclusions of the manuscript in 200 words or less. It should be able to stand apart from the article while being concise and factual. References and uncommon abbreviations should be avoided. Opting for a graphical abstract, a concise illustration summarizing the article, is encouraged.

Keywords

Immediately below the abstract provide 3 to 6 American English keywords. Footnotes placed on the first page of the article may be used to define lesser-known abbreviations that form part of the keywords.

3.3 References

As a new submission, there are no specific guidelines for references as long as the rules used are kept consistent throughout the manuscript. Single spacing is preferable in the reference list.

The use of DOI and where appropriate addition of authors' names, journal title/book title, chapter title/article title, year of publication, volume number/book chapter and the article number are encouraged. In text references will provide the author(s) and publication date, for example (Author *et al.*, 2021). Below are a few examples of references for the reference list:

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In vitro permeation of potassium hexachloroplatinate through full thickness human abdominal skin

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Abstract

During the processing of platinum, exposure to platinum salts may occur. Respiratory sensitization has been strongly associated with inhalation exposure to platinum salts. However, the dermal route was proposed as a viable route of exposure and was substantiated by results reporting *in vitro* dermal permeation of potassium tetrachloroplatinate through human skin. This study focused on quantifying the *in vitro* permeation of potassium hexachloroplatinate by utilizing 0.3 mg/mL K_2PtCl_6, full thickness human abdominal skin, and the static Franz diffusion cell methodology. The receptor solution was removed at 2-, 4-, 6-, 8-, 10-, 12-, 14- and 24-hour intervals and analyzed by Inductively Coupled Plasma-Mass Spectrometry. The cumulative platinum mass that permeated through the skin increased with time. The significant increase of 68 % in permeation between 8- and 12-hours is an example thereof. Results indicated a significant dermal retention of platinum (3.11 ± 0.25 %) compared with the mass that was absorbed through the skin (1.8×10^{-3} %). A short lag time of 2.26 hours was calculated. The permeation parameters indicate the likelihood of platinum salt dermal exposure leading to systemic absorption in exposed workers and significant dermal retention that may add to the total body burden.

Keywords

Franz diffusion cells; occupational health; dermal exposure; platinum group metals; platinum salts; skin retention.

1. Introduction

South Africa possess 80 % of the platinum deposits in the world and provided a stable 73 % of the total worldwide platinum demand between 2017 and 2019 (JM, 2018, 2019, 2020). Platinum, as part of the platinum group metals (PGMs), possesses group characteristics including resistance to corrosion, a high melting point and an ability to form multiple oxidative states (Cotton *et al*., 1999; Xiao and Laplante, 2004). In addition to platinum, the other PGMs are palladium (Pd), rhodium (Rh), ruthenium (Ru), osmium (Os) and iridium (Ir) (Wiseman and Zereini, 2009; Iavicoli *et al*., 2012). Due to platinum's unique characteristics, it is utilized in multiple industries such as in the manufacturing of automotive catalysts, dentistry instruments and jewelry as well as in antitumor treatments (Hunter *et al*., 1945; Hunt, 1982; Rauch and Peucker-Ehrenbrink, 2015). The mining, refining, processing, recycling and usage of platinum may lead to workers being exposed to toxic substances, including platinum in various forms (Linnett and Hughes, 1999; Brook, 2006). Exposure to platinum at refineries and recycling plants are often in the form of platinum salts and often elevated in nature (Calverley *et al.*, 1995; Linnett and Hughes, 1999; Linde *et al*., 2017, 2018). These platinum salts may cause respiratory sensitization, along with dermal manifestations such as itching, urticaria, and

dermatitis (Hunter *et al*., 1945; Lown *et al*., 1980; Calverley *et al*., 1995; Merget *et al*., 2000). Inhalation of platinum salts has been positively associated with the development of respiratory sensitization (Calverley *et al.*, 1995; Linnett and Hughes, 1999; Heederik *et al*., 2016). However, the dermal route of exposure was suggested when the respiratory sensitization reactions were not justified by inhalation exposure alone. This was indicated by low levels of inhalation exposure, although with no change in the occurrence of sensitization reactions reported in workers in the areas of exposure (Maynard *et al*., 1997). The dermal route of exposure was further implicated after sensitization reactions manifested following exclusive *in vivo* dermal exposure of animals (Schuppe *et al*., 1997; Kimber and Dearman, 2002). Furthermore, dermal exposure to sodium hexachloroplatinate (Na_2PtCl_6) positively associated to the exacerbation of existing respiratory sensitization. Thus, proving that respiratory sensitization towards platinum salts in animals is not exclusively limited to inhalation exposure (Schuppe *et al.*, 1997). Subsequent human studies led to the *in vitro* experimentation with potassium tetrachloroplatinate (K_2PtCl_4) resulting in permeation through full thickness human abdominal skin (Franken *et al*., 2014, 2015).

Tetra- and hexachloroplatinate are considered as the most toxic platinum salts (Ravindra *et al*., 2004; Bencs *et al*., 2011). The toxicity is a manifestation of the number of halide bonds (Cl^-) in the compound as the harmfulness is directly associated with increases in halide bonds (Cleare *et al*., 1976). Thus hexachloroplatinate, containing four halide bonds, is more toxic than its tetra counterpart containing only two. However, not all halide containing platinum compounds are sensitizing (Cleare *et al*., 1976; Di Gioacchino *et al*., 2004). Furthermore, the bond with ammonium and potassium as alternatives to, for example sodium, has indicated increased toxicity as well (Ravindra *et al*., 2004; Bencs *et al*., 2011). The compound composition dictates the speciation of platinum, which is of the utmost importance when considering the permeation profile of the compound and the development of adverse health effects (Linnett and Hughes, 1999; Boscolo *et al*., 2004; Di Gioacchino *et al*., 2004; Ravindra *et al*., 2004; Van Briesen *et al*., 2010; Bencs *et al*., 2011). Speciation is associated, for example, to the oxidative state, with a higher oxidative state often relating with increased dermal permeation (Hostýnek, 2003; Van Lierde *et al*., 2006). Although studies have been conducted on tetrachloroplatinate, the importance of speciation (Linnett and Hughes, 1999; Van Briesen *et al*., 2010) together with the positive association between the number of halide bonds and the toxicity of platinum salts (Cleare *et al*., 1976) justifies the *in vitro* dermal permeation investigation of the more toxic, hexachloroplatinate. The aim of this study was to quantify the *in vitro* dermal permeation of potassium hexachloroplatinate (K_2PtCl_6) through full thickness human skin.

2. Materials and Methods

The methodology was based on published studies using the Franz diffusion cell to investigate the dermal permeation of metals such as nickel, cobalt, chromium and gold (Larese Filon *et al*., 2004, 2007, 2009, 2011). The methodology was modified for PGMs in collaboration with Anglo American, Technical Solutions - Research. The methodology in this section is based on the adapted methodology used to determine the *in vitro* permeation of potassium tetrachloroplatinate (K_2PtCl_4) (Franken *et al.*, 2014, 2015).

Chemicals

All chemicals were purchased as analytical grade. Sodium chloride (NaCl; > 99.5 %), ammonia (32 %), lactic acid (90 %), potassium hexachloroplatinate (K_2PtCl_6; > 99 %), disodium hydrogen phosphate (Na_2HPO_4; 99.8 %) and potassium dihydrogen phosphate (KH_2PO_4; 99.5 %) were purchased from Merck, South Africa. Urea (99.3 %), acetone (99.9 %), hydrogen peroxide (H_2O_2; 30 %), nitric acid (HNO_3; 65 %) and hydrochloric acid (HCl; 32 %) were purchased from De Bruyn Spectroscopic Solutions (South Africa). All solutions were prepared with water obtained from an ultrapure water (type 1) purification system (RephiLe Bioscience, China).

The synthetic blood plasma (physiological solution) used as the receptor solution consisted of 0.9 % NaCl, 0.238 % Na_2HPO_4, 0.019 % KH_2PO_4 dissolved in 2 L type 1 water and adjusted to a pH of 7.35 with HCl. The synthetic sweat solution comprised of 1 L of water (type 1) in which 0.5 % NaCl, 0.1 % urea and 0.1 mL lactic acid were dissolved (6.5 pH adjusted with ammonia). The donor solution for the experimental cells was prepared by dissolving 0.0374 g K_2PtCl_6 in 50 mL synthetic sweat solution and adjusted to a pH of 6.5 with ammonia. This ensured a concentration of 0.3 mg Pt/mL of K_2PtCl_6 donor solution. A 0.9 % NaCl solution was used for the skin integrity testing.

Collection and Preparation of skin samples

This study was approved by the North-West University Health Research Ethics Committee (ethics approval no. NWU-00202-15-A1-02). Caucasian full thickness abdominal skin was obtained from abdominoplasty procedures following informed consent from participating surgeons and their female patients. Thereafter the skin was labeled and stored at -18 °C for a period not exceeding eight months (see **Table 1**). Studies have shown that freezing skin for *in vitro* purposes for a period of 12 months or more did not have a significant effect on the skin's integrity or permeation characteristics (Barbero and Frasch, 2016). At the time of experimentation, the skin was allowed to thaw and, with the subcutaneous fat removed, cut into circular pieces of 2.4 cm in diameter. Care was taken to exclude damaged skin (e.g. visible signs of stretch marks) and skin of ≥ 1 mm in thickness was rejected (OECD, 2004a). The skin

pieces were subjected to skin integrity testing before and after the experiment by means of a 0.9 % NaCl solution and a Precision LCR-Meter (LCR-800; GW-Instek) set at 1 kHz in a parallel equivalent circuit. Skin pieces with a resistance below 10 kΩ were excluded and the resistance of selected pieces were within a range of 8 kΩ of each other (Fasano and Hinderliter, 2004). The selected skin pieces were then sorted so that the Franz cells for experimentation comprised of an equal number of both donors in each of the two experiments. The ratio of experimental cells (exposed to K_2PtCl_6) to blanks was 2:1.

Table 1:
Summary of skin donor information.

	Experiment 1		*Experiment 2*	
Donor	A	B	C	D
Age of donor (years)	41	42	46	48
Freeze period (months, days)	7m, 12d	7m, 23d	3m, 26d	7m, 2d
Number of experimental cells (*number of blanks*)	12 (*7*)		12 (*5*)	

Experimentation

Static 2 mL Franz cells were utilized in which the selected skin pieces were clamped between the donor and receptor compartments with the epidermis towards the donor compartment. The Franz system with the clamped skin pieces will be referred to as cells. Prior to experimentation 2 mL of receptor solution (preheated to 37 °C) and 1 mL synthetic sweat solution (preheated to 32 °C) were placed into the respective cell compartments for blank cells. Experimental cells received the receptor solution and 1 mL preheated donor solution (32 °C) containing the 0.3 mg Pt/mL K_2PtCl_6. The cells were then placed in a water bath set at 37 °C and the activation of the magnetic stirrer was marked as time 0. The temperature and magnetic stirrer were used to simulate the *in vivo* environment (Franz, 1975; OECD, 2004a). At time intervals of 2-, 4-, 6-, 8-, 10-, 12-, 14- and 24-hours, 2 mL of receptor solution was extracted, and the compartment was rinsed with 2 mL receptor solution to ensure that all platinum was removed at each interval. Thereafter, the compartment was refilled with fresh receptor solution. After completion of the experiment, at 24 hours of exposure, the donor compartment solution was extracted, and the compartment rinsed four times with 1 mL synthetic sweat solution. The rinse solution and the extracted solutions were placed into clearly labeled vials for analysis. After post-integrity testing the cells were taken apart and the skin pieces placed into separate labeled vials for the standard digestion process as described in Franken *et al.* (2014, 2015).

Skin digestion

The skin digestion process began by weighing the individual skin pieces to estimate the volume of chemicals necessary. Acetone (1 mL) was used to rinse each vial into labeled glass beakers to ensure the entire sample was transferred. The beaker was then placed on a hotplate and covered with a watch glass for the remainder of the process. HNO_3 and H_2O_2 were added, 5 mL and 2 mL respectively, and evaporated separately. HCl and HNO_3 were added to the dry beaker in quick succession, in a ratio commonly known as aqua regia (3:1), to ensure all platinum in the sample was dissolved. After only a little liquid was left 2 mL of HCl was added and left to evaporate until a small amount of liquid was left. The dissolved platinum solution was hereafter transferred to a labeled Falcon® tube. Rinsing of the beaker and watch glass with 0.07 M HCL ensured the entire sample was transferred into the tube. The sample solution volume was then adjusted with 0.07 M HCl to a sample volume of 10 mL.

Chemical analysis

The receptor and donor solutions were analyzed by means of Inductively Coupled Plasma-Mass Spectrometry (ICP-MS; Agilent 8900 Triple-quad) and digested skin solutions were analyzed by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES; Agilent 5110) to quantify the platinum concentrations. Yttrium and Thulium were used as internal standards to correct potential instrument drift. The ICP-MS was calibrated to the range of 10 and 2000 ng/L and the ICP-OES to the range of 0.05 - 2 mg/L and matched to the matrix where possible. Samples were diluted in 1 % HCl by factor of 10 or 20, depending on the matrix. External quality controls were performed with certified concentrations to evaluate the accuracy of the calibrations. A 100 ng/L detection limit was established originally and 500 ng/L for the repeat analysis. The repeat analysis was conducted on 6.4 % of the samples to improve the accuracy and reliability of the data.

Data analysis and statistics

The equation to calculate the mass of a contaminant permeating through a membrane at a specific time was developed in 1991 by Díez-Sales *et al.* (1991), Eq. (1):

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

$Q(t)$: Mass passing through membrane at specific time interval (t)

A: Actual surface diffusion area

K: Partition coefficient formed between the skin and donor solution

h: Membrane thickness

C_v: Concentration of the contaminant in the donor solution

D: Diffusion coefficient of the contaminant in the membrane

The exponential term becomes insignificant as the time interval approaches infinity thus the formation of Eq. (2):

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

In the case where K, D and h are unknown, as was in the case of this particular study, Kh and D/h^2 could be replaced by α and β respectively which leads to the formation of Eq. (3) and (4) for calculating the permeability coefficient (k_p) and flux (J):

$$k_p = \frac{KD}{h} = \alpha\beta$$

$$J = k_p C_v$$

Calculating the permeation parameters (i.e. mean permeation, total mass retained in the skin, permeability coefficient, flux and lag time), the mean blank values were subtracted from the experimental data to compensate for potential contamination during experimentation. The platinum concentration in the receptor solution (ng/L) was converted to the cumulative concentration that permeated through the exposed dermal area (ng/cm^2). The total exposed dermal area was 1.02 cm^2. The amount of permeate through the skin is known as the cumulative concentration. The cumulative concentration was plotted against the extraction time intervals (0, 2, 4, 6, 8,10, 12, 14, and 24-hour) to represent the total mass of platinum that permeated through the skin per time unit. Flux permeation was calculated from the steady state area of the cumulative mass over time graph, previously curve fitted. Whereas the point at which the curve fitted graph intercepted the x-axis was used to calculate the lag time. The curve fit was done by means of inputting Eq. (2), in Spiral software – EasyPlot (Aerious Limited) version 4.0.5. to derive values equal to α and β. These results were used to calculate the flux value (J) using Eq. (3) in Microsoft Excel version 2105.

The statistical analysis was based on what was previously described by Franken *et al.* (2014, 2015) and confirmed by Statistical Consultation Services of the NWU. Statistical analysis was performed by means of Statistica (Statsoft) version 14.0.0.15. Descriptive statistics, such as mean and standard error of mean (SEM), were calculated by utilizing the original data. Normal distribution was achieved by means of implementing a Box-Cox transformation onto the data (Li, 2005). The afore-mentioned transformation was preferred due to the size of the groups. The Box-Cox transformed data was then used for dependent t-testing of the cumulative concentrations between all consecutive time intervals and between combinations of 8-, 12- and 24-hour exposure intervals. To enable the evaluations of probable

dissimilarity between the two experiments due to different skin donors and batches of solution, an independent t-test was performed. An Analysis of variance (ANOVA) test was applied to the data to identify calculated parameters with large variance between donors. An independent t-test was performed on all the parameters identified during the ANOVA to determine which individual donor pair expressed the variance. All p-values ≤ 0.05 were considered as statistically significant.

3. Results

Platinum permeated through the intact full thickness Caucasian female abdominal skin. The permeation increased over several time intervals, as illustrated in **Fig. 1**. Results indicated permeation of platinum as early as 2 hours after exposure commenced. At 8 hours of exposure the mean permeation through the skin was 1.87 ± 0.32 ng/cm^2, after which the mean permeation increased to 3.14 ± 0.53 ng/cm^2 at 12 hours (68 % increase). The 24-hour interval mean permeation was 5.08 ± 0.63 ng/cm^2 and the mean total mass diffused was 1293 ± 160 ng/L. Therefore, the mean permeation increased by 62 % from 12 to 24 hours of exposure. Statistically significant increases in cumulative mass permeation were indicated between all consecutive intervals (p-values ranging between < 0.001 and ≤ 0.01) except between 14- and 24-hour intervals. After 24 hours of exposure the mean total platinum mass retained in the skin was 1861.64 ± 147.07 ng/cm^2 (8848.03 ± 699.02 ng). Thus, 3.11 ± 0.25 % of the total mass (dose) applied in the donor solution was retained in the skin, whereas 1.8 x 10^{-3} ± 0.06 x 10^{-3} % (5.17 ± 0.64 ng) mean platinum permeated through the skin and was present in the reception solution. The mass balance recovery calculations were performed utilizing the mean platinum mass retained in the skin, and in the receptor-, and donor solutions. This mass balance indicated a range of 83 % to 108 %, with a mean of 102 %. There were no statistically significant differences between donors A to D or between experiment 1 and 2 as determined by ANOVA and subsequent independent t-tests.

Table 2:
Summary of calculated parameters quantifying the permeation profile of K_2PtCl_6.

Parameter	*Mean ± SEM*
Permeation after 8 hours of exposure	1.87 ± 0.32 ng/cm^2
Permeation after 12 hours of exposure	3.14 ± 0.53 ng/cm^2
Permeation after 24 hours of exposure	5.08 ± 0.63 ng/cm^2
Total percentage of Pt permeated	180.39 x 10^{-5} ± 5.47 x 10^{-5} %
Total percentage of Pt in skin	3.11 ± 0.25 %
Permeability coefficient	0.11 x 10^{-5} ± 0.02 x 10^{-5} cm/h
Flux	0.32 ± 0.05 ng/cm^2/h
Lag time	2.26 ± 0.31 h

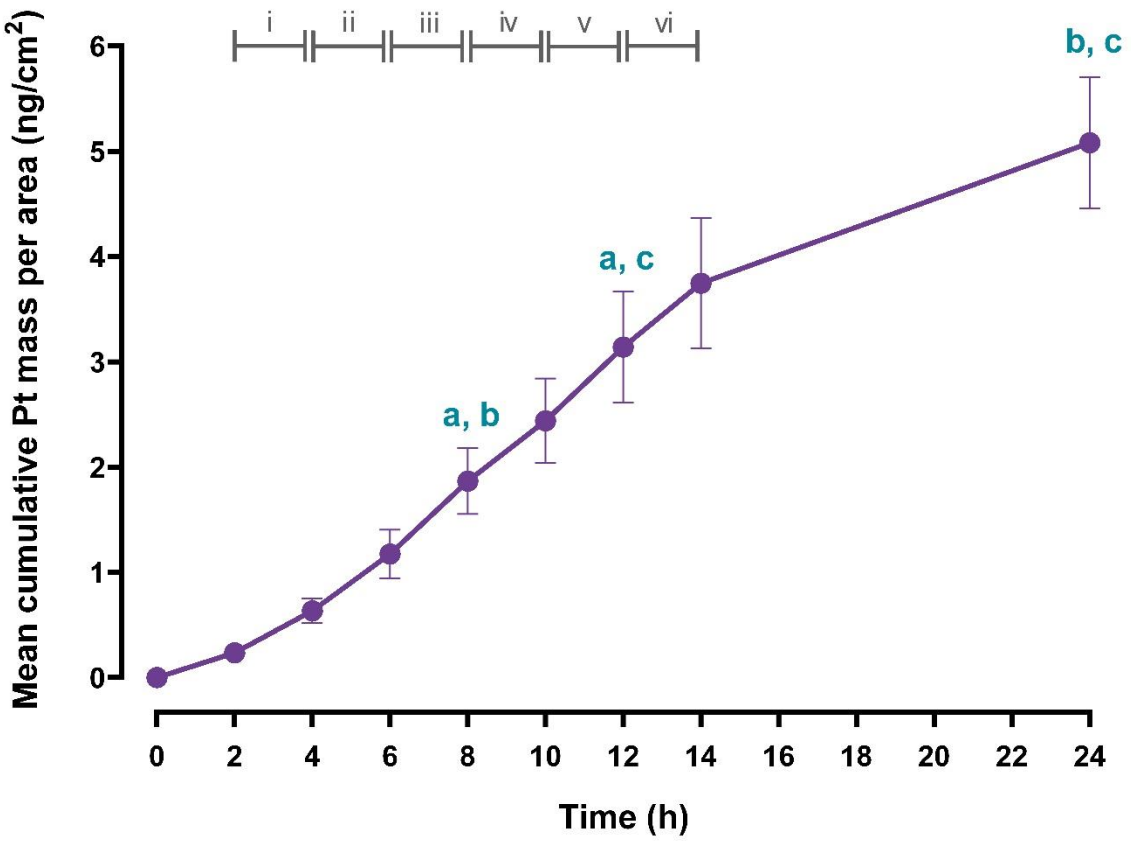

Fig. 1: Mean cumulative mass of platinum permeated, per dermal area through full thickness human abdominal skin (n = 24) (mean ± SEM). **i to vi** indicates significant differences in the cumulative mass permeation between consecutive time intervals (p values ranging from < 0.001 to 0.01). **a – c** indicates significant differences in the cumulative mass permeation between (**a**) 8- and 12-hour ($p < 0.001$); (**b**) 8- and 24-hour ($p < 0.001$); and (**c**) between 12- and 24-hour ($p = 0.04$) exposure intervals.

4. Discussion

In this study the *in vitro* permeation of platinum through full thickness human abdominal skin was investigated while utilizing potassium hexachloroplatinate (K_2PtCl_6). Platinum permeation through the skin was confirmed from the donor compartment and the permeation increased with prolonged exposure time (**Fig. 1**). The effect of time is clearly demonstrated by the increase in mean permeation between intervals 8-, 12- and 24-hours. According to the results of this study, extended exposure from 8 to 12 hours led to a significant increase of 68 % ($p < 0.001$) in the mean permeation of platinum. Normal circumstances in the workplace dictate a standard eight hour work shift although extension to 12 hours is common (Franken *et al*., 2015). Furthermore, the 62 % increase in mean permeation between 12 and 24 hours ($p = 0.04$), and 172 % increase between 8 and 24 hours ($p < 0.001$), are of importance when the 'take-home' effect is considered. This known phenomenon is a consequence of poor personal hygiene, which is

often observed amongst exposed workers (Franken *et al*., 2014). This effect describes a continuous dermal exposure due to lack of decontamination of deposited contaminant from the dermal surface after the work shift and subsequent permeation of exogenous compounds even after the worker has left the potential exposure area (Schneider *et al*., 1999; Franken *et al*., 2014).

In addition, this may cause contamination 'layering' where fresh deposition forms on top of the previous contamination still present due to the lack of decontamination, loading the dermal surface. As this study used infinite dosing, defined as a dose large enough to ensure that no amount of permeation or evaporation from the donor compartment could lead to depletion (Selzer *et al.*, 2013), the dosing represents the layering effect if insufficient decontamination routines are observed. Additional layering of contamination due to insufficient decontamination in industry could occur from airborne platinum salts being deposited onto the skin or other surfaces, such as PPE or clothing, or directly from touching the source of contamination (Schneider *et al*., 1999). Thus, during a risk assessment regarding workers' platinum salt exposure, airborne exposure, dermal deposition and potential surface contamination from various sources should be considered. A recent study at a South African refinery investigating worker exposure to platinum, confirmed inhalation exposure with a geometric mean (GM) of 0.301 µg/m^3, as well as dermal exposure with a GM of 0.008 µg/cm^2. Dermal exposure was detected on workers' hand (palm), wrist, neck and forehead. Absorption of platinum into the body was confirmed by platinum detected in the urine of exposed workers (GM = 0.212 µg/g creatinine). In addition to investigating personal exposure, surface sampling indicated detectable levels of platinum salts on a variety of surfaces with concentrations ranging from 0.001 to 145.470 µg/cm^2 (Linde *et al*., 2018).

Furthermore, when assessing the measured dermal exposure (Linde *et al.*, 2018), the risk towards dermal absorption, as reported here, adding to the body's platinum burden should be considered (Franken *et al*., 2014, 2015; Van Nieuwenhuizen, 2016). Dermal permeation could potentially add to the existing body burden (absorption) due to multiple routes and repetitive exposure over subsequent work shifts if the next exposure occurs before complete clearance (excretion) of the contaminant from the body. Half-life is a common parameter describing the amount of time it takes to clear 50 % of the compound out of the systemic circulation (Eaton and Gilbert, 2013). A long half-life of approximately 50 hours has been reported after once-off inhalation exposure to ammonium hexachloroplatinate ($(NH_4)_2PtCl_6$) (Schierl *et al*., 1998). Furthermore, observations of workers being exposed to higher levels of platinum salts on a regular basis (as seen by the increased levels in their urine; 6270 ng/g creatinine) indicate a longer half-life than the general population (5 ng/g creatinine) (Brook, 2006). This increases the risk of dermal exposure and layering of contamination

possibly adding to the body burden before the previous platinum concentration could be cleared (excreted) from the body.

Considering the potential for workplace exposure and the long half-life of platinum, the mass retained in the skin, the lag time as reported here (2.26 ± 0.31 h), and further speciation considerations may further implicate the risk factor towards the permeation of potassium hexachloroplatinate and the possible addition of platinum to the body burden. The results obtained indicated a high mean dermal retention of 8848.03 ± 699.02 ng (3.11 % of the dose applied) in relation to the mean mass absorbed through the full thickness of the skin (5.17 ± 0.64 ng; 1.8 x 10^{-3} % of the dose applied). Thus, the mean mass retained within the skin was 1711 times the absorbed mass in the receptor solution. This retained mass may form a reservoir from where continuous permeation could occur with time, leading to absorption into the circulatory system (Selzer *et al*., 2013; Franken *et al*., 2014, 2015; Van Nieuwenhuizen, 2016; Jansen van Rensburg *et al*., 2017).

The substantial retained mass in the skin is supplemented by the quick saturation of the skin (2.26 h i.e. ≈ 2 h 15 min), commonly known as the lag time. Lag time is defined as the estimated time required for permeation of a compound to reach a steady state in which the rate of permeation is constant (McDougal and Boeniger, 2002; OECD, 2004b). Thus, this is an indication of time elapsed until the membrane (skin) becomes saturated with the compound (platinum) (McDougal and Boeniger, 2002). The lag time in this study (≈ 2 h 15 min) was shorter than any of the other PGMs studies published to date (see **Table 3**). The next shortest lag time is 2.5 h (2 h 30 min) obtained with potassium tetrachloroplatinate permeation through full thickness Caucasian abdominal skin (4.5 pH) (Van Nieuwenhuizen, 2016). The lag time of the published PGM literature could be represented as $Pd_{(powder)}$ > $Rh_{(6.5\ pH)}$ > $PtCl_{4\ (African\ skin)}$ > $Pd_{(nano)}$ > $PtCl_{4\ (Caucasian\ skin)}$ > Rh > $Rh_{(4.5\ pH)}$ > $PtCl_4$ > $PtCl_{4\ (6.5\ pH)}$ > $PtCl_{4\ (4.5\ pH)}$ > $PtCl_{6}$ (**this study**). Reference to the specific study could be found in the 'Notes' section of **Table 3** and results obtained by the utilization of damaged skin was excluded from the comparisons mentioned here. The differences in results between the studies utilizing the same compounds (two rhodium chloride and two potassium tetrachloroplatinate studies) could be ascribed to inter-donor variability or subtle differences in solution concentrations. However, lag time is extremely sensitive to variation in membrane (skin sample) thickness and is thus improbable to replicate and validate, thus should be interpreted with caution (Kierstan *et al*., 2001). Lag time is therefore considered together with the other parameters such as the permeation coefficient.

Table 3:
Summary of current published PGM *in vitro* permeation study results through human skin.

Author	PGM	*Dose*	*Retained in skin*	*Permeability coefficient*	*Diffusion coefficient*	*Partition coefficient*	*Flux*	*Lag time*	*Notes*
		mg/mL	*ng/cm²*	*cm/h*	*cm/h*		*ng/cm²/h*	*h*	
Franken *et al.* (2014)	K_2PtCl_4	0.3	1459.47	0.4×10^{-6}	0.048	9×10^{-6}	0.12	3.5	
	$RhCl_3$		757.04	0.2×10^{-6}	0.23	4×10^{-6}	0.05	4.4	
Franken *et al.* (2015)	K_2PtCl_4	0.3	1486.32	0.93×10^{-6}	-	-	0.27	4.5	*Caucasian skin*
			3064.13	6.6×10^{-6}	-	-	1.93	4.9	*African skin*
Mauro *et al.* (2015)	*nano*-Na_2PtCl_6	2	800	-	-	-	BDL	-	
			1740	-	-	-	BDL	-	*Damaged skin*
	nano-$RhCl_3$		430	-	-	-	BDL	-	
			7410	-	-	-	40	7.9	*Damaged skin*
Larese Filon *et al.* (2016)	*nano*-$PdCl_2$	2	98	-	-	-	5	4.8	
			1060	-	-	-	57	4.2	*Damaged skin*
Van Nieuwenhuizen (2016)	K_2PtCl_4	0.3	1771.30	-	-	-	0.8	3.4	*pH 6.5*
			2118.90	-	-	-	1.07	2.5	*pH 4.5*
Jansen van Rensburg *et al*. (2017)	$RhCl_3$	0.3	1029.90	-	-	-	0.12	5	*pH 6.5*
			1428.70	-	-	-	0.16	4.1	*pH 4.5*
Crosera *et al.* (2018)	*powder* Pd	50	108200	-	-	-	20	6	
			25600	-	-	-	100	2	*Damaged skin*
This study	K_2PtCl_6	0.3	1861.64	1.11×10^{-6}	-	-	0.32	2.3	

- Not reported
BDL Below Detection Limit

The permeability coefficient is a general value used in permeation research, that quantifiably expresses the affinity of the exogenous compound towards the membrane (the skin) and the vehicle, which in this particular case was the synthetic sweat solution in the donor compartment (Watkinson and Brain, 2002). It, therefore, represents the rate at which the compound moves through the skin (OECD, 2011). The calculated permeability coefficient was three times higher for potassium hexachloroplatinate ($0.11 \times 10^{-5} \pm 0.02 \times 10^{-5}$ cm/h) than that of tetrachloroplatinate. Furthermore, comparing the cumulative mass permeation of potassium tetrachloroplatinate (2.57 ± 0.57 ng/cm^2) with the mean permeation of hexachloroplatinate of this study (5.08 ± 0.63 ng/cm^2), it is evident that the mean permeation of Pt from hexachloroplatinate was nearly twice the mass of mean Pt permeation from tetrachloroplatinate (**Table 4**).

Table 4:
Summary of chemical properties and permeation results between potassium tetra- and hexachloroplatinate.

Compound	*Complete dissolution*	*Hydrophilicity*	*Toxicity*	*Molecular weight (g/mol)*	*Cumulative concentration (ng/cm^2)*	*Permeability coefficient (cm/h)*
	Brook, 2006	Lindell, 1977	Cleare *et al*., 1976	Franken *et al*., 2014; **this study**		
K_2PtCl_4	Pt^{2+}	slightly less	less	415.09	2.57	0.4×10^{-6}
K_2PtCl_6	Pt^{4+}	slightly more	more	485.98	5.08	1.11×10^{-6}

These permeation profile differences between compounds with very similar chemical structures, even when utilizing the same methodologies, emphasize the importance of speciation when considering dermal exposure and subsequent health effects (Cleare *et al*., 1976; Linnett and Hughes, 1999; Van Briesen *et al*., 2010). The speciation referred to is often depicted in differences in the oxidative state of the metal compound which is in relation to the compound composition itself and molecular weight (Ravindra *et al*., 2004; Van Lierde *et al*., 2006; Williams, 2018). The oxidative state and molecular weight are important in the permeation profile and the compound composition is influential in the development of adverse health effects. The importance of speciation becomes apparent as higher levels of permeation are associated with higher oxidative states (Hostýnek, 2003; Van Lierde *et al*., 2006). Potassium hexachloroplatinate is associated with a higher oxidative state and a higher oxidative state has been associated with higher dermal permeation such as when referring to the oxidative state and permeation differences between potassium hexa- and tetrachloroplatinate (**Table 4**). However, higher molecular weight has an inverse relationship to

permeation, although, in compounds with a molecular weight less than 500 g/mol this phenomenon's effect becomes negligibly small (Bos and Meinardi, 2000; Williams, 2018).

In order to translate the results of any *in vitro* permeation research to real-world workplace applications, the workplace conditions and worker population should be taken into account. As **Table 3** suggests, influential factors on permeation such as sex, race, pH, particle size and the integrity of the skin should be considered. The effects of dermal structural differences between the sexes and races on permeation of exogenous compounds are still controversial (Chen *et al*., 2010; Muizzuddin *et al*., 2010). An example of this is indicated by the differences in permeation parameters between African and Caucasian skin (**Table 3**) after *in vitro* exposure to potassium tetrachloroplatinate (Franken *et al.*, 2015).

Furthermore, the general environment in refineries is more acidic in nature affecting the dermal surface pH. This was indicated among South African refinery workers in a study conducted in 2010 (Du Plessis *et al*., 2010). The skin pH facilitates the barrier function of the skin and ensures the skin's integrity, regulation of epidermal barrier homeostasis and the optimum functioning of lipid-producing enzymes (Stefaniak *et al*., 2013). Furthermore, the pH has a considerable influence on permeation by chemically changing the oxidation levels of compounds (Konda *et al.*, 2012) thus increasing absorption (Hostýnek, 2003; Van Lierde *et al*., 2006). Studies evaluating the influence of sweat pH on the permeation of PGMs indicated a positive relation between more acidic sweat and rhodium ($RhCl_3$) (Jansen van Rensburg *et al*., 2017) and platinum (K_2PtCl_4) (Van Nieuwenhuizen, 2016) permeation. Other real-world workplace considerations that may influence the permeation of compounds is the integrity of the skin. The skin may be physically or chemically damaged throughout the work shift, and this decrease in the integrity of the skin barrier my increase dermal permeation of contaminants (Kezic and Nielsen, 2009; Larese Filon *et al*., 2016; Crosera *et al*., 2018). Increased permeation has been found through damaged skin (Mauro *et al*., 2015; Larese Filon *et al*., 2016; Crosera *et al*., 2018) and with common observation among refinery workers having skin abrasions and dermatitis on their exposed skin (Hunter *et al*., 1945; Santucci *et al*., 2000) this may increase dermal permeation levels.

5. Conclusions

Potassium hexachloroplatinate permeated through full thickness human abdominal skin with a cumulative increase in permeation with prolonged exposure time. The significant increase in mean permeation between 8 and 12 hours ($p < 0.001$) proves to be substantial, considering that extended work shifts are common. Over the full 24 hours period, the mean mass retained in the skin, forming a potential reservoir, was considerably higher than the mean mass that permeated

through to the receptor solution. Therefore, prolonged exposure and the potential for reservoir formation in the skin, could possibly lead to continuous platinum absorption. The presence of platinum mass in the receptor solution at the 2-hour interval suggest that even short periods of exposure to potassium hexachloroplatinate presents a risk of permeation into the body and possible addition of platinum to the total body burden. With extrapolation of the *in vitro* results to the worker and the workplace, it is important to consider the various factors that could influence permeation through the skin. It is, therefore, important to protect workers against dermal exposure to platinum salts in an effort to prevent permeation through the skin, and potential absorption into the body.

6. Funding

This study was financially supported by the IPA (International Platinum group metals Association) and the Scientific Task Force that it governs. Furthermore, collaboration with Johnson Matthey Technology Centre based in Sonning Common (United Kingdom) provided all the necessary chemical analyses of the samples collected. The NWU (North-West University, South Africa) provided financial support in part and the facilities needed to execute this study.

7. Conflict of interest

No conflicts of interest existed.

8. Acknowledgements

The authors would like to express their gratitude for the immense support from and collaboration with the IPA and JM's Technology Centre.

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CHAPTER 4: CONCLUDING CHAPTER

The intention of this chapter is to provide an overall conclusion to this study and to discuss possible limitations and recommendations based on the methodology and results obtained. Specific reference is given to the aims, objectives, and hypothesis, as emphasized in Chapter 1. The limitations and recommendations are discussed as a means of providing critical insight into real-world workplace application of the results, as stated in Chapter 3, as well as to recommend further research on the permeation of PGM's through human skin.

4.1 Conclusions

During the mining and refining of PGMs, workers are exposed to platinum and platinum compounds, such as platinum salts (Hunter *et al.*, 1945; Kielhorn *et al.*, 2002; Ravindra *et al*., 2004; Linde *et al.*, 2017, 2018a, 2018b). These platinum salts cause respiratory sensitization reactions as well as dermal manifestations such as urticaria and dermatitis (Hunter *et al*., 1945; Calverley *et al*., 1995; Merget *et al*., 2000). The association between inhalation exposure and the sensitization reactions towards these platinum salts, of which tetra- and hexachloroplatinate are the most toxic (Ravindra *et al*., 2004; Bencs *et al*., 2011), has been sufficiently proven throughout the years (Maynard *et al.*, 1997; Ravindra *et al*., 2004; Cristaudo *et al*., 2007; Colombo *et al*., 2008; Wiseman and Zereini, 2009; Heederik *et al*., 2016). However, studies reporting incidences of sensitization in workers with no or very low inhalation exposure to platinum questioned the possible addition of a dermal route of exposure (Maynard *et al*., 1997). *In vitro* research provided insight into the dermal route of exposure and reported the permeation of potassium tetrachloroplatinate (K_2PtCl_4) through full thickness human abdominal skin (Franken *et al.*, 2014, 2015) and the effects of a lower pH on platinum permeation (Van Nieuwenhuizen, 2016). However, the potential dermal permeation of the most potent sensitizer, hexachloroplatinate, has not yet been investigated.

Therefore, the aim of this particular study was to investigate the permeation of potassium hexachloroplatinate (K_2PtCl_6) through full thickness human abdominal skin and included the following objectives: Objective *1,* to quantify the permeation, Objective *2,* to calculate the percentage of platinum retained in the skin after 24 hours and Objective *3,* to evaluate the specific influence of exposure duration on the permeation at intervals of 8-, 12- and 24-hours. The successful use of static Franz diffusion cells with full thickness human abdominal skin enabled the investigation as indicated in the aim and objectives. The chemical analysis, by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS), provided the mass of platinum present in the various matrices (receptor- and donor solutions) that was used to calculate the permeability coefficient, flux and lag time values. These values are commonly used to

quantifiably describe the permeation profile of an exogenous compound (Díez-Sales *et al.,* 1991). Furthermore, digesting the skin after 24 hours of exposure and analyzing the solution by means of Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES), provided the values used to calculate the percentage of platinum retained in the skin. Lastly, the frequent extraction of receptor solution at intervals of 2, 4, 6, 8, 10, 12, 14, and 24 hours provided a cumulative permeation curve to indicate the influence of time on the permeation. Therefore, the aim and the objectives of this study were achieved.

Results and subsequent discussion of the analyzed data indicated that platinum permeated through full thickness human abdominal skin with an increase in permeation associated with exposure time (24-hour cumulative mass of 5.08 ng/cm^2; 1293 ± 160 ng/L total mass diffused). Thus, the hypothesis '*that potassium hexachloroplatinate (K_2PtCl_6) permeates through intact human abdominal skin at a quantifiable level (> 1 ng/L) when utilizing in vitro methods*' is accepted. Considering all the results collectively, the possible permeation of platinum after dermal exposure to K_2PtCl_6 should be included during risk assessments for the potential of dermal contamination contributing to exposure and subsequent potential adverse health effects. This conclusion is based upon the presence of platinum in the receptor solution after only two hours of exposure (0.24 ± 0.05 ng/cm^2) and the short lag time. The lag time calculated is short compared to other PGM lag times through full thickness intact human skin, indicating that the skin is saturated with platinum 2 hours and 15 minutes after exposure starts in an *in vitro* setting. The lag time thus represents the time at which optimal permeation of the exogenous compound occurs (OECD, 2011). Furthermore, the considerable platinum reservoir that forms in the skin layers is also cause for concern as this could possibly lead to further permeation, eventually leading to absorption into the circulatory system. This could possibly continue even after exposure has ended and the dermal surface was decontaminated. The reservoir retained inside the skin was quantified as 6890 times more (3.11 %) of the total dosage percentage than the percentage that diffused through the skin (45.14 x 10^{-5} %).

Real-world workplace circumstances incorporate factors such as the potential workers' poor personal hygiene, a 'take-home' effect, co-exposures and the employment population, that should be considered when extrapolating from laboratory results. The lack of effective personal hygiene after the work shift may result in improper decontamination of the skin thus leading to a 'layering' effect where new contamination is piled on to previous contamination (Franken *et al*., 2014). Poor decontamination of the skin in this manner has been observed in South African refineries by contamination of dermal areas being analyzed before the work shift started. The use of previously worn hardhats that were not thoroughly cleaned as a contaminated source, was one example observed (Du Plessis *et al*., 2010). Together with the

layering effect described, ineffective decontamination could lead to a 'take-home' effect where continuous contamination of the skin may proceed even after leaving the potential exposure area (Franken *et al*., 2014). Furthermore, the layering and 'take-home' effect contamination could lead to continuous permeation that could be facilitated by the co-exposure to other chemicals. A common example of co-exposure is other PGMs, such as observed by Linde *et al*. (2018b) in a platinum exposure study at a South African refinery. These co-exposures included dermal exposure to rhodium (Rh), iridium (Ir), ruthenium (Ru) and osmium (Os) (Linde *et al*., 2018b). Exposure to rhodium and osmium cause dermatitis (Muris *et al*., 2014; Friedova *et al*., 2020) which is known to have detrimental effects on the barrier function of the skin causing a potential increase in permeation of certain compounds (Rerknimitr *et al*., 2018).

Lastly, bearing in mind the circumstances under which exposure might occur, it is imperative to consider the exposed workers and how worker characteristics may affect permeation and subsequent development of adverse health effects. The South African workforce is composed of approximately 75 % African workers, of which 56 % are male (STATS SA, 2020) and as several PGM refineries are based in South Africa, the associated exposed population forms part of this statistic. Published literature is still ambiguous about the anatomical differences between races and sex and the effect thereof on the permeation of various compounds (Chen *et al*., 2010; Muizzuddin *et al*., 2010; Franken *et al*., 2015; Rahrovan *et al*., 2018). However, a study comparing the permeation of potassium tetrachloroplatinate (K_2PtCl_4) through full thickness African and Caucasian skin found higher permeation of platinum through African skin (Franken *et al.*, 2015). Thus, all the above-mentioned real-world factors should be considered when contemplating protection of the workers exposed to platinum salts and/or other PGMs. Sufficient training, which include decontamination of themselves and PPE, effective protective wear and the prevention of spread from the exposure source are examples of such protective measures.

4.2 Recommendations towards workplace exposure

Based on the results and real-world workplace considerations discussed previously, this section of recommendations is for future consideration to protect workers from the adverse health effects of PGM dermal exposure. Research indicates that the highest-ranking risk factor in the development of adverse health effects of exposure to chloroplatinate salts is a high level of exposure (risk increasing from low respiratory exposure levels of ≤ 49 ng/m^3 and reaching a plateau at > 200 ng/m^3) dermal or otherwise. The plateau in risk development was considered to indicate saturation of all possible individual sensitive workers to reach sensitization at that exposure level (Heederik *et al.*, 2016).

Furthermore, merely removing a sensitized worker from the exposed area does not guarantee the prevention of further asthma attacks in future (Merget *et al*., 2017). Thus, the recommendations given here aim at reducing the level and duration of exposure and are based on the hierarchy of control measures commonly used in the Occupational Hygiene community. The hierarchy of control measures (in order of effectiveness) recommends the total removal (elimination) of the toxic compound as the most effective control measure to prevent exposure. However, due to complications and often financial implications of such a removal, substituting the compound with a less toxic compound or form of the compound instead of complete removal of that element in processes, is also highly effective. An example of this would be substituting a powder form of the contaminant with a pellet and/or solution form reducing the presence of airborne particulates in the workplace. As elimination and/or substitution is not always feasible; such as in platinum refineries and recycling plants, other control measures are considered such as engineering controls, administrative controls, and as a last resort, personal protective equipment (PPE) (Lombaard *et al*., 2015).

4.2.1 Engineering controls

In order to effectively decrease dermal exposure to platinum salts all possible routes of exposure and exposure models such as depicted by Schneider *et al*. (1999) should be considered when implementing control measures. This ensures the anticipation and limitation of possible contamination from the source, the air, air deposition onto surfaces, cross-contamination between different surfaces and redeposition into the air from surfaces (Schneider *et al*., 1999). The implementation of engineering controls is a prime example of how the entire system should be considered, as discussed in this section.

4.2.1.1 Air deposition and cross-contamination

Installing engineering controls, such as total enclosure of certain procedures, encapsulates the airborne particles at the source. Installing proper local exhaust ventilation that incorporates the various components of the exposure, such as particle size and sedimentation, may lead to less deposition of particles onto surfaces, preventing contamination from the source and cross-contamination between different surfaces (Schneider *et al*., 1999). The ventilation systems, such as local exhaust ventilation (LEV) systems, should be placed close to the source of exposure and fitted with appropriate hoods. The Health and Safety Executive's (HSE, UK) guide for LEV systems (HSG258) is a valuable standard to use when considering the installation of these ventilation systems (visit https://www.hse.gov.uk/pubns/books/hsg258.htm). The use of high efficiency particulate air (HEPA) filters in conjunction with the ventilation systems may increase the immediate removal of the toxic particles from the air. However, in instances where

close fitted ventilation systems may cause a high degree of product loss and thus may lead to excessive detrimental economic complications for the company, other measures should be considered. Furthermore, where applicable, the simple selection of instruments causing less dust being introduced into the working environment and utilizing wet processing of dust could decrease the risk towards airborne exposure. The installation of mist sprayers could induce particulates to settle on a specific surface. The procedure of settling dust by means of wet processes such as mists reduces the risk of product loss as well and could justify the financial expenses involved.

4.2.1.2 Contamination from the source

Considering dermal exposure from physical handling platinum and platinum salts, such as found in the removal of ammonium hexachloroplatinate salts from the calcination furnaces' filter (Seymour and O'Farrelly, 2012) more specific engineering controls are required. The use of automation, barriers and contained transfer systems are examples of such controls.

Automating a task, and eliminating workers being physically involved may reduce accidental spills and worker exposure. The use of autonomized transporting devices that are enclosed, for example an enclosed conveyer belt, could lead to less dust formation and less physical handling of the contaminant by a worker. The physical handling of platinum and platinum salts could also be decreased using barriers and contained transfer systems. The use of polyurethane or PVC film as glovebags could be used as a highly efficient short-term barrier between the worker's skin and platinum of various forms (IPA, 2017) that does not negatively affect viewing of a proses. However, during the removal of these bags for decontamination purposes, redistribution of contaminant into the system is an often occurrence.

The use of isolators of negative internal pressure with access granted via built-in gloves, decreases the likelihood of redisposition. The negative pressure provides an efficient barrier between the worker and the platinum compound being handled and is thus a good control measure for example the process of filtering chloroplatinate suspensions. An isolator is most effective when coupled with contained transfer systems to introduce the compound in and out of the cabinet. Contained transfer systems refer to the use of various methods to connect equipment/containers used during a process to each other causing a closed loop of transferring the compound from one step in a process to the next. Simple examples of these transfer systems are the bag-over-bag method and the use of a continuous liner. The bag-over-bag method is the use of multiple bags instead of pouring a compound into a single bag and releasing a lot of dust. The compound is poured into a second bag that is placed around the first

and manipulated by means of o-rings and the bag itself, decreasing dermal contact with the compound and the risk towards cross-contamination.

As each situation and process differs, the selection of suitable engineering controls would vary as well. The use of guidelines such as HSG 262 '*Managing skin exposure risks at work*' (https://www.hse.gov.uk/pubns/priced/hsg262.pdf) (HSE, 2015) and literature such as '*Safe use of platinum group metals in the workplace*' written by the IPA (IPA, 2017) as guidance material to the PGM industry are valuable tools in this selection process

4.2.2 Administrative policies

Simple administrative policies that could protect workers include the implementation of a management plan. In the South African mining industries, which include refineries, these policies and their implementation could be included in their code of practice (COP) as required by the MHSA section 9(2) (DMR, 1996). This plan should include a policy against the growth of a beard combined with wearing of a respirator, which may cause ill-fitting respirators not sealing against the face properly. The prohibition of eating, drinking and smoking in any area other than designated locations outside the exposure areas should be strictly enforced. Further administrative procedures recommended here include shift regulations, providing proper washing facilities, dermal exposure monitoring, good housekeeping, and the implementation of training programs.

4.2.2.1 Shift and access regulations

Regulating the number of workers allowed to work in a high exposure area at a specific time and implementing regular shift changes are important to ensure that less workers are exposed for a shorter duration of time. This could also mean having to ask certain workers to leave the adjacent area while a specific high exposure task is being performed and training more workers to operate certain machinery related to a high risk towards exposure to enable shift rotation schedules. Shift rotation establishes an interchangeable team of workers scheduled to work on a high exposure risk activity only for short periods of time during the week, thus decreasing exposure time. Other policies may include restricted entry of certain workers into some high-risk areas. Atmospheric pressure differences and the use of HEPA filters in conjunction with a ventilation system in these restricted areas are engineering control measures that would facilitate the encapsulation of contamination in such areas. Lastly the physical placement of certain tasks further away from contamination sources could help reduce overall worker exposure.

4.2.2.2 Washing facilities

The employer should provide appropriate washing facilities where strict rules apply to implore workers to wash their hands and arms before exiting certain high-risk areas. These washing facilities should be located between adjacent areas to ensure passing through them before the possibility of cross-contamination of other often-touched surfaces, for example doorknobs. This implementation would also decrease the likelihood of accidental ingestion of platinum compounds during lunch hours. To further decrease the likelihood of dermal cross-contamination, the provision of sensors at doors adjacent to high-risk areas instead of doorknobs and the use of a foot lever at ground level instead of tap handles should be considered.

Training sessions and visual notices demonstrating the 'take-home' effect and how to efficiently wash their hands should be implemented alongside verbal encouragements. The provision of disposable towels instead of reusable ones is preferred to ensure a clean surface after every wash with no previous contamination. This should also encourage workers not to dry their washed hands on possibly contaminated clothing. Showering facilities should include proper containers in close proximity of the showers to deposit contaminated PPE. Effective washing habits before leaving for home should be encourage to decrease the 'take-home' effect. Contaminated clothing could add to the deposition of the contaminant onto the skin and these contaminated dermal areas could spread as the worker touches him-/herself (Schneider *et al*., 1999). The regular implementation of cleaning and maintenance schedules of facilities and PPE are important administrative measures in any work environment. Clear labeling of containers storing clean or dirty PPE would facilitate effective maintenance of PPE. This will also ensure that only cleaned PPE is set out for future use after a shift ends.

By providing skin lotion at the washing facilities and encouraging the workers to use it, after efficient cleaning, the employer ensures the maintenance and protection of workers' dermal health. Creams hydrate the skin and decrease the formation of cracks in the skin thus increasing dermal resistance to permeation of exogenous compounds on clean hands. However, applying creams on contaminated hands may cause entrapment of the contaminant on the skin. Thus, proper instruction should be given along with the creams.

4.2.2.3 Dermal exposure monitoring

Administrative procedures that would prove helpful is the implementation of a dermal and surface sampling protocol enabling the evaluation of real-time dermal and surface contamination. This could help to identify problem areas and workers that are especially at risk

for exposure. The use of GhostWipes™ is commonly recommended by the ISO standard (ISO/TR 14294:2011) for dermal exposure monitoring and analysis by ICP-MS. To ensure that all workers are sufficiently protected from the dermal exposure route it would be beneficial to act as if all workers have damaged skin. Damaged skin is less resistant to permeation of various compounds and as skin abrasions and/or dermatitis are often found on refinery workers' skin (Hunter *et al*., 1945; Santucci *et al*., 2000), this assumption may facilitate successful risk assessments. Referring the high at-risk workers, such as those with abrasions or dermatitis, for further medical surveillance may prove essential to determine why the dermal condition is present and treat the condition (Schoeman *et al*., 2015). Medical surveillance should use biological monitoring protocols, such as assessing the platinum concentration in the urine, to determine the total body burden from all routes of exposure. The results from assessing the total body burden could be used as an indicator whether control measures are effective to protect workers from exposure or not (Aitio *et al.,* 2006; Angerer *et al.,* 2007). Urine analysis has proven effective in the evaluation of platinum salts exposure (Cristaudo *et al.*, 2007; Linde, 2018; Linde *et al*., 2018a, 2018b). In order to explain all routes of possible dermal contamination, frequent air and surface sampling protocols could be implemented. The standardized method compiled by the American Society for Testing and Materials (ASTM) for surface sampling titled *Surface sampling of metals and metalloids for worker protection* (ASTM D7659 https://www.astm.org/Standards/D7659.htm) could be a helpful guide.

4.2.2.4 Good housekeeping

The surface sampling may supply results indicating that certain areas need improved general cleaning. As a rule, using mechanized methods are more successful than the simple task of sweeping certain surfaces. Mechanized methods (such as vacuuming or localized ventilation) remove the contaminant from the environment without the risk of exposing a worker. In contrast, sweeping causes resuspension of particles into the air and possible redeposition as it settles on a worker's skin, clothing, or other surfaces (Schneider *et al*., 1999; IPA, 2017). The redeposition of particles may lead to cleaners being exposed to a potentially high concentrations of the contaminant at regular intervals (IPA, 2017). A good example includes the risk of short-term exposure to potential high levels of platinum dust when cleaning the vacuum cleaner that was used during cleaning processes. Even though mechanized methods are preferred, the use of compressed air in the cleaning process should be prohibited for it only redistributes the contamination into the air.

To evaluate the effectiveness of cleaning processes previous studies have recommended the use of colorimetric techniques such as the utilization of 1 % sodium borohydride ($NaBH_4$) solution. The solution is sprayed onto surfaces converting all platinum species present into non-

toxic metallic platinum which will leave a black stain with a grainy texture on tissue paper when wiped. The presence of the black stain would indicate contamination still present even after cleaning and would suggest cleaning of the surface again (Franken, 2014; Linde, 2018).

4.2.2.5 Training

Proper training programs including information on the health effects and routes of exposures of platinum salts may guide the workers to be more mindful of their actions. These programs should contain a guideline where to report certain risk activities, or control malfunctions and apparent health effects detected. Furthermore, how to use machinery and PPE to avoid contamination are important skills as well as emphasis on personal hygiene explaining the so called 'take-home' effect. Training regarding the use of PPE should contain specific instructions on how to handle contaminated PPE to decrease the spread of contamination. This includes specific instructions on how to remove gloves as to avoid dermal contact and the proper way to wash hands. A helpful tool to help with the visualization of how contamination spreads and/or how effective washing procedures are, is the use of fluorescent soap and UV lamps. Teaching proper hand washing procedures to workers in the health care industry, is an example where this visualizing technique was used (Škodová *et al*., 2015). Applying a fluorescent test lotion onto the outside of a glove, such as where deposition of contaminants could probably take place and asking the worker to put the gloves on and take them off again, could illustrate the spread of contamination. Using an UV lamp, any dermal contact with the fluorescent lotion would become visible. Lastly, workers involved in cleaning should also be trained on all health effects of contamination and how to clean with reduced contamination in mind. If the $NaBH_4$ solution testing is implemented, then it should be accompanied by sufficient training of the cleaning crew on how to utilize the method and how to interpret its findings.

Training should accompany the start of every new task or commencement of work in a new area. If anything changes or spikes in exposure are experienced, then new training sessions should be attended by all workers at risk. Hard copy booklets written in a selection of well-used languages handed to the workers after every training session and some strategically located visual representations, such as posters, explaining critical aspects of control measures, would facilitate the effectiveness of the training courses.

4.2.3 PPE

PPE must be implemented as a last resort after all other measures have been considered and implemented, due to the simple fact that incorrect fit and/or incorrect use of PPE leads to inefficient protection and often increased exposure (Boeniger, 2003; Geer *et al*., 2007). The 'human element' is the complicating factor that most of the other control measures avoid

because it results in the less efficient protection (Hunter *et al.,* 1945; Geer *et al.*, 2007). The wearing of PPE could cause worker behavior to be driven by a false sense of security although the sole use of PPE is not sufficient to protect the worker. This behavior may lead to an increase in exposure due to unnecessary contact with contaminants (Semple, 2004). In contrast, workers with a more careful mindset tend to be more cautious when handling toxic material (Drexler, 2003).

The use of disposable coveralls in addition to regular overalls, has shown to prevent dermal exposure to platinum salts considerably (Linde *et al*., 2018a). These coveralls should not have pockets in any form to prevent the placement of contaminated hands or items into them. The removal of anything from these contaminated pockets may cause contamination onto the skin from the pockets (cross surface contamination). Filtering Face Piece 3 (FFP3) respirators specially designed for metal dusts, used in addition to double layered nitrile PVC elbow length gloves would be beneficial in protecting workers from inhalation and dermal exposure to PGMs (Franken, 2014). Effective decontamination of PPE is important to prevent any continuous contamination, even before the work shift starts. The use of barrier creams to protect from dermal exposure could be useful provided that the barrier cream is given enough time to be absorbed so that the worker's hands are not greasy. Greasy hands may increase the risk of a safety incident occurring (Sadhra *et al*., 2014). However, the choice of cream should be done carefully as some creams have been proven unfavorable to the skin's natural protective functions (Vermaak, 2014).

4.3 Limitations

As described in the previous section, the methodology proved valuable in the recommendation towards workplace control measures and allowed the evaluation of the aim and objectives put forth in this study. Multiple other studies have used the *in vitro* methodology to report valid results used in the extrapolation of risk assessments of *in vivo* worker exposure (Larese Filon *et al*., 2004; Liu *et al*., 2011; Franken *et al*., 2014; Crosera *et al*., 2018; Frasch and Barbero, 2018). However, in order to provide recommendations on possible further PGM permeation research, it is crucial to critically evaluate and discuss identified limitations the experimentation may have revealed.

4.3.1 Donor skin and population

The effect of race (Muizzuddin *et al*., 2010; Franken *et al*., 2015) and sex (Chen *et al*., 2010; Rahrovan *et al*., 2018) on exogenous compound permeation is still a controversial topic in literature. Considering the population of mostly African men in the South African workplace (STATS SA, 2020), the use of female Caucasian skin might not portray all the influential factors

towards the permeation of platinum salts. However, the use of female abdominal skin is widely accepted although there are significant differences in anatomical areas across the human body (Hostýnek, 2003; Finnin *et al*., 2012). Nevertheless, the advantages of utilizing female Caucasian abdominal skin with such as a large surface area (abdominal) and the availability of donor skin (Hostýnek, 2003; Finnin *et al*., 2012) has proven statistically valuable in a variety of studies (Vallet *et al*., 2007; Franken *et al*., 2014, 2015; Van Nieuwenhuizen, 2016; Jansen van Rensburg *et al*., 2017). Thus, the use of female abdominal skin is a known limitation but an acceptable one due to the many advantages.

4.3.2 Skin storage

The effect of using previously frozen skin for *in vitro* permeation studies has been investigated and delivered controversial results. Several studies report no significant difference between water permeation (Bronaugh *et al*., 1986; Harrison *et al*., 1984) or lag times when comparing frozen and non-frozen skin (Barbero and Frasch, 2016) and others report significant variance of flux and lag time (Hawkins and Reifenrath, 1986; Ahlstrom *et al*., 2007). The use of skin that was not frozen for storage would be optimal. However, the availability and practicality thereof are extremely taxing. Thus, the use of frozen stored skin has been identified as a valid method during *in vitro* experimentation (OECD, 2011).

4.3.3 Applied dosage

As dosage has been identified as an influencing factor on permeation (Selzer *et al*., 2013; Zsikó *et al*., 2019), the use of a high applied dosage, such as utilized in this particular study, should be carefully evaluated. This study utilized a 0.3 mg/mL (approximately 300 µg/cm^2) K_2PtCl_6 dosage although a recent investigation into the dermal exposure of South African refinery workers indicated a dermal exposure of 0.008 µg/cm^2 (Linde *et al*., 2018). The elevated applied dose was implemented due to detection limitations of current Inductively Coupled Plasma (ICP) analysis methodology (i.e. 100 ng/L). In order to deliver concentrations detectable in the receptor solution above set detection limits, applied dosage should compensate for the dosage remaining in the donor solution and skin. A higher dose compensating for detection limitations has been common practice in many *in vitro* studies (Mauro *et al*., 2015; Larese Filon *et al*., 2016; Crosera *et al*., 2018). Furthermore, the applied dosage permitted the results comparison with previous studies such as Franken *et al*. (2014, 2015), of which a 0.3 mg/mL concentration was utilized as well.

4.4 Recommendations towards further PGM dermal permeation research

Although the insights into the permeation profile of potassium hexachloroplatinate (K_2PtCl_6) generated with this study are valuable, the complexity of the permeation influencing factors, as

discussed in Chapter 2, necessitates further research. The following recommendations are formulated to address these complexities.

4.4.1 Race and Sex

As the influence of race and sex of the donor on dermal permeation of PGMs and/or exogenous compounds in general is still highly controversial, it would be beneficial to investigate these avenues further. Although the availability of male donor skin is low, some studies have been able to gather enough for statistically significant results (Mauro *et al*., 2015; Larese Filon *et al*., 2016; Crosera *et al*., 2018). Thus, provided that an opportunity arises to acquire enough male donor skin, experimentation on male skin could become a possibility. Furthermore, as previously discussed, due to the majority of workforce in SA refineries being of an African race, it would be beneficial to pursue contributing research with African skin, should the opportunity arise.

4.4.2 Dermal reservoir for platinum

Microscope technology and tape strip analysis could be used to determine in which layer of the skin the postulated platinum reservoir is formed. This may provide more insight into the permeation profile of platinum salts and how it may contribute to the associated adverse health effects. The standardization of these methods would also be beneficial, to eliminate issues such as the 'smudging' effect when slicing the sample for the microscopy analysis. This effect describes the cross-contamination to different layers by the instrument used to slice the sample, thus leaving the test results invalid. The problematic 'smudging' effect was discussed during the Johnson Matthey Technology Centre (Sonning Commons, UK) research visit (Dr Briceno, oral communication, 2019). An example where these types of insights provided better understanding of the permeation/penetration mechanism of an exogenous compound is the conclusions of experimentation with caffeine. These conclusions stated that caffeine in larger particle forms would not permeate deeper than the outer-most layers of the stratum corneum (Abd *et al*., 2018) comprising of corneocytes. This layer is readily shed and thus contamination is lost during the normal corneocytes shedding cycle of 14 days (Rice and Mauro, 2013).

4.4.3 Dissolution and Agglomeration

Understanding the dissolution of K_2PtCl_6 in the donor solution would provide perspective into how much of the platinum was available for permeation. Theoretically, dissolution or in other words dissociation from the attachments to other elements in the compound, is an essential step for a metal to diffuse into and through the skin barrier (ICMM, 2007). Other dissolution composition insights such as the tendency towards agglomeration and ionization of the

compound could explain permeation parameters further (Knorr *et al*., 2009; Franken *et al*., 2014). Transmission Electron Microscopy (TEM) imagery analysis (Franken *et al*., 2014; Larese Filon *et al*., 2016), nano-particle tracking analysis (Lebedová *et al*., 2018) and Dynamic Light Scattering (DLS) (Larese Filon *et al*., 2016) have previously been used to assess the agglomerates of compounds.

4.4.4 Investigation into the follicular route

The follicular route of dermal permeation has been reported to be size-dependent (Knorr *et al*., 2009) and has been suggested to play a role in the permeation of potassium tetrachloroplatinate (K_2PtCl_4) due to small agglomerates (Franken *et al*., 2014). Some studies reported the degree of the follicular route's involvement during permeation by using an epidermis and stratum corneum sandwich (Essa *et al*., 2002). The utilization of differential stripping described as a combination of tape-stripping and cyanoacrylate superglue tape-stripping (Teichmann *et al*., 2005; Desai *et al*., 2013; Abd *et al*., 2018) and follicular closure techniques have proven valuable as well in the evaluation of the follicular route *in vitro* (Stahl *et al*., 2012; Desai *et al*., 2013). Investigating this phenomenon may provide an explanation for the high degree of dermal retention as indicated in the results of Chapter 3. The aggregation of particles in the follicles would cause a high degree of storage of the compound and is indicated by a high concentration of compound retained in the skin compared to being absorbed through to the receptor solution (Knorr *et al*., 2009).

4.4.5 Speciation

Determining the speciation of the platinum salt after dissolution in the donor solution or in the different phases of the Franz diffusion system (skin or receptor solution) may provide insight into how the various factors influence permeation. These factors may include molecular shape and oxidative state. To illustrate this, consider the ions Pt^{4+} and Rh^{3+} as an example i.e., the ions that would be present in the complete dissolution of K_2PtCl_6 and $RhCl_3$ (rhodium chloride). These ions are similar in molecular shape and possess different oxidative states although how the interaction of shape and oxidative state may affect their permeation profiles is unknown. The above-mentioned example was discussed during a symposium between the Johnson Matthey Technology Centre (Sonning Commons, UK) (Dr Ash, oral communication, 2019) and researchers of this study. Various methods to speciation analysis can be found in Vitkova *et al.* (2003).

4.4.6 Effect of dermal movement (flexing)

Currently, there is little research on the effect of flexing, and the kinetic energy produced by the movement of the skin on the permeation of PGMs. Studies evaluating the effect of this movement on the permeation of particles have found that in some circumstances this kinetic

energy is necessary for permeation (Tinkle *et al.,* 2003; Rouse *et al.,* 2007). Methods for flexing of the skin include the use of a so called ‘flexing device’ that moves the skin at a 45° angle and rotation of 20 flexes per minute (Tinkle *et al.,* 2003; Rouse *et al.,* 2007). In both these methods, the use of flexing the skin increased permeation compared to skin that was kept static. Rouse *et al*. (2007) referenced the device description to http://pubs.acs.org.

4.4.7 Report on adverse health effects

The description of the manifestations of dermatitis in published exposure studies is varied based on type and diagnosis process. Thus, it could be beneficial if future studies would be more consistent in how the dermatitis symptoms are reported. Recommendations on reporting on the type of dermatitis (irritant, contact, erythematous, or eczematous dermatitis) observed after exposure and the symptoms that led to the specific classification would provide more accurate understanding concerning the information given. Due to the various types of dermatitis and the different mechanisms associated with the development of related symptoms, it is important to know which type is specifically reported to understand the influence of exposure on the development of the symptoms. Present classification systems such as the SCORAD system, available at https://dermnetnz.org/topics/scorad/, could be a starting point.

4.5 References

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ANNEXURE

1 DECLARATION OF LANGUAGE EDITING

WOORDE WAT WERK
WORKING WORDS

Venita de Kock
BA HONS. • PEG
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12 August 2021

LANGUAGE EDITING STATEMENT

I, Jannetje Levina De Kock hereby declare that the dissertation

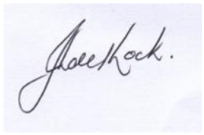
In vitro permeation of potassium hexachloroplatinate through full thickness human abdominal skin

by
Barbara Bosch

for submission to the NWU
in the Niche area Occupational Hygiene and Health Research Initiative (OHHRI)

- has been edited for language correctness and spelling.
- has been edited for consistency (repetition, long sentences, logical flow)

No changes have been made to the document's substance and structure (nature of academic content and argument in the discipline, chapter and section structure and headings, order and balance of content, referencing style and quality).



J L DE KOCK

2 PLAGIARISM REPORT

10101268:Barbara_Bosch_Draft_8_27072021_Turn_it_In.pdf

ORIGINALITY REPORT

8%	4%	6%	1%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	dspace.nwu.ac.za Internet Source	1%
2	Submitted to University of Cape Town Student Paper	1%
3	"Agache's Measuring the Skin", Springer Science and Business Media LLC, 2017 Publication	1%
4	Stephanus J.L. Linde, Anja Franken, Johannes L. du Plessis. "Urinary excretion of platinum (Pt) following skin and respiratory exposure to soluble Pt at South African precious metals refineries", International Journal of Hygiene and Environmental Health, 2018 Publication	1%
5	Submitted to North West University Student Paper	<1%
6	"Kanerva's Occupational Dermatology", Springer Science and Business Media LLC, 2020 Publication	<1%

3 ETHICS APPROVAL

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South Africa 2520

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Fax: 018 299-4910
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Research Ethics Regulatory Committee
Tel: 018 299-4849
Email: nkosinathi.machine@nwu.ac.za

20 March 2019

ETHICS APPROVAL LETTER OF STUDY

Based on approval by the North West University Health Research Ethics Committee (NWU-HREC) on 20/03/2019, the NWU Health Research Ethics Committee hereby approves your study as indicated below. This implies that the North-West University Research Ethics Regulatory Committee (NWU-RERC) grants its permission that, provided the special conditions specified below are met and pending any other authorisation that may be necessary, the study may be initiated, using the ethics number below.

Study title: In vitro permeation of potassium hexachloroplatinate through full thickness human abdominal skin.
Study Leader/Supervisor (Principal Investigator)/Researcher: Prof A Franken
Student: BS Bosch

Ethics number:

N	W	U	-	0	0	2	0	2	-	1	5	-	A	1	-	0	2
Institution				Study Number						Year			Status				

Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation

Application Type: Single Study
Commencement date: 20/03/2019 **Risk:** Minimal
Expiry date: 31/03/2020
Approval of the study is initially provided for a year, after which continuation of the study is dependent on receipt and review of the annual (or as otherwise stipulated) monitoring report and the concomitant issuing of a letter of continuation.

Special in process conditions of the research for approval (if applicable):

General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, the following general terms and conditions will apply:

- *The study leader/supervisor (principle investigator)/researcher must report in the prescribed format to the NWU-HREC:*
  - *annually (or as otherwise requested) on the monitoring of the study, whereby a letter of continuation will be provided, and upon completion of the study; and*
  - *without any delay in case of any adverse event or incident (or any matter that interrupts sound ethical principles) during the course of the study.*
- *The approval applies strictly to the proposal as stipulated in the application form. Should any amendments to the proposal be deemed necessary during the course of the study, the study leader/researcher must apply for approval of these amendments at the NWU-HREC, prior to implementation. Should there be any deviations from the study proposal without the necessary approval of such amendments, the ethics approval is immediately and automatically forfeited.*
- *Annually a number of studies may be randomly selected for an external audit.*
- *The date of approval indicates the first date that the study may be started.*
- *In the interest of ethical responsibility the NWU-RERC and NWU-HREC reserves the right to:*
  - *request access to any information or data at any time during the course or after completion of the study;*
  - *to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process;*
  - *withdraw or postpone approval if:*

- *any unethical principles or practices of the study are revealed or suspected;*
- *it becomes apparent that any relevant information was withheld from the NWU-HREC or that information has been false or misrepresented;*
- *submission of the annual (or otherwise stipulated) monitoring report, the required amendments, or reporting of adverse events or incidents was not done in a timely manner and accurately; and / or*
- *new institutional rules, national legislation or international conventions deem it necessary.*

- *NWU-HREC can be contacted for further information or any report templates via Ethics-HRECApplv@nwu.ac.za or 018 299 1206.*

The NWU-HREC would like to remain at your service as scientist and researcher, and wishes you well with your study. Please do not hesitate to contact the NWU-HREC or the NWU-RERC for any further enquiries or requests for assistance.

Yours sincerely

Digitally signed by Wayne Towers
Date: 2019.04.12 09:03:40 +02'00'

Prof Wayne Towers
Chairperson NWU Health Research Ethics Committee

Original details: (22051930) C:\Users\22051930\Desktop\ETHICS APPROVAL LETTER OF STUDY.docm
6 November 2018

Current details: (22051930) M:\CSS\1463\Monitoring and Reporting Cluster\Ethics\Certificates\Templates\Research Ethics Approval Letters\9.1.5.4.1 HREC Ethical Approval Letter.docm
3 December 2018

File reference: 9.1.5.4.2

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Health Sciences Ethics Office for Research, Training and Support

North-West University Health Research Ethics Committee (NWU-HREC)
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Prof A Franken
Occupational Hygiene
OHHRI

7 April 2020

Dear Prof Franken

APPROVAL OF YOUR AMENDMENT REQUEST BY THE NORTH-WEST UNIVERSITY HEALTH RESEARCH ETHICS COMMITTEE (NWU-HREC) OF THE FACULTY OF HEALTH SCIENCES

Ethics number: NWU-00202-15-A1-02

Kindly use the ethics reference number provided above in all future correspondence or documents submitted to the administrative assistant of the North-West University Health Research Ethics Committee (NWU-HREC) secretariat.

Study title: *In vitro* permeation of potassium hexachloroplatinate through full thickness human abdominal skin

Study leader: Prof A Franken

Student: BS Bosch-24118338

You are kindly informed that your amendment request (Addition of specific analytical methods study) to the aforementioned project has been approved. Any future amendments to the proposal or other associated documentation must be submitted to the NWU-HREC, Faculty of Health Sciences, North-West University, prior to implementing these changes. These requests should be electronically submitted to Ethics-HRECApply@nwu.ac.za, for review BEFORE approval can be provided, with a cover letter with a specific subject title indicating, "Amendment request: NWU-XXXXX-XX-XX". The letter should include the title of the approved study, the names of the researchers involved, the nature of the amendment/s being made (indicating what changes have been made as well as where they have been made), which documents have been attached and any further explanation to clarify the amendment request being submitted. The amendments made should be indicated in yellow highlight in the amended documents. The *e-mail*, to which you attach the documents that you send, should have a *specific subject line* indicating that it is an amendment request e.g. "Amendment request: NWU-XXXXX-XX-XX". This e-mail should indicate the nature of the amendment. This submission will be handled via the expedited process.

We wish you the best as you conduct your research. If you have any questions or need further assistance, please contact the Faculty of Health Sciences Ethics Office for Research, Training and Support at Ethics-HRECApply@nwu.ac.za.

Yours sincerely

Digitally signed by Prof Petra Bester
Date: 2020.04.07 16:09:46 +02'00'

Chairperson: NWU-HREC