

Dermal exposure and skin barrier function of workers exposed to copper sulphate at a chemical industry

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PREFACE

This mini-dissertation is submitted in partial fulfilment of the requirements of the degree Magister Scientiae in Occupation Hygiene, and is structured as follows:




- Chapter 1 – Gives a general introduction to the study, states the objectives as well as the Hypothesis.
- Chapter 2 – Gives a detailed literature review on topics relevant to this study.
- Chapter 3 – Presents a research article, as for potential publication in the journal, *Annals of Occupational Hygiene*.
- Chapter 4 – Concludes the study by means of a discussion, recommendations, limitations and suggestions for future studies.
- Chapter 5 – Contains Annexure A-D, presenting the questionnaires used in this study as well as figures indicating the designated dermal sampling and skin barrier function measurement areas.

For the sake of uniformity throughout this mini-dissertation, the reference style used is that of the journal, *Annals of Occupational Hygiene*. A detailed discussion of the reference requirements as stipulated by the journal can be found at the beginning of Chapter 3.

AUTHOR'S CONTRIBUTIONS

This study was conducted by a team of researchers whose contributions are depicted in Table 1.

Table 1: Authors contributions and consent.

Author	Contribution	Consent*
Ms. C. Steynberg	<ul style="list-style-type: none"> • Compilation of the research proposal • Literature research • Data collection by means of personal and environmental sampling • Analysis of data and interpretation of results • Writing the mini-dissertation 	
Prof. F.C. Eloff Supervisor	<ul style="list-style-type: none"> • Planned and designed the study • Approved the protocol • Assisted in interpretation of results • Supervised the writing of the mini-dissertation • Reviewed all documentation, the article and mini-dissertation 	
Prof. J.L. du Plessis Co-supervisor	<ul style="list-style-type: none"> • Assisted with the planning and design of the study, as well as approved the protocol • Assisted with analysis and interpretation of results • Reviewed documentation of study 	

*I hereby declare that I have approved the mini-dissertation and that my role in the study as indicated is representative of my actual contribution, and I hereby give consent that it may be published as part of Ms. C. Steynberg's M.Sc. (Occupational Hygiene) mini-dissertation.

ABSTRACT

Title: Dermal exposure and skin barrier function of workers exposed to copper sulphate at a chemical industry

Copper exposure is known to be a rare cause of skin irritation and allergic reactions and according to our knowledge occupational dermal exposure to copper sulphate has not yet been characterised. As a result, the *objectives* of this study were to assess the dermal exposure of workers at a chemical industry to copper sulphate and to characterise the change in their skin barrier function from before to the end of the work shift, as the skin's barrier function can greatly influence the permeation of chemical substances.

Methods: The change in skin barrier function of reactor workers, crystal and powder packaging workers at the chemical industry were assessed by measuring their dominant hand's palm, back and wrist as well as their foreheads' skin hydration, transepidermal water loss (TEWL) and skin surface pH before and at the end of the work shift. Commercial GhostwipesTM were used to collect dermal exposure samples from the same four anatomical areas before and at the end of the shift. Additional dermal exposure samples were collected from the palm and back of hand, prior to breaks 1 and 2. Surface wipe sampling was also conducted at several work and recreational areas of the chemical industry. Wipe samples were analysed by an accredited analytical laboratory, according to NIOSH method 9102 by means of Inductively Coupled Plasma-Atomic Emission Spectrometry.

Results: Changes in skin hydration of the workers and anatomical areas at the end of the work shift were highly variable, while in general TEWL increased and skin surface pH decreased. Copper was collected from the skin of all workers before the shift commenced, and dermal exposure increased throughout the work shift. All of the work and recreational areas from which surface samples were taken, were contaminated with copper.

Conclusion: As a result of intermittent use of inadequate protective gloves and secondary skin contact with contaminated surfaces and work clothing, workers at the chemical industry are exposed to copper sulphate via the dermal exposure route. The decrease in the workers' skin barrier function (increased TEWL) and skin surface pH is most likely the result of their dermal

exposure to sulphuric acid, and may lead to enhanced dermal penetration. The low account of skin irritation or reaction incidences among these workers is contributed to their ethnicity as well as to the low sensitisation potential of copper. Recommendations on how to lower dermal exposure and improve workers' skin barrier function are made.

Key words: dermal exposure, skin barrier function, copper sulphate, skin hydration, transepidermal water loss, skin surface pH.

OPSOMMING

Titel: Dermale blootstelling en velgrensfunksie van werkers blootgestel aan kopersulfaat by 'n chemiese bedryf

Koper blootstelling is bekend as 'n seldsame oorsaak van velirritasie en allergiese reaksies en volgens ons kennis is beroepsdermale blootstelling aan kopersulfaat nog nie gekarakteriseer nie. As gevolg hiervan, was die *doelwitte* van hierdie studie om die dermale blootstelling van werkers aan kopersulfaat by 'n chemiese bedryf te evalueer en om die verandering in hul velgrensfunksie van voor tot en met die einde van die werkskof te karakteriseer, omdat die vel se grensfunksie 'n groot invloed op die deurlaatbaarheid van chemiese stowwe kan hê.

Metode: Die verandering in die velgrensfunksie van reaktorwerkers, kristal- en poeierverpakkingwerkers in die chemiese industrie is bepaal deur metings van hul dominante hand se palm, bokant van hand en pols, asook hulle voorkop se velhidrasie, trans-epidermale waterverlies (TEWV) en veloppervlak-pH voor en aan die einde van die werkskof te neem. Kommersiële GhostwipesTM is gebruik om dermale blootstellingsmonsters te versamel van dieselfde vier anatomiese areas voor en aan die einde van die skof. Bykomende dermale blootstellingsmonsters van die palm en die bokant van die hand is ingesamel voor pouses 1 en 2. Oppervlakveegmonsterneming is ook by verskeie werk- en ontspanningsareas van die chemiese bedryf uitgevoer. Veegmonsters is ontleed deur 'n geakkrediteerde analitiese laboratorium, volgens NIOSH metode 9102 deur middel van Induktiewe Plasma-Atomiese Emissiespektrometrie.

Resultate: Verandering in die velhidrasie van die werkers en anatomiese areas aan die einde van die werkskof was hoogs veranderlik, terwyl TEWV in die algemeen toeneem en veloppervlak-pH verlaag het. Koper is versamel vanaf die vel van al die werkers voor die skof begin het, en dermale blootstelling het toeneem met die verloop van die werkskof. Al die werk- en ontspanningsareas waarvan oppervlakmonsters geneem is, was gekontamineer met koper.

Gevolgtrekking: As gevolg van die afwisselende gebruik van onvoldoende beskermende handskoene en sekondêre velkontak met gekontamineerde oppervlaktes en werksklere, is die werkers by die chemiese bedryf blootgestel aan kopersulfaat deur die dermale

blootstellingsroete. Die afname in die werkers se velgrensfunksie (verhoogde TEWV) en veloppervlak-pH is hoofsaaklik die gevolg van hul dermale blootstelling aan swaelsuur, en kan lei tot verhoogde dermale penetrasie. Die lae voorkoms van velirritasie of reaksievoorvalle onder hierdie werkers is toegeskryf aan hul etnisiteit, sowel as die lae sensitiseringspotensiaal van koper. Aanbevelings is gemaak oor hoe om dermale blootstelling te verlaag en werkers se velgrensfunksie te verbeter.

Sleutelwoorde: dermale blootstelling, velgrensfunksie, kopersulfaat, velhidrasie, trans-epidermale waterverlies, veloppervlak-pH.

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CHAPTER 1

GENERAL INTRODUCTION

GENERAL INTRODUCTION

1.1 INTRODUCTION

Copper was one of the first metals excavated by humans (Doeblich, 2009) and is primarily used as metal or alloy in building construction and the manufacturing of electric and electronic products, transportation equipment and other metal products (Edelstein, 2013). Copper compounds of which copper sulphate is the most common, are used in agriculture to treat plant diseases or for water treatment and as preservatives for wood, leather and fabrics (ATSDR, 2004).

Copper forms part of a small group of metals considered as essential nutrients indispensable to sustain normal functioning of many proteins (Cushing *et al.*, 2007; Jo *et al.*, 2008). The dose-response relationship of all essential nutrients is U-shaped, thus indicating that a deficiency as well as an excess would result in adverse health effects (Moffett *et al.*, 2007; Oguz *et al.*, 2010). Such effects pose a challenge to conventional risk assessment as efforts to reduce exposure as far as possible might elicit negative health effects as a result of a deficiency (ICMM, 2007).

Health effects due to accidental or suicidal ingestion of large quantities of copper salts are well described and are mainly gastrointestinal of nature (Ellingsen *et al.*, 2007; Rana, 2008; De Romaña *et al.*, 2011). A daily dietary intake of 0.9, 1 and 1.3 mg of copper has been recommended for adults, pregnant and lactating women, respectively (De Romaña *et al.*, 2011).

In comparison to ingestion exposure, less is known about health effects following occupational inhalation exposure to copper dust and fumes but is limited by most countries to 1 mg/m³ and 0.2 mg/m³ respectively (Department of Labour, 1995; Hostýnek and Maibach, 2004a; Ellingsen *et al.*, 2007). Copper is considered a respiratory tract irritant (ATSDR, 2004) and high exposures may result in metal fume fever and pulmonary structural changes (Ellingsen *et al.*, 2007).

Copper, unlike other metal compounds such as nickel, chromium and cobalt is not a recognised skin sensitiser (Forte *et al.*, 2008) and has no skin notation indicating that it should not contribute significantly to systemic toxicity (Sartorelli *et al.*, 2007).

It is, however, considered to be a rare cause of allergic and irritant contact dermatitis and of growing concern among dermatologists (Hostýnek and Maibach, 2004a; Forte *et al.*, 2008). Contact dermatitis, allergies or skin problems following copper exposure have been reported by Rademaker (1998), Wöhrl *et al.* (2001), Hostýnek and Maibach (2004b), Ellingsen *et al.* (2007) and Forte *et al.* (2008).

In the general population, dietary intake or ingestion is considered the main exposure source to copper (Sadhra *et al.*, 2007), where inhalation is more common in occupational settings (Jo *et al.*, 2008). The skin was previously considered an impermeable barrier and overlooked as route of exposure (Hostýnek *et al.*, 2006; Du Plessis *et al.*, 2010). A now recognised port of entry for exogenous agents, dermal exposure studies have gained increased interest since 2008 (Lidén *et al.*, 2008).

Many methods have been developed to assess dermal exposure and were described by Du Plessis *et al.* (2010) as interception methods, removal of the contaminant methods and detection methods. Dermal exposure studies, especially on liquid pesticides, have been performed extensively in the past, but more recently some focus has fallen on dermal exposure to metallic compounds. Studies based on metals such as antimony, beryllium, chromium, cobalt, lead, nickel and zinc have been reported, but to our knowledge, occupational dermal exposure to copper sulphate has not been characterised (Day *et al.*, 2007; ICMM, 2007; Lidén *et al.*, 2008; Day *et al.*, 2009; Du Plessis *et al.*, 2010; Hughson *et al.*, 2010; Julander *et al.*, 2010; Du Plessis *et al.*, 2013a).

Dermal permeation of substances is complex and affected by numerous intrinsic and extrinsic factors of which contaminant physico-chemical properties and exposure conditions have been extensively studied. Regrettably, the effect of skin barrier function has received less attention (Cohen and Rice, 2003; Winder, 2004; Sartorelli *et al.*, 2007; Kezic and Nielsen, 2009).

The stratum corneum (SC) is the uppermost layer of the skin and considered the principal physical barrier (Agache, 2004; Chou *et al.*, 2004; Proksch *et al.*, 2008; Imhof *et al.*, 2009, Du Plessis *et al.*, 2013b) that not only protects the skin from exogenous agents but also helps maintain the body's internal homeostasis (Cohen and Rice, 2003; Imhof *et al.*, 2009). Impairment of this barrier function may not only lead to enhanced SC penetration but also to the

penetration of substances otherwise not possible through intact skin (Nielsen, 2005; Nielsen *et al.*, 2007; Kezic and Nielsen, 2009; Du Plessis *et al.*, 2013b). Because of the latter mentioned fact, the assessment of skin barrier function in conjunction with dermal exposure measurements is becoming the norm (Du Plessis *et al.*, 2013b).

Multiple non-invasive bioengineering methods have been developed to quantify skin barrier function by measuring different skin parameters (Darlenski *et al.*, 2009; Sotoodian and Maibach, 2012). Skin hydration and transepidermal water loss (TEWL) are two parameters described by Du Plessis *et al.* (2013b) as extensively used in skin barrier function assessment. Skin hydration indicates the skin's surface moisture level (Du Plessis *et al.*, 2010) where TEWL represents the quantity of water that passively diffuses from the viable epidermis through a unit area of the SC to the surrounding atmosphere over a certain period (Agache and Black, 2004; Imhof *et al.*, 2009). In addition to skin hydration and TEWL measurements Darlenski *et al.* (2009) suggested that skin surface pH is essential for the integral evaluation of the skin barrier function. The quality of the SC is dependent on skin surface acidity and an acidic pH is very important for the correct functioning of this tissue (Rawlings *et al.*, 2008). Furthermore changes in skin surface pH can affect the dissolution and/or penetration of substances in contact with the skin (Stefaniak *et al.*, 2013).

Skin parameters are influenced by numerous factors, hence manufacturers' instructions of measuring equipment and exclusion criteria for subjects are used to eliminate most environmental, measurement, endogenous and exogenous factors in clinical studies (Du Plessis *et al.*, 2013a). In workplace studies the above are seldom viable, posing numerous challenges for the researcher (Kütting *et al.*, 2010; Du Plessis *et al.*, 2013b; Stefaniak *et al.*, 2013). As a result Du Plessis *et al.* (2013b) and Stefaniak *et al.* (2013) developed international guidelines for the *in vivo* assessment of transepidermal water loss, skin hydration and pH in non-clinical settings.

Studies making use of these three parameters in the assessment of skin barrier function in an occupational setting are limited, and those making use of the international guidelines to report skin barrier function results even more so. Furthermore the assessment of skin barrier function coupled with the real time dermal exposure to copper sulphate of workers at a chemical industry has not been done.

1.2 RESEARCH OBJECTIVES

The objectives of this mini-dissertation were

- to assess dermal exposure of workers at a chemical industry, to copper sulphate;
- to characterise the change in the same workers' skin barrier function from before the onset of the work shift to the end by measuring skin hydration, TEWL and skin surface pH; and
- to assess the workers' subjective skin condition by letting them complete a skin assessment questionnaire.

1.3 HYPOTHESIS

Hypothesis 1: Workers at the chemical industry are exposed to copper sulphate via the dermal exposure route.

Hypothesis 2: Skin barrier function of workers at the chemical industry shows impairment by the end of the work shift, with a decrease in skin hydration and pH levels and an increase in TEWL.

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CHAPTER 2
LITERATURE REVIEW

LITERATURE REVIEW

The following chapter gives an overview on available literature relevant to this study. Copper in both elemental and copper sulphate form will be discussed briefly along with the role of copper as an essential metal. Focus will then shift to copper ingestion, inhalation and dermal exposure as well as the resulting health effects. As dermal exposure was assessed in this study, an overview of the different dermal exposure sampling techniques will be given. Lastly the skin, its barrier function as well as the parameters used to assess the barrier function will be discussed.

2.1 COPPER

As first element of Group IB in the periodic table copper displays four oxidation states: Cu (0), Cu (I), Cu (II) and Cu (III); giving it the ability to readily form complexes (ATSDR, 2004; Hostýnek and Maibach, 2006). Copper in elemental form, in compound with sulphur and its role as an essential metal are discussed below.

2.1.1 ELEMENTAL COPPER

Copper is a reddish brown metal which occurs naturally in all parts of the ecosystem from rock and water to plants and mammals (ATSDR, 2004; Ellingsen *et al.*, 2007). As one of the first metals excavated by humans, it is resistant to corrosion, easy to mould and has high ductility and malleability (Ellingsen *et al.*, 2007; Doebrich, 2009). Due to its useful properties, copper has been exploited since 8000 Before Christ and an estimated 20 million tons were used worldwide in 2008 alone (Doebrich, 2009). Modern day uses of copper are primarily as metal or alloy in building construction and the manufacturing of electric and electronic products, transportation equipment and other metal products (Edelstein, 2013). Copper compounds are most commonly used in agriculture, or for water treatment and as preservatives for wood, leather, and fabrics (ATSDR, 2004).

2.1.2 COPPER SULPHATE

Acclaimed as the most important and commonly used compound of copper, copper sulphate is primarily produced via an electrochemical reaction of copper with sulphuric acid, or as a by-product of copper production by means of ore leaching (ATSDR, 2004; CDAA 2010). The majority of products containing copper sulphate are used in agriculture as fungicides and algaecides, or in the industry as metal finish, wood preservative and mineral froth flotation where the remainder would go to water treatment (ATSDR, 2004).

2.1.3 COPPER – AN ESSENTIAL METAL

Copper is part of a small group of metallic compounds that are considered as essential nutrients (Cushing *et al.*, 2007). Essential nutrients are involved in a variety of biological processes as they form part of several enzymes and participate in oxygen transport, gene transcription, nerve conduction and redox-reactions (Ellingsen *et al.*, 2007; Jo *et al.*, 2008; Rana, 2008). Apart from its essentiality, in excess these nutrients are toxic and numerous regulating homeostatic control mechanisms have evolved to control the absorption, distribution, storage and excretion of these nutrients (Ellingsen *et al.*, 2007; De Romaña, 2011).

Similar to a dietary deficiency or the excessive intake of copper, a disruption in the homeostatic control mechanisms will result in illnesses or negative health effects (Goering and Barber, 2010). The manifestation of health effects due to a deficiency or high doses of copper are well indicated by the U-shape dose-response relationship (Hostýnek and Maibach, 2004a; Moffett *et al.*, 2007; Oguz *et al.*, 2010).

A deficiency of copper is most common amongst infants, and can lead to health effects such as integumentary and skeletal abnormalities, defects in growth and development, neurodegenerative symptoms, anaemia, reproductive failure, poor cardiovascular health, impaired immunity and pigmentation as well as hair structure defects (Hostýnek and Maibach, 2004a; Qian *et al.*, 2005; Cushing *et al.*, 2007; De Romaña, 2011). Most of these symptoms are evident in patients with Menkes disease, caused by mutations in the copper transporting alpha polypeptide (ATP7A) export pump. The mutations result in copper not being delivered to important enzymes causing a

severe brain copper deficiency with symptoms often resulting in death (Ellingsen *et al.*, 2007; De Romaña, 2011).

The most common known example of chronic copper toxicity is Wilson disease, where a mutation of the copper transporting beta polypeptide (ATP7B) homologous transporter is present. Wilson disease mainly causes an accumulation of copper in the liver, brain and/or cornea and depending on the area of manifestation, results in liver disease, neurological impairment and kidney malfunction. Other examples of copper toxicity are Indian Childhood Cirrhosis, Tyrolean Infantile Cirrhosis and Idiopathic Chronic Toxicosis which are caused by extremely high chronic copper exposures (Ellingsen *et al.*, 2007; De Romaña, 2011).

Apart from individuals suffering from Wilson disease, acute toxic effects associated with copper are rare and have mainly been described after accidental or suicidal ingestion of large quantities of copper salts with health effects mainly being gastrointestinal (Ellingsen *et al.*, 2007; Rana, 2008; De Romaña *et al.*, 2011).

For a more extensive review of adverse health effects following ingestion as well as inhalation and dermal exposure, please refer to Section 2.4 of this chapter.

2.2 EXPOSURE TO COPPER

Copper exposure can occur through the environment, as a consumer or occupationally (ATSDR, 2004).

2.2.1 ENVIRONMENTAL EXPOSURE

Copper is widespread in the environment and can be found in the ground, water and air as well as in plants and animals (ATSDR, 2004; Ellingsen *et al.*, 2007). Apart from entering the environment via natural sources such as volcanic eruptions and decaying vegetation, environmental copper also originates from anthropogenic sources such as mining activities and factories (ATSDR, 2004).

2.2.2 CONSUMER EXPOSURE

The main sources of human copper exposure are food, drinking water and copper containing supplements (De Romaña, 2011) with the average daily dietary intake of adults varying between 1-2.5 mg (Ellingsen *et al.*, 2007).

Apart from ingestion of copper, consumers can be exposed to copper on a daily basis by means of a wide array of products for example cosmetics, garden products such as fungicides, and jewellery (ATSDR, 2004; Winder, 2004a).

For many centuries it has been believed that wearing copper jewellery would relieve inflammatory and musculoskeletal disorders. To date there are no studies concluding the matter, and the medicinal value of copper jewellery remains a folk remedy (Hostýnek and Maibach, 2006).

Copper is also regularly used in dental and medical devices such as dental restorations and intrauterine devices (IUD) (Hostýnek and Maibach, 2004a; Hostýnek and Maibach, 2004b). The amount of copper released from a commonly used dental casting alloy was measured at 0.15 $\mu\text{g}/\text{cm}^2/\text{day}$ over a 10 months period and 11.4 $\mu\text{g}/\text{ml}$ of copper was present in the intrauterine fluid of women 6 months following implantation of an IUD (Hostýnek and Maibach, 2004a). This steady release and presence of copper can lead to adverse local effects or cytotoxicity resulting in reactions of distal tissues of which the most common is dermal allergic reactions (Hostýnek and Maibach, 2004a; Hostýnek and Maibach, 2004b).

2.2.3 OCCUPATIONAL EXPOSURE

In the general population diet is the most common route of exposure to copper, where occupational exposure is mainly through skin contact and inhalation, with the latter being predominant (ATSDR, 2004; Hostýnek and Maibach, 2004a; Jo *et al.*, 2008).

Occupational exposure is common among miners, copper smelters and workers in other industries that utilise copper (Jo *et al.*, 2008). Ellingsen *et al.* (2007) reported occupational inhalation exposures to copper at a copper smelter, turbine production company, copper refinery, nickel refinery and a copper casting industry.

Reports of dermal exposure to copper in occupational settings are limited. However, the prevalence of skin problems amongst furniture polishers, farmers and workers exposed to fungicides was attributed to the dermal exposure of copper (Rademaker, 1998; Forte *et al.*, 2008).

2.3 TOXICOKINETICS OF COPPER

Free copper ions are kept in balance throughout the body by a number of homeostatic mechanisms controlling the absorption, compartmental distribution, storage and excretion thereof (ATSDR, 2004; Ellingsen *et al.* 2007). The above toxicokinetic properties following ingestion, inhalation and dermal exposure are discussed below.

2.3.1 TOXICOKINETICS OF COPPER FOLLOWING INGESTION

Copper is mainly absorbed through the mucosal membrane of the stomach and duodenum, and depending on the dietary intake ranges between 12.4 – 79%. After absorption, copper is systemically distributed attached to albumin, ceruloplasmin, transcuprein or low-molecular-weight components in the portal circulation. Copper is stored mainly in the liver, brain, skeleton and muscles as a complex with metallothioneins and glutathione (Tapiero *et al.*, 2003; ATSDR, 2004; Ellingsen *et al.*, 2007). These metal binding metallothioneins and glutathione are considered the most important homeostatic mechanisms for the control of free intracellular copper ions (ATSDR, 2004; Cornelis and Nordberg, 2007; Ellingsen *et al.*, 2007). Cellular uptake of copper is mediated through energy-independent copper transporters CTR1 and CTR2 (ATSDR, 2004; Ellingsen *et al.*, 2007).

Excretion through bile accounts for 98% of copper excretion whereas the remaining 2% is through urine. The amount of copper lost through perspiration is negligible. A biological half-life of 13-33 days was established for copper with a generally shorter half-life in females than males (Ellingsen *et al.*, 2007).

2.3.2 TOXICOKINETICS OF COPPER FOLLOWING INHALATION

No literature is currently available on the extent and rate of copper absorption, distribution, and excretion following inhalation exposure in humans (ATSDR, 2004). Copper oxide was however found in the alveolar capillaries of rats following exposure to copper welding fumes (ATSDR, 2004). Ellingsen *et al.* (2007) also reported pulmonary half-lives of 7.5 and 37 hours respectively, after intratracheal installation of copper sulphate and copper oxide in Wistar rats, thus suggesting a faster pulmonary clearance of more soluble copper compounds.

2.3.3 TOXICOKINETICS OF COPPER FOLLOWING DERMAL EXPOSURE

Skin absorption is very complex and affected by numerous intrinsic and extrinsic factors (Cohen and Rice, 2003; Winder, 2004b; Kezic and Nielsen, 2009). Hostýnek (2003) stated that some factors are closely interrelated and the effect due to this combination is not predictable or entirely understood. Factors influencing skin absorption are age, gender, skin barrier function, exposure conditions and homeostatic controls to name a few. These factors are discussed in studies by Stanton and Jeebhay (2001), Cohen and Rice (2003), Hostýnek (2003), Hostýnek and Maibach (2004a), Winder (2004b) Hostýnek and Maibach (2006) and Hostýnek *et al.* (2006).

Before copper can be absorbed by the skin, it has to be oxidised (Hostýnek and Maibach, 2004a) and copper was awarded an oxidation potential of +0.23 eV by Hostýnek *et al.* (2006). Copper is oxidised by skin exudates, sebum and sweat, enabling ions to penetrate the skin barrier (Hostýnek and Maibach, 2004b; Hostýnek and Maibach, 2006; Forte *et al.*, 2008).

Metal ions form lipophilic soaps and hydrophilic ionised salts respectively with free fatty acids and amino acids in the sebum and sweat on the skin surface (Hostýnek and Maibach, 2004a; Hostýnek and Maibach, 2004b; Hostýnek and Maibach, 2006).

It is only after these complexes have formed that copper becomes diffusible through the skin barrier via the three penetration pathways: the intercellular route, the trans cellular route across cornified cells and lipid bilayer and the shunt route, where chemicals diffuse along the hair follicles and sweat glands (Hostýnek and Maibach, 2004b; Hostýnek and Maibach, 2006).

Copper deposits have been found in the stratum corneum (SC), but also have the ability to penetrate the skin beyond the viable epidermis indicating that it may become locally and systemically available (Hostýnek *et al.*, 2006).

Data on the rate and degree of copper absorption through the skin *in vivo* and *in vitro* are limited (ATSDR, 2004; Hostýnek and Maibach, 2006). A permeability coefficient of the order 10^{-6} cm/h was calculated for copper salts following an *in vitro* study by Hostýnek and Maibach (2006), where ATSDR (2004) described copper absorption via the skin as poor following an absorption degree of less than 6% *ex vivo*. The impact of a compromised skin barrier on the penetration of copper is not known. A study on the absorption of other metals by Larese Filon *et al.* (2009) however, concluded that the risk of skin absorption increases a great deal once the skin barrier is damaged.

The rate and degree of copper distribution and excretion following dermal exposure are unknown (ATSDR, 2004).

2.4 HEALTH EFFECTS

The main characteristic that makes copper toxic is its ability to readily donate and accept electrons (Jo *et al.*, 2008). Copper can participate in Fenton reactions to generate highly reactive oxygen species (ROS) (ATSDR, 2004; Jo *et al.* 2008) which in turn is responsible for oxidation of proteins, lipid peroxidation in membranes and cleavage of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) molecules (Tapiero *et al.*, 2003). The development of various pathologies such as neurological diseases (Tapiero *et al.*, 2003; Qian *et al.*, 2005), cancer, aging (Tapiero *et al.*, 2003) and skin diseases (Fuchs *et al.*, 2001) have been linked to the generation and action of ROS. In addition to oxidative damage, another mechanism said to contribute to copper's toxicity is that copper displaces other metal co-factors such as zinc from their natural ligands (Tapiero *et al.*, 2003).

Some health effects following the three major exposure pathways are discussed in Section 2.4.1, 2.4.2 and 2.4.5.

2.4.1 HEALTH EFFECTS FOLLOWING INGESTION

Ingestion exposure is the most common route of exposure to copper amongst the population (Sadhra *et al.*, 2007).

Acute toxicity has mainly been described after accidental or suicidal consumption of large quantities of copper (ATSDR, 2004; Cushing *et al.*, 2007; De Romaña *et al.*, 2011). Nausea and vomiting are the predominant health effects but diarrhoea, stomach pain, dizziness and respiratory difficulty have also been reported (ATSDR, 2004; Cushing *et al.*, 2007; Ellingsen *et al.*, 2007; De Romaña *et al.*, 2011). Fatalities following acute ingestion have been attributed to central nervous system depression, renal and hepatic failure (ATSDR, 2004).

Chronic ingestion of high levels of copper is potentially fatal and can produce hepatic and renal damage, hematuria and gastrointestinal bleeding (ATSDR, 2004; Cushing *et al.*, 2007). A possible relationship between the prevalence of coronary heart disease and elevated serum copper levels have also been explored (ATSDR, 2004).

To prevent possible toxic effects from copper ingestion exposure, the daily oral intake of copper for adults, pregnant and lactating woman should be limited to 0.9, 1 and 1.3 mg respectively (De Romaña *et al.*, 2011).

2.4.2 HEALTH EFFECTS FOLLOWING INHALATION

Copper inhalation exposure limits are well defined and most countries, including South Africa, limit copper dust and copper fume exposures to 0.1 and 0.2 mg/m³ respectively (Department of Labour, 1995; Hostýnek and Maibach, 2004a; Ellingsen *et al.*, 2007).

Copper is considered as a respiratory tract irritant (ATSDR, 2004; Ellingsen *et al.*, 2007) and workers exposed to copper dust reported symptoms such as coughing, sneezing and runny noses (ATSDR, 2004). High exposure levels can also lead to metal fume fever (ATSDR, 2004; Rana *et al.*, 2008) which is said to be an influenza-like syndrome with fever, myalgias and profuse perspiration (Rana *et al.*, 2008).

Ellingsen *et al.* (2007) suggested structural changes to the mucous membranes of the nose, dyspnoea, thoracic pain, emphysema and pulmonary fibrosis resulting from chronic copper inhalation exposure.

Although suggested, substantial proof on an increased cancer risk in humans following copper dust, mist and fume exposure has yet to be demonstrated (Ellingsen *et al.*, 2007).

2.4.3 HEALTH EFFECTS FOLLOWING DERMAL EXPOSURE

A limited number of chemicals have a skin notation, indicating that they can contribute significantly to systemic toxicity following dermal exposure (Hostýnek and Maibach, 2004a; Sartorelli *et al.*, 2007). Copper has not been awarded a skin notation, but this does not mean it imposes no danger following contact with the skin.

After SC penetration, copper can lead to irritation (Hostýnek and Maibach, 2004a), or due to its electrophilic nature, be haptenised and recognised by the immune system as foreign resulting in allergic reactions (Hostýnek and Maibach, 2004a; Hostýnek and Maibach, 2004b; Hostýnek *et al.*, 2006).

Reports following dermal exposure to copper also imply that health effects are mainly immunological and lymphoreticular in nature (ATSDR, 2004).

Allergies and a form of contact dermatitis associated with copper have been reported following skin contact to copper dust and salts (Ellingsen *et al.*, 2007). Cushing *et al.* (2007) continuously stated that the development of allergic contact dermatitis during these prevailing circumstances is possible. Forte *et al.* (2008) reported dermal problems in workers exposed to fungicide and contact dermatitis in furniture polishers using a commercial spirit both of which contained copper sulphate. All of the previous mentioned patients developed erythema, itching and vesiculopustular areas on either their face, neck, forearms or hands. In a study by Rademaker (1998) on contact dermatitis amongst farmers, 5 of the 46 test subjects reacted to a copper sulphate patch test, indicating their dermatitis was secondary to occupational exposure. It was also stated by Wöhrl *et al.* (2001) that positive patch test results for copper are often obtained in their laboratory.

2.5 ASSESSMENT OF DERMAL EXPOSURE

Dermal exposure can occur via direct contact, secondary contact with contaminated surfaces and/or airborne particles settling on the skin (Schneider *et al.*, 2000; Stanton and Jeebhay, 2001; Badenhorst *et al.*, 2007; Du Plessis *et al.*, 2008).

Traditionally human exposure assessments focused on inhalation and ingestion exposures (Beamer *et al.*, 2009), overlooking and underrating the skin as route of exposure, as it was considered an impermeable barrier (Hostýnek *et al.*, 2006; Du Plessis *et al.*, 2010). A now recognised port of entry for exogenous agents, dermal exposure has been brought to attention and relevant studies have gained increased interest since 2008 (Lidén *et al.*, 2008). The importance of dermal exposure assessment has been stressed by Soutar *et al.* (2000) and Crosera *et al.* (2009) as dermal absorption of toxins can present a substantial health risk (Hostýnek and Maibach, 2006).

Dermal exposure assessment was labelled complex (Schneider *et al.*, 2000) and a wide array of dermal exposure assessment methods have been developed (Soutar *et al.*, 2000; Badenhorst *et al.*, 2007; Du Plessis *et al.*, 2008; Du Plessis *et al.*, 2010). These methods were summarised by Du Plessis *et al.* (2010) as interception methods, removal of the contaminant methods and detection methods.

2.5.1 INTERCEPTION METHODS

Interception methods were previously described as surrogate skin methods (Du Plessis *et al.*, 2008), and determine the amount of contaminant deposited on skin or clothing (Badenhorst *et al.*, 2007; Du Plessis *et al.*, 2008).

Patches, gloves and whole body suits are used to collect and trap contaminants in a similar manner as skin (Badenhorst *et al.*, 2007; Du Plessis *et al.*, 2008), and are placed on body areas or worn by the employee during working operations. Thereafter the collection media can be analysed to determine the expected dermal exposure of a certain body area/whole body or the amount of chemical breakthrough through protective clothing (Soutar *et al.*, 2000; Badenhorst *et al.*, 2007; Du Plessis *et al.*, 2008).

According to Soutar *et al.* (2000), interception methods are frequently used to determine dermal exposure. Du Plessis *et al.* (2008) continuously stated that these methods have been used to determine dermal exposure to mostly pesticides, but also to chromium, metal working fluids, copper oxide, polyaromatic hydrocarbons and dust.

2.5.2 REMOVAL OF THE CONTAMINANT METHODS

Removal of the contaminant methods in assessing dermal exposure have been used extensively (Brouwer *et al.*, 2000). These methods involve the removal of the contaminant present on the skin (Brouwer *et al.*, 2000) during the sampling period, and can be done by means of wiping, washing/rinsing, tape stripping or by suction (Du Plessis *et al.*, 2008).

As mentioned by Du Plessis *et al.* (2008), these methods are best suited for substances that have a low volatility, and hence present on the skin for a significant period after contamination. When conducting a removal method, it is also of utmost importance to establish the sampling and recovery efficiencies of the media used to collect the contaminant from the skin. The latter is conducted under controlled circumstances, and respectively refers to the sampling media's ability to capture the contaminant during sampling, and the extent to which it can be removed for analysis (Du Plessis *et al.*, 2008).

2.5.2.1 SKIN WIPE SAMPLING

Skin wipe sampling has been used to monitor dermal exposure to a wide array of contaminants (Du Plessis *et al.*, 2008), and the exclusive usage thereof for the assessment of dermal metal exposure was proposed by the ICMM (2007).

This sampling technique is based on surface sampling methods described by US EPA and OSHA (Du Plessis *et al.*, 2008), and contaminants are collected by wiping sampling media over the skin's surface.

Sampling media vary in material, shape and size and can be wetted with a chemical, to enhance sampling efficiency, or used dry. Sampling media are also commercially available and moist wipes named Jeyes 'sticky fingers' wet ones and GhostwipesTM were used successfully for

dermal sampling of nickel compounds by Hughson *et al.* (2010) and Du Plessis *et al.* (2010) respectively.

In studies by Lidén *et al.* (2008), Day *et al.* (2009), Du Plessis *et al.* (2010), Hughson *et al.* (2010) and Du Plessis *et al.* (2013a) dermal metal exposure assessment was carried out on body areas considered most exposed, and included the fingers, hands, chest, neck and face.

Wipe sampling can be conducted over an entire body area staying in between anatomical markers (Day *et al.*, 2009; Du Plessis *et al.*, 2010) or templates with an open aperture can be used to sample only a specific region of a body area (Lidén *et al.*, 2008; Du Plessis *et al.*, 2010; Hughson *et al.*, 2010; Du Plessis *et al.*, 2013a).

The sampling efficiency of the media will determine the number of times a single wipe is passed over the sampling area, as well as the number of consecutive wipes used per area (Lidén *et al.*, 2008; Du Plessis *et al.*, 2010; Hughson *et al.*, 2010).

The amount of pressure applied to wipe the media over the skin, influences sampling efficiency and therefore to rule out inter-operator variability, it is recommended a single operator collect all samples. During each sampling session, as precaution for sample contamination, the operator should wear a clean pair of disposable gloves and make use of a clean template, if applicable (Du Plessis *et al.*, 2008).

2.5.2.2 SKIN WASH SAMPLING

Du Plessis *et al.* (2008) identified three skin wash sampling methods: hand washing, hand rinsing and finger immersion sampling. In certain studies skin wash sampling has proved to be more efficient in contaminant removal than wipe sampling and with the exception of finger immersion sampling, these techniques have been used extensively in dermal exposure assessment (Brouwer *et al.*, 2000; Du Plessis *et al.*, 2008).

Hand wash sampling was described by Brouwer *et al.* (2000) as similar to skin wipe sampling in the manner that both techniques remove the contaminant from the skin via a combination of chemical and mechanical action. When conducting hand wash or rinse sampling a predetermined amount of chemical is used to either wash or rinse over the hands, and in doing so traps the

contaminant present for exposure analysis (Brouwer *et al.*, 2000; Du Plessis *et al.*, 2008). Immersion sampling differs from the latter as only a finger of an employee is placed into the trapping liquid for a given period (Du Plessis *et al.*, 2008).

2.5.2.3 TAPE STRIP SAMPLING

Tape stripping is a minimally invasive sampling method (Cullander *et al.*, 2000; Nylander-French, 2000) commonly used in dermatopharmacokinetic and toxicological research, as well as in dermal exposure assessment (Cullander *et al.*, 2000; Hostýnek and Maibach, 2006; Du Plessis *et al.*, 2008, Liljelind *et al.*, 2010).

During sampling, the SC is removed by means of stripping adhesive tape applied to predetermined skin areas (Nylander-French, 2000; Du Plessis *et al.*, 2008). The number of consecutive tape strip samples taken differ depending on the sampling efficiency of the adhesive tape used (Du Plessis *et al.*, 2008; Liljelind *et al.*, 2010). After sampling, the tape strips are analysed for the contaminant individually or as consecutive tape strips pooled together (Du Plessis *et al.*, 2008).

2.5.2.4 SUCTION METHODS

The use of suction methods to determine dermal exposure is both limited and expensive. More commonly used to determine surface contamination (Ashley, 2010), suction methods work on the principle that the contaminant is removed by the suction power generated by either a vacuum or Smair sampler and then recollected on a filter for analysis (Du Plessis *et al.*, 2008).

2.5.3 DETECTION METHODS

This method was previously described as the fluorescent tracer method and works on the principle of adding a fluorescent tracer to the contaminant during a production process. Dermal exposure as well as surface contamination can then be qualitatively and quantitatively determined making use of a long wave UV light and a video camera. Although proved effective in assessing dermal exposure, this method is expensive and sometimes impracticable or impossible to execute (Cherrie *et al.*, 2000; Du Plessis *et al.*, 2008).

2.6. SKIN ANATOMY AND FUNCTION

The skin is the largest human organ and covers an area of 2 - 2.3 m² (Winder, 2004b), and accounts for more than 10 per cent of body mass (Crosera *et al.*, 2009). Its most important function as highlighted by Proksch *et al.* (2008) is “to form an effective barrier between the outside and inside of an organism”.

The skin consists of four layers: the SC as the uppermost layer, followed by the viable epidermis, dermis and hypodermis. In addition, the skin hosts sweat and sebaceous glands, hair follicles and nails, otherwise known as skin appendages (Agache, 2004a; Hostýnek and Maibach, 2006).

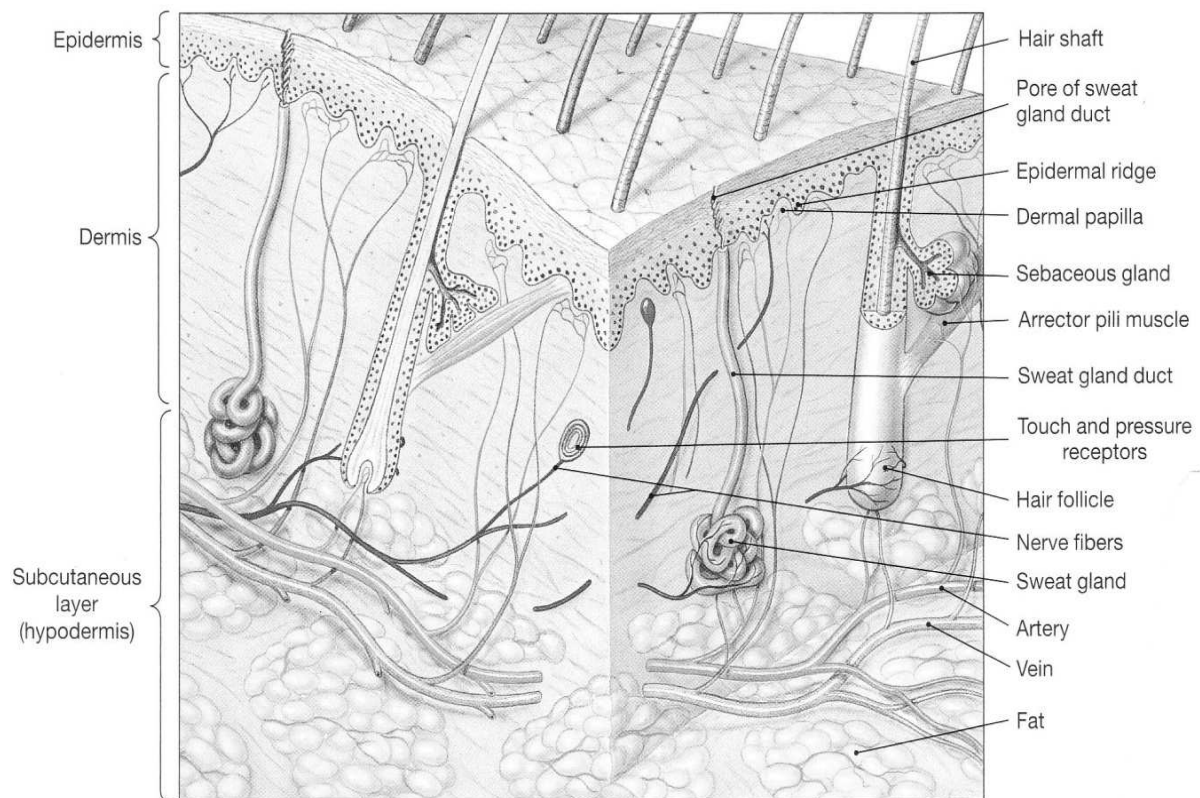


Fig. 1. The Structure of the skin, indicating the *epidermis* (consisting of the stratum corneum and viable epidermis), *dermis* and the *hypodermis* (Martini and Bartholomew, 2003).

The SC is considered the principal physical barrier of the skin (Proksch *et al.*, 2008; Imhof *et al.*, 2009; Kezic and Nielsen, 2009; Du Plessis *et al.*, 2013b), limiting the uptake and loss of exogenous and endogenous agents through the skin (Hostýnek and Maibach, 2006). The structure of the SC is often described as a “brick and mortar” structure, consisting of dead keratinocytes (corneocytes) embedded in a lipid bilayer (Hostýnek and Maibach, 2006; Kezic and Nielsen, 2009).

Adjacent to the SC is the strata of the viable epidermis, which in descending order consists of the: stratum lucidum, stratum granulosum, stratum spinosum and the stratum germinativum or stratum basale (Martini and Bartholomew, 2003; Hostýnek and Maibach, 2006). The primary function of the viable epidermis is the formation of the SC (Gentilhomme and Neveux, 2004). This is accomplished as keratinocytes formed in the basal layer transition outwards into the superficial layers of the viable epidermis, gradually undergoing keratinisation by means of differentiation, finally resulting in the dead cells of the SC. In addition Langerhans cells and melanocytes are found throughout the viable epidermis respectively contributing to the skin’s immune response and pigmentation (Cohen and Rice, 2003; Martini and Bartholomew, 2003; Gentilhomme and Neveux, 2004; Hostýnek and Maibach, 2006).

The dermis is the thickest of the skin layers (Agache, 2004b) and is divided from the epidermis by means of the basement membrane (Martini and Bartholomew, 2003). Consisting of mostly connective tissue, the dermis has a superficial papillary and a deeper reticular layer (Martini and Bartholomew, 2003; Agache, 2004b). The papillary layer functions as support system to the epidermis, supplying it with nutrients through its vascular network, and is also involved in the immune function of the skin. The reticular layer, where most skin appendages originate from, has elastic properties and mainly serves as architectural protection for cells and blood vessels (Agache, 2004b; Hostýnek and Maibach, 2006).

With no distinct barrier between the dermis, the hypodermis contains few capillaries, hosts no vital organs and is mainly made up of connective and adipose tissue. The hypodermis’s main function is to stabilise the superior skin layers with regard to deeper tissue and organs, but also serves as energy resource, shock absorber and body insulation (Martini and Bartholomew, 2003).

2.7 SKIN BARRIER FUNCTION AND THE ASSESSMENT THEREOF

As mentioned in Section 2.6, the primary function of the skin is to act as protective barrier (Proksch *et al.*, 2008; Rawlings *et al.*, 2008), limiting the rate of penetration of exogenous substances into the skin as well as control the loss of water, proteins and plasma components through the skin (Hostýnek and Maibach, 2006; Darlenski *et al.*, 2009).

It is, therefore, evident that the quality of the skin barrier will greatly affect the extent of chemical penetration into the skin (Cohen and Rice, 2003; Hostýnek, 2003; Winder, 2004b; Kezic and Nielsen, 2009). Studies by Nielsen (2005), Nielsen *et al.* (2007) and Kezic and Nielsen (2009) showed that an impaired skin barrier might not only lead to enhanced SC penetration, but also to the penetration of chemicals and particles that could not have permeated through intact skin. Therefore a person with a damaged skin barrier is more susceptible to both local and systemic toxicity (Nielsen *et al.*, 2007; Kezic and Nielsen, 2009). Because of the latter mentioned, the assessment of skin barrier function in conjunction with dermal exposure measurements are becoming the norm (Du Plessis *et al.*, 2013b).

Skin barrier disruption is a result of skin protein alteration, lipid removal or lipid disorganisation (Charbonnier *et al.*, 2007) and is commonly caused by prolonged wet work, chemical and mechanical damage and medical skin conditions such as irritation and eczema to mention a few (Nielsen, 2005; Nielsen *et al.*, 2007; Kezic and Nielsen, 2009).

Darlenski *et al.* (2009), Du Plessis *et al.* (2010) and Sotoodian and Maibach (2012), stated that the measurement of skin parameters such as SC hydration, transepidermal water loss (TEWL), skin surface acidity and transepidermal oxygen, carbon dioxide and ion flux are effective to evaluate skin barrier function. Darlenski *et al.* (2009) continued by stating that the use of a single parameter for skin barrier function assessment would be insufficient, and suggested a multi parametric approach.

The three parameters used for the assessment of skin barrier function and of concern for this mini-dissertation are discussed in turn, followed by a brief description of factors that can affect the skin, its barrier function and the measurement results thereof.

2.7.1 SKIN HYDRATION

Skin hydration indicates the skin's surface moisture level (Du Plessis *et al.*, 2010) and optimal skin function is dependent on adequate hydration (Kezic and Nielsen, 2009). The water capacity of the SC influences its physical, functional and regulating properties as well as its viscoelastic characteristics (Darlenski *et al.*, 2009; Sotoodian and Maibach, 2012).

Dehydration of the SC induces dryness and results in an impaired barrier function (Rawlings *et al.*, 2008; Darlenski *et al.*, 2009) due to reduced mechanical flexibility, scaling (Sotoodian and Maibach, 2012) and physiological changes of the skin such as alterations in the lipid composition (Rawlings *et al.*, 2008). Denda (2000) and Rawlings *et al.* (2008) continuously stated that skin surface dryness is a prelude to cutaneous dermatoses and other skin diseases like eczema.

As with dehydration of the skin surface, excessive SC hydration can lead to a reduced skin barrier function resulting in enhanced percutaneous absorption (Hostýnek and Maibach, 2006; Kezic and Nielsen, 2009). As discussed in Section 2.7.4.2 excessive SC hydration frequently occurs as a result of prolonged wet work, regular contact with water (Kezic and Nielsen, 2009) and skin occlusion by means of wearing protective clothing that prevents normal sweat evaporation (Cohen and Rice, 2003; Hostýnek and Maibach, 2004a; Kezic and Nielsen, 2009).

2.7.2 TRANSEPIDERMAL WATER LOSS (TEWL)

The assessment of skin barrier function frequently involves measurement of TEWL (Pirost and Falson, 2004; Darlenski *et al.*, 2009; Kezic and Nielsen, 2009), which can be indicative of epidermal barrier disruption (Sotoodian and Maibach, 2012), permeability (Darlenski *et al.*, 2009) and skin irritancy (Charbonnier *et al.*, 2007; Darlenski *et al.*, 2009). Agache and Black, (2004), Imhof *et al.* (2009) and Du Plessis *et al.* (2013b) stated that TEWL represents the quantity of water that passively diffuses from the viable epidermis through a unit area of the SC to the surrounding atmosphere over a certain period.

A low TEWL usually denotes an intact skin barrier (Darlenski *et al.*, 2009), as a large portion of the diffusing water is retained by natural moisturising factors within the SC (Sotoodian and Maibach, 2012). A damaged SC will therefore result in less water retention and hence an

elevated TEWL (Proksch *et al.*, 2008; Rawlings *et al.*, 2008; Kezic and Nielsen, 2009; Sotoodian and Maibach, 2012).

An inverse relationship is said to exist between TEWL and SC hydration (Proksch *et al.*, 2008; Darlenski *et al.*, 2009; Du Plessis *et al.*, 2013b) with high TEWL values frequently correlating with low SC hydration. Inconsistency in the relationship has, however, been observed in extremely damaged skin, during early stages of irritancy reaction and in certain body regions (Darlenski *et al.*, 2009).

2.7.3 SKIN SURFACE pH

The pH of the skin is said to increase with depth, and ranges from 4 - 6.3 on the surface of the SC (Hostýnek and Maibach, 2006; Kim *et al.*, 2009; Schreml *et al.*, 2010), to 7.4 in deeper skin tissue (Hostýnek and Maibach, 2006; Kim *et al.*, 2009). The variability in reported skin surface pH levels is due to the fact that numerous factors as discussed in Section 2.7.4 can affect skin surface pH.

The lower pH of the skin's surface is referred to as the "acidic mantle", and together with the concomitant pH gradient over the SC, is crucial for the normal functioning of the skin barrier tissue (Lambers *et al.*, 2006; Rawlings *et al.*, 2008). It supports epidermal barrier homeostasis, controls the presence of both resident and transient skin micro flora, and regulates corneocyte desquamation (Waller and Maibach, 2005, Hostýnek and Maibach, 2006; Lambers *et al.*, 2006; Rawlings *et al.*, 2008; Darlenski *et al.*, 2009).

The origin of the acidic skin surface pH, is still unclear but is said to stem from amino acids, alpha-hydroxy acids and acidic lipids that either originate in the epidermis or as products of sebaceous gland activity (Hostýnek and Maibach, 2006; Lambers *et al.*, 2006).

2.7.4. FACTORS AFFECTING SKIN AND SKIN BARRIER FUNCTION

Numerous endogenous, exogenous, environmental and measurement related factors can influence skin hydration, TEWL and skin surface pH (Du Plessis *et al.*, 2013a). The most important factors affecting these three parameters will be discussed in short.

2.7.4.1 ENDOGENOUS FACTORS

Age, gender, ethnicity, anatomical position, skin temperature and sweating, circadian rhythm as well as the presence of a skin ailment are endogenous factors that influence the skin.

2.7.4.1.1 AGE

Skin hydration is said to decrease at a slow and steady pace with age (Du Plessis *et al.*, 2013b). Sotoodian and Maibach (2012) stated that TEWL is usually elevated in premature babies that have underdeveloped skin barriers. Otherwise, TEWL stays constant and unaffected by age until 60 years, whereafter it decreases slightly (Du Plessis *et al.*, 2013a). During the first month after birth (Lambers *et al.*, 2006) and from 70-95 years of age, skin surface pH is less acidic (Stefaniak *et al.*, 2013).

2.7.4.1.2 GENDER

Darlenski *et al.* (2009) and Du Plessis *et al.* (2013b) stated that gender does not have an effect on skin hydration and TEWL. Lambers *et al.* (2006) reported that in comparison to males, women have less acidic skin surface pH. In contrast Ehlers *et al.* (2001) reported the exact opposite, making the effect of gender on skin surface pH conflicting (Lambers *et al.*, 2006; Darlenski *et al.*, 2009; Stefaniak *et al.*, 2013).

2.7.4.1.3 ETHNICITY

The influence of ethnicity on skin hydration, TEWL (Darlenski *et al.*, 2009; Du Plessis *et al.*, 2013b) and skin surface pH are conflicting (Lambers *et al.*, 2006; Darlenski *et al.*, 2009; Stefaniak *et al.*, 2013).

A study by Muizzuddin *et al.* (2010) reported black skin to be scallier and less hydrated when compared to Caucasian and East Asian skin. Repeatedly Du Plessis *et al.* (2013b) reported several studies that suggested an effect of race on skin hydration, but also reported controversial studies that might be as a result of different anatomical measurement positions.

The latter is also of concern for TEWL with Darlenski *et al.* (2009) and Du Plessis *et al.* (2013b) highlighting other controversial studies. On the other hand, Sotoodian and Maibach (2012) reported the effect of ethnicity on TEWL, were TEWL for white > Asian > Hispanic > black.

Darlenski *et al.* (2009) stated that ethnicity has an effect on skin surface pH, and Lambers *et al.* (2006) reported the skin of Caucasians' to be less acidic than that of black people. In contrast Stefaniak *et al.* (2013) and earlier reports by Lambers *et al.* (2006) found the effect of ethnicity on skin surface pH to be conflicting.

2.7.4.1.4 ANATOMICAL POSITION

Sufficient evidence exists to conclude that skin hydration, TEWL (Darlenski *et al.*, 2009; Du Plessis *et al.*, 2013b) and skin surface pH (Darlenski *et al.*, 2009; Stefaniak *et al.*, 2013) differ among anatomical positions as well as among sites of a certain position.

In general the skin of the forehead and palm are more hydrated than that of the abdomen, thighs and lower legs (Du Plessis *et al.*, 2013b). Sotoodian and Maibach (2012) stated that research shows TEWL of the palm and sole to be higher than forearm, abdomen and back. Du Plessis *et al.* (2013b) concluded that the palm's TEWL tend to be higher than other anatomical areas.

Studies have reported the difference in the skin surface pH between forearm areas, the forearm and the elbow and the forearm of the dominant and non-dominant hand (Stefaniak *et al.*, 2013). Lambers *et al.* (2006) continuously stated that significant differences in skin surface pH between body areas have been observed, and certain more occluded body areas have a considerable higher skin surface pH.

2.7.4.1.5 SKIN TEMPERATURE AND SWEATING

Skin hydration and TEWL are affected by skin temperature and sweating (Darlenski *et al.*, 2009; Plessis *et al.*, 2013b), where sweating increases both skin hydration and TEWL (Du Plessis *et al.*, 2013b). Darlenski *et al.* (2009) stated that sweating has an effect on skin surface pH and Stefaniak *et al.* (2013) suggested that excess sebum be wiped from the skin surface before taking skin surface pH measurements. In contrast Darlenski *et al.* (2009) reported conflicting data on the effect of skin temperature on skin surface pH.

2.7.4.1.6 CIRCADIAN RHYTHM

Darlenski *et al.* (2009) and Sotoodian and Maibach (2012) stated that circadian rhythm does have an effect on TEWL. Du Plessis *et al.* (2013b) also reported studies confirming the effect of circadian rhythmicity on TEWL as well as studies dismissing it. Reports on the effect of circadian rhythm on skin hydration are conflicting (Darlenski *et al.*, 2009; Du Plessis *et al.*, 2013b).

Circadian rhythm is known to exercise an impact on skin surface pH levels during the course of the day (Darlenski *et al.*, 2009; Schreml *et al.*, 2010; Stefaniak *et al.*, 2013). Lambers *et al.* (2006) and Schreml *et al.* (2010) reported peak skin surface pH values in the afternoon and low pH values in the evening.

2.7.4.1.7 SKIN DISEASE

Substantial evidence exists that skin hydration, TEWL (Proksch *et al.*, 2008; Du Plessis *et al.*, 2013b) and skin surface pH (Lambers *et al.*, 2006, Selander *et al.*, 2006; Stefaniak *et al.*, 2013) are influenced by various pathological skin conditions like eczema, ichthyosis, atopic and irritant contact dermatitis. In general TEWL and skin surface pH levels are higher and skin hydration lower in involved skin (Du Plessis *et al.*, 2013b; Stefaniak *et al.*, 2013).

2.7.4.2 EXOGENOUS FACTORS

Skin washing and wet work, the use of topical products, contact with solvents, detergents and irritants, occlusion, mechanical skin damage, smoking and caffeine consumption are exogenous factors that influence the skin.

2.7.4.2.1 SKIN WASHING AND WET WORK

Regular skin washing and prolonged wet work do influence skin hydration, TEWL and skin surface pH (Du Plessis *et al.*, 2013b; Stefaniak *et al.*, 2013). Increased skin hydration (Kezic and Nielsen, 2009; Du Plessis *et al.*, 2013b) and TEWL (Du Plessis *et al.*, 2013b) are common following frequent or prolonged exposure to water. Depending on the pH of the water and soaps used in skin washing, skin surface pH can either increase or decrease for a time period thereafter (Lambers *et al.*, 2006; Du Plessis *et al.*, 2013b).

2.7.4.2.2 USE OF TOPICAL PRODUCTS

The use of hydro and barrier creams is reported to lower TEWL (Du Plessis *et al.*, 2013b). As with hand washing, the use of topical products influences skin surface pH for a period after application with skin surface pH returning to baseline values in as little as 90 - 120 minutes (Stefaniak *et al.*, 2013).

2.7.4.2.3 CONTACT WITH SOLVENTS, DETERGENTS AND IRRITANTS

Contact with certain chemicals has an impact on skin hydration, TEWL (Du Plessis *et al.*, 2013b) and skin surface pH (Stefaniak *et al.*, 2013). Dermal exposure to surfactants will decrease skin hydration whilst exposure to organic solvents, sodium lauryl sulphate (Du Plessis *et al.*, 2013b) and lactic acid (Darlenski *et al.*, 2009) may increase TEWL.

2.7.4.2.4 OCCLUSION

Occlusion of the skin by protective gloves restricts the evaporation of moisture from the skin's surface, and can have an effect on the skin's barrier function (Todd and Carman, 2001; Cohen and Rice, 2003; Hostýnek and Maibach, 2004a; Kezic and Nielsen, 2009). Du Plessis *et al.* (2013b) reported increased skin hydration, hyper-hydration of the SC and increased TEWL as a result of occlusion. Data on the short term effect of occlusion on skin surface pH are limited, yet occlusion of the skin for 3 - 4 days resulted in an increased skin surface pH (Stefaniak *et al.*, 2013).

2.7.4.2.5 MECHANICAL DAMAGE, SMOKING AND CONSUMPTION OF CAFFEINE

Skin damage as a result of mechanical friction, smoking and the consumption of caffeine occurs often in the workplace and can affect skin barrier function (Du Plessis *et al.*, 2013b). Du Plessis *et al.* (2013b) reported elevated TEWL values and decreased skin hydration for smokers when compared with non-smokers.

2.7.4.3 ENVIRONMENTAL AND MEASUREMENT FACTORS

Air movement, ambient temperature, relative humidity, season and the presence of direct sunlight during measurements can influence skin barrier function and the results of the measurement thereof.

2.7.4.3.1 MEASUREMENT CONDITIONS

Ambient temperature, relative humidity, air convection and the presence of direct sunlight can influence skin barrier function and the results of the measurements thereof (Gabard and Treffel, 2004; Chou *et al.*, 2005; Darlenski *et al.*, 2009; Du Plessis *et al.*, 2013b; Stefaniak *et al.*, 2013).

Skin hydration is said to increase with a rise in temperature and relative humidity (Goh, 2006), and whilst TEWL also increases at higher temperatures (Tupker and Pinnagoda, 2006) it decreases with a rise in relative humidity (Gabard and Treffel, 2004).

Due to the effect temperature and relative humidity have on skin barrier function parameters the European Group on Efficacy Measurement of Cosmetics and Other Topical Products (EEMCO) (Berardesca, 1997; Rogiers, 2001; Parra and Paye, 2003) and manufacturers' instructions of measuring equipment recommends that skin barrier function measurements be carried out at certain temperatures and relative humidities. In workplace studies, however, the above are seldom viable, therefore Du Plessis *et al.* (2013b) and Stefaniak *et al.* (2013) developed international guidelines for the *in vivo* assessment of transepidermal water loss, skin hydration and pH in non-clinical settings.

The impact of air convection and direct sunlight on skin barrier function measurements can be avoided by doing measurements in a designed enclosure (Gabard and Treffel, 2004).

2.7.4.3.2 SEASONALITY

Seasonality influences skin hydration, TEWL (Darlenski *et al.*, 2009; Du Plessis *et al.*, 2013b) and skin surface pH (Darlenski *et al.*, 2009; Stefaniak *et al.*, 2013). Barel and Clarys (2006) suggested that skin hydration increases during summer months due to higher temperatures and relative humidity. Denda (2000) stated that skin barrier function declines during winter months when relative humidity is low. Although reports on seasonal variation in skin surface pH are

limited, Abe *et al.* (1980) reported results that suggested skin surface pH can be influenced by seasonality.

2.7.5. MEASUREMENT OF SKIN BARRIER FUNCTION

Numerous non-invasive bioengineering methods have been developed (Darlenski *et al.*, 2009; Sotoodian and Maibach, 2012) over the last three decades (Rawlings *et al.*, 2008) to quantify skin barrier function. These methods have been extensively used to assess skin barrier function in cosmetology, pharmaceutical, toxicological and clinical studies (Sotoodian and Maibach, 2012), yet workplace studies are limited.

Goh and Gan (1994), Smith *et al.* (1994), Chou *et al.* (2004), Chou *et al.* (2005), Chou *et al.* (2008) and Kütting *et al.* (2010) reported changes in TEWL of workers in occupational settings. In conjunction with TEWL, Berndt *et al.* (1999) and Du Plessis *et al.* (2010) reported skin hydration. Recently Du Plessis *et al.* (2013a) assessed changes in the skin hydration, TEWL and skin surface pH of workers at a base metal refinery.

Similar to Du Plessis *et al.* (2013a) skin hydration, TEWL and skin surface pH were measured in this study and used to assess changes in the skin barrier function of workers exposed to copper sulphate at a chemical industry. A brief description of the methods used in the assessment of the above parameters is given below.

2.7.5.1 SKIN HYDRATION

Skin hydration can be quantified by numerous methods (Rawlings *et al.*, 2008; Sotoodian and Maibach, 2012) such as microwave, thermal, nuclear magnetic resonance, infrared and Raman spectroscopy (Darlenski *et al.*, 2009). The most common method, however, indirectly indicates the SC hydration by measuring electrical properties of the skin like impedance, conductance or capacitance (Pirrot and Falson, 2004; Rawlings *et al.*, 2008; Darlenski *et al.*, 2009; Sotoodian and Maibach, 2012).

The Corneometer used during this study is a capacitance-based device (Heinrich *et al.*, 2003), which measures the change in the dielectric constant due to SC hydration changing the

capacitance of a precision capacitor (Darlenski *et al.*, 2009). This device is also efficient due to its easy handling, economy, short measuring period and reproducibility (Heinrich *et al.*, 2003).

2.7.5.2 TRANSEPIDERMAL WATER LOSS (TEWL)

TEWL can be measured *in vivo* by means of open chamber, closed chamber and ventilated chamber methods (Darlenski *et al.*, 2009; Imhof *et al.*, 2009; Sotoodian and Maibach, 2012). Both open and closed chamber methods deliver reliable TEWL measurements (De Paepe *et al.*, 2005), which according to Darlenski *et al.* (2009) correlate significantly with absolute skin water loss rates, assessed gravimetrically.

The Vapometer (Delfin, Finland) used in this study is a closed chamber method. This method works on the principal that water vapour lost from the skin accumulates inside the closed chamber when applied directly to the skin. A hygrosensor fitted inside the chamber then measures the percentage change in relative humidity over time and calculates the TEWL (Sotoodian and Maibach, 2012).

A problem associated with the use of closed chamber methods, however is the potential occlusion of the skin. This can easily be compensated for by shortening the measurement time, such as the case with the Vapometer (Darlenski *et al.*, 2009).

2.7.5.3 SKIN SURFACE pH

Skin surface pH can be measured by making use of pH-sensitive dyes (Darlenski *et al.*, 2009) or flat glass electrodes fitted to a potentiometer (Waller and Maibach, 2005; Fluhr *et al.*, 2006; Darlenski *et al.*, 2009). The latter is more commonly utilised (Schreml *et al.*, 2010) due to its ease of use, simplicity and reproducibility (Darlenski *et al.*, 2009). The functioning of these instruments is based on the potential difference created over a thin glass slide separating the skin surface and the solution inside the glass electrode (Waller and Maibach, 2005).

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CHAPTER 3

ARTICLE

ARTICLE

Traditionally, occupational exposure assessment focused on inhalation as route of exposure. As a result, reports on dermal exposure assessment are scant and according to our knowledge occupational dermal exposure to copper sulphate has not yet been characterised. The dermal exposure of workers at a chemical industry producing copper sulphate was therefore assessed. In addition, it was decided to characterise the change in the workers' skin barrier function from before the work shift to the end by measuring certain skin parameters.

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In conclusion the following should be taken into consideration:

- Throughout the article copper sulphate and copper are used interchangeably, but would refer to the same substance as the copper detected was primarily in the form of copper sulphate.
- The questionnaires that were presented to the workers, whether referred to in this study or not, are captured in Annexures A, B and C.
- The designated areas on the workers' palm, back of hand, wrist and forehead from which skin barrier function measurements and dermal samples were taken are indicated in Annexure D.

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or Jones *et al.* (1995) if there are more than two authors. For example: Jones and Brown (1995) observed total breakdown of control...or

Total breakdown of control has sometimes been observed (Jones and Brown, 1995).

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DERMAL EXPOSURE AND SKIN BARRIER FUNCTION OF WORKERS EXPOSED TO COPPER SULPHATE AT A CHEMICAL INDUSTRY

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ABSTRACT

Objectives: The objectives of this study were to assess the dermal exposure of workers at a chemical industry to copper sulphate and to characterise the change in their skin barrier function from before the onset of the work shift to the end through measuring skin hydration, transepidermal water loss (TEWL) and skin surface pH. **Methods:** Skin hydration, TEWL and skin surface pH measurements were taken before and at the end of the work shift from the palm (except for TEWL), back and wrist of workers, from three different work categories', dominant hand as well as their foreheads. Commercial GhostwipesTM were used to collect dermal exposure samples from the same four anatomical areas before and at the end of the shift. Additional dermal exposure samples were collected from the palm and back of hand, prior to break 1 and 2. Surface wipe sampling was also conducted at several work and recreational areas of the chemical industry. Wipe samples were analysed by an accredited analytical laboratory, according to NIOSH method 9102 by means of Inductively Coupled Plasma-Atomic Emission Spectrometry. **Results:** In general the skin hydration of the reactor and powder packaging workers decreased by the end of the work shift, whilst that of the crystal packaging workers increased. With a few exceptions, TEWL of the workers increased and skin surface pH decreased. All anatomical areas of workers from the different work categories experienced dermal copper exposure throughout the shift with mean dermal exposures ranging from 0.14 $\mu\text{g}/\text{cm}^2$ (reactor workers, forehead, end of shift) to 18.11 $\mu\text{g}/\text{cm}^2$ (crystal packaging workers, palm, end of shift). All areas from which surface samples were taken, were contaminated with copper sulphate. **Conclusion:** The changes in skin barrier function of the workers were in part secondary to their dermal exposure to sulphuric acid and possibly copper sulphate, which was mainly contributed to inadequate protective gloves and due to secondary skin contact with contaminated surfaces. The decreased skin barrier function and skin surface pH of workers can lead to enhanced dermal permeation, which in turn may result in further skin barrier deterioration. The low account of skin irritation or reaction incidences was contributed to workers' ethnicity as well as to the low sensitisation potential of copper. Recommendations on how to lower dermal exposure and improve workers skin barrier function were made.

INTRODUCTION

Unlike other metal compounds such as nickel, chromium and cobalt, copper is not a well-recognised skin sensitiser (Forte *et al.*, 2008) and has no skin notation indicating that it should not contribute significantly to systemic toxicity (Sartorelli *et al.*, 2007). However, contact dermatitis, allergies and skin problems have been reported following dermal and systemic exposure to copper (Rademaker, 1998; Wöhrl *et al.*, 2001; Hostýnek and Maibach, 2004a; Ellingsen *et al.*, 2007; Forte *et al.*, 2008). Copper is therefore labelled a rare allergen (Wöhrl *et al.*, 2001) and cause of allergic (Forte *et al.*, 2008) and irritant contact dermatitis (Hostýnek and Maibach, 2004b).

As the largest organ (Winder, 2004; Crosera *et al.*, 2009) and “envelope” of the human body, (Hostýnek and Maibach, 2006) the skin is often exposed to mechanical, chemical, physical and biological hazards (Todd and Carman, 2001). Upon skin contact, chemicals can penetrate the skin’s primary barrier known as the stratum corneum (SC) (Proksch *et al.*, 2008; Kezic and Nielsen, 2009), to cause local toxic (irritant) damage, allergic reactions or contribute to systemic toxicity (Todd and Carman, 2001; Du Plessis *et al.*, 2010). Notwithstanding the previous, it was only recently that the skin was recognised as a significant route of exposure (Hostýnek and Maibach, 2006; Hostýnek *et al.*, 2006) and since then dermal exposure assessment has gained increased interest (Lidén *et al.*, 2008).

Numerous methods have been developed to assess dermal exposure (Soutar *et al.*, 2000; Badenhorst *et al.*, 2007; Du Plessis *et al.*, 2008; Du Plessis *et al.*, 2013a) and were summarised by Du Plessis *et al.* (2010) as interception methods, removal of the contaminant methods and detection methods.

Dermal exposure studies, especially on liquid pesticides, have been performed extensively (Du Plessis *et al.*, 2010), and lately some focus has fallen on dermal exposure to metals. Studies based on metals such as antimony, beryllium, chromium, cobalt, lead, nickel and zinc have been reported (Day *et al.*, 2007; ICMM, 2007; Lidén *et al.*, 2008; Day *et al.*, 2009; Du Plessis *et al.*, 2010; Hughson *et al.*, 2010; Julander *et al.*, 2010; Du Plessis *et al.*, 2013a), yet “contact by copper and its compounds with the skin potentially represent a route of exposure that is not well characterized” (Hostýnek and Maibach, 2006).

On skin contact copper is oxidised by skin exudates, sebum and sweat, (Hostýnek and Maibach, 2004a; Hostýnek and Maibach, 2006; Forte *et al.*, 2008) resulting in the formation of lipophilic soaps and hydrophilic ionised salts (Hostýnek and Maibach, 2004a; Hostýnek and Maibach, 2004b; Hostýnek and Maibach, 2006). These compounds are able to penetrate the skin barrier via three penetration pathways: the intercellular route, the trans cellular route across cornified cells and lipid bilayer as well as the shunt route where they diffuse along the hair follicles and sweat glands (Hostýnek and Maibach, 2004a; Hostýnek and Maibach, 2006). The permeation of copper through the skin is said to be time dependent with a permeability coefficient in the order of 10^{-6} cm/h (Hostýnek and Maibach, 2006).

Skin permeation is, however, very complex and is affected by numerous intrinsic and extrinsic factors such as contaminant physico-chemical properties, exposure conditions, as well as the state of the skin barrier (Cohen and Rice, 2003; Winder, 2004; Sartorelli *et al.*, 2007; Kezic and Nielsen, 2009). An impaired skin barrier function may not only lead to enhanced SC penetration and penetration of substances otherwise not possible through intact skin, but may also be indicative of skin damage or reactions secondary to chemical exposure (Nielsen, 2005; Nielsen *et al.*, 2007; Kezic and Nielsen, 2009).

Skin hydration and TEWL are two parameters often used to quantify skin barrier function (Du Plessis *et al.*, 2013b), where skin hydration indicates the skin's surface moisture level (Du Plessis *et al.*, 2010) and TEWL represents the quantity of condensed water that passively diffuses through the SC (Agache and Black, 2004; Imhof *et al.*, 2009; Du Plessis *et al.*, 2013b). In addition to these mentioned parameters, Darlenski *et al.* (2009) stated that the quantification of skin surface pH is essential for the integral evaluation of skin barrier function as it supports epidermal barrier homeostasis, controls the presence of resident and transient skin micro flora, and regulates corneocyte desquamation (Waller and Maibach, 2005, Hostýnek and Maibach, 2006; Lambers *et al.*, 2006; Rawlings *et al.*, 2008; Darlenski *et al.*, 2009, Du Plessis *et al.*, 2013a).

In 2010 Du Plessis *et al.* reported skin barrier function measurements (skin hydration and TEWL) in conjunction with the dermal exposure of base metal refinery workers to nickel species (Du Plessis *et al.*, 2010). Since then international guidelines for the *in vivo* assessment of TEWL,

skin hydration and pH in non-clinical settings have been developed (Du Plessis *et al.*, 2013b; Stefaniak *et al.*, 2013) and the assessment thereof in conjunction with dermal exposure measurements are becoming the norm (Du Plessis *et al.*, 2013b).

The objectives of this study were to assess the dermal exposure of workers at a chemical industry to copper sulphate, as well as to characterise the change in their skin barrier function by measuring skin hydration, TEWL and skin surface pH.

METHODS

Workplace description

The study was performed at a chemical industry based in South Africa. The chemical industry produces copper sulphate by means of a chemical method from scrap copper obtained from various other industries including the mining sector. Before entering a steam-powered chemical reactor filled with sulphuric acid (H₂SO₄), copper is cut into smaller pieces by a guillotine on site. Once inside the reactor, the metallic copper dissolves to form copper sulphate in reaction with sulphuric acid. The fluid based copper sulphate is then pumped from the reactor to the crystallisation area where it is left to dry. On completion of the drying process, the copper sulphate is packed in crystal, or after further processing, powder form.

Thirteen workers with the perceived highest exposure risk were identified, and worked either in the reactor, crystal packaging or powder packaging areas. The workers, all African males, gave informed consent to partake in the study that stretched over a time period of four days. Exposure controls at the chemical industry were in the form of personal protective equipment only, and no engineering or administrative measures were in place. Personal protective equipment (PPE) used by these workers differed according to their tasks at hand, but generally consisted of long sleeve overalls, hard hats, polyvinyl chloride (PVC) chemical protective gloves, FFP2 respirators, ear plugs and protective eye wear.

Skin barrier function measurement

Changes in skin barrier function of the workers were determined by measuring skin hydration, TEWL and skin surface pH in accordance with the international guidelines for the *in vivo* assessment of skin properties in non-clinical settings (Du Plessis *et al.*, 2013b; Stefaniak *et al.*, 2013). Skin hydration and skin surface pH were measured by using the Derma Unit SSC 3 (Courage Khazaka electronic GmbH, Köln, Germany) fitted with a hydration and pH probe respectively. A Vapometer (Delfin, Finland) was used to measure TEWL. All above-mentioned equipment and measuring instruments were used, stored and calibrated in accordance with manufacturers' instructions. Designated areas on the palm (except for TEWL), back and wrist of workers' dominant hand, as well as their foreheads were measured before the onset and at the end of the work shift. Due to time constraints two skin barrier function measurements instead of 3, as recommended by the guidelines (Du Plessis *et al.*, 2013b; Stefaniak *et al.*, 2013), were taken to determine the average value per anatomical area.

Due to the fact that skin parameters (skin hydration, TEWL and skin surface pH) are influenced by numerous factors and consensus with regard to reference values for normal skin are lacking, results are reported as percentage change relative to before shift measurements and not as change in absolute values.

Skin condition questionnaire

A validated questionnaire regarding skin condition, developed by Dalgard *et al.* (2003), was given to the workers in both their native language Setswana and English to complete. This questionnaire indicates the subjective risk the workers have for developing skin diseases and consists of 10 questions. The answers of the questions were scored on a scale from 1-4: 1 being no; 2: yes, a little; 3: yes, quite a lot; and 4: yes, very much. The mean of the answers was calculated and a score above 1.3 indicated an increased risk for developing skin diseases.

Additional questions were presented to the subjects regarding personal factors and basic hygiene that could possibly be related to the occurrence of dermatitis and other skin problems. Van Wendel De Joode *et al.* (2007) previously used these and other questions in a study.

Dermal sampling

A removal method similar to that described by Du Plessis *et al.* (2013a) was used to assess the workers' dermal exposure to copper. Commercial moist wipes (Ghostwipes™) were used to wipe four consecutive times over a predetermined anatomical sampling area restricted by an acetate template with an open aperture of 10 cm². Sampling was done in a horizontal direction folding the exposed side of the wipe inward after each wipe.

Using one wipe for each sample taken, skin wipe sampling was conducted four times daily: before the shift, prior to tea (break 1) and lunch break (break 2) and at the end of the shift. Baseline and end of the shift samples were taken from the workers' palm, back and wrist of dominant hand as well as their foreheads. The samples prior to the tea and lunch breaks were taken only from the palm and back of the dominant hand.

Twelve dermal samples were collected from each worker, as well as 1.25 field blanks per day, to make provision for any adventitious contamination that might have occurred. Field blanks were handled in the same manner as the dermal exposure samples, except for being wiped over the workers' skin.

Samples were collected by the same researcher, hence applying similar pressure, whilst wearing a new pair of disposable vinyl gloves and making use of a clean acetate template. On completion Ghostwipes™ were placed in individually labelled storage vials, and sent to a laboratory accredited for such purposes of analysis.

Dermal exposure was expressed as skin surface loading (micrograms of copper per square centimetre of skin) after all masses had been blank corrected. Where the mean mass of copper on the blanks were higher than detected on the skin's surface, skin surface loading was considered to be zero.

Surface sampling

On each of the sampling days, three surface areas likely to be contaminated with copper, and with which workers were expected to come into contact were selected for wipe sampling. A total

of twelve surface contamination samples were taken from different working and recreational areas at the chemical industry.

The surface samples were collected, according to the manner described by Badenhorst (2007), using commercial moist wipes (Ghostwipes™) and acetate templates with an open aperture of 100 cm². As with the dermal sampling, the same researcher, wearing a clean pair of vinyl gloves and using a clean template conducted all the surface sampling.

In the case of irregularly shaped surfaces, a similar sampling strategy was followed with the exception of the use of acetate templates.

All samples were individually packed in storage vials, and sent for analysis.

After being blank corrected results were expressed in micrograms per square centimetre, and micrograms per sample for flat and irregularly shaped surfaces respectively.

Analysis of dermal and surface samples

Dermal and surface wipe samples were analysed according to NIOSH method 9102 by an accredited analytical laboratory, using Inductively Coupled Plasma-Atomic Emission Spectrometry. The limit of detection for copper in the analysis used by the laboratory is 0.57 µg.

Dermal and surface sampling method validation

The Occupational Safety and Health Administration's method ID-125G (OSHA, 2002) reported the overall efficiency of Ghostwipes™ in the surface sampling of metals.

The sampling efficiency for copper with Ghostwipes™ averaged at 96.15% and was obtained by liquid spiking six glass templates with a known amount of copper. After a drying period, a single wipe was wiped three consecutive times over the glass surface. The sampling method was also found to be reproducible with an average 3.90% difference in copper recovery when executed by two different operators. The analytical method had 99.60% efficiency in the recovery of copper.

The authors acknowledge the fact that the sampling efficiency of this method from the skin and other surfaces from which samples were taken, can differ from that of the test surface glass.

However, in order to uphold the highest sampling efficiency, the GhostwipesTM were wiped four consecutive times over the workers' skin, and numerous times over the surface sampling areas contrary to the three times during the study by OSHA (2002). The above mentioned should therefore be taken into consideration when interpreting the dermal and surface sampling results.

Statistical analysis

The results obtained were statistically analysed by using Statistica Version 11 (Statsoft Inc., 2013) and SPSS Statistics Version 18.0 (SPSS, 2009).

Descriptive statistics of the results were summarised in terms of number of measurements (n), arithmetic mean (Mean), standard deviation (SD) and range (maximum and minimum values) and were determined by using Microsoft Excel 2003. The data were tested for normality and log-transformed where necessary.

Repeated measures analysis (ANOVAs) with a Tukey HSD or Bonferroni post hoc test or dependant sample t-tests were used to determine any statistically significant effect of anatomical area, measurement time and their interaction on dermal exposure results. Due to the small number of skin barrier function measurements, non-parametric Friedman ANOVAs were used to analyse the skin barrier function data. Results with a p value ≤ 0.05 were considered statistically significant.

Spearman's rank order correlations coefficient were obtained to evaluate relationships among the workers' dermal exposure, skin condition score and the number of years they have been employed. This particular coefficient is a non-parametric correlation coefficient and does not rely on assumptions such as normality (Field, 2009), which makes it more appropriate than repeated measures analysis. Due to the small sample size, the correlation coefficients were considered with caution, and therefore Scatter plots were also explored. Results with an r value of approximately 0.5 were considered to indicate a practically significant correlation.

RESULTS

The thirteen workers monitored during the course of the study had been working at the chemical industry for an average of 4.47 ± 5.13 years. Three workers were employed at the reactor area of the chemical industry and of the remaining ten workers, five performed their daily tasks at each of the crystal and powder packaging areas.

The study was performed during late winter in a non-climate controlled environment where climatic conditions were similar to that inside the working areas.

Mean percentage change in skin hydration of the different anatomical areas and between the work categories varied noticeably (Fig.1a) with a statistically significant difference ($p = 0.041$) in percentage change between the four anatomical areas. In addition there was a large variation in percentage change in skin hydration amongst workers within the same work category. By the end of the work shift skin hydration of the reactor workers' palm, back of hand and wrist decreased to values 2.79%, 16.71% and 37.62 % lower than the initial hydration levels before the shift, whilst the forehead skin hydration increased by 18.65%. In general the crystal packaging workers' skin hydration increased from the onset of the work shift, with the palm 29.38%, back of hand 11.75%, wrist 14.75% and forehead 39.93% more hydrated by the end of the shift. With the exception of a 14.27% increase in the palm's skin hydration, the skin hydration of the powder packaging workers decreased by 28.13% for the back of hand, 26.10% for the wrist and 10.02% for the forehead by the end of the shift.

Percentage change in TEWL of workers was highly variable (Fig.1b), but in general TEWL of the back of hand and wrist increased while the foreheads' TEWL decreased by the end of the work shift. Statistically significant differences were found to exist between the change in TEWL of the different anatomical areas of all work categories ($p = 0.001$), and between that of the anatomical areas of the crystal packaging workers ($p = 0.015$). Mean TEWL of the back of hand increased by 63.67% for the reactor worker, 69.57% for crystal packaging workers and 108.22% for powder packaging workers. With the exception of a 22.51% decrease in TEWL for the powder packaging workers, wrist TEWL of the reactor and crystal packaging workers increased by 11.27% and 56.05% respectively. The decrease in forehead TEWL by the end of the work shift ranged from 10.44% (powder packaging workers) to 68.22% (reactor worker).

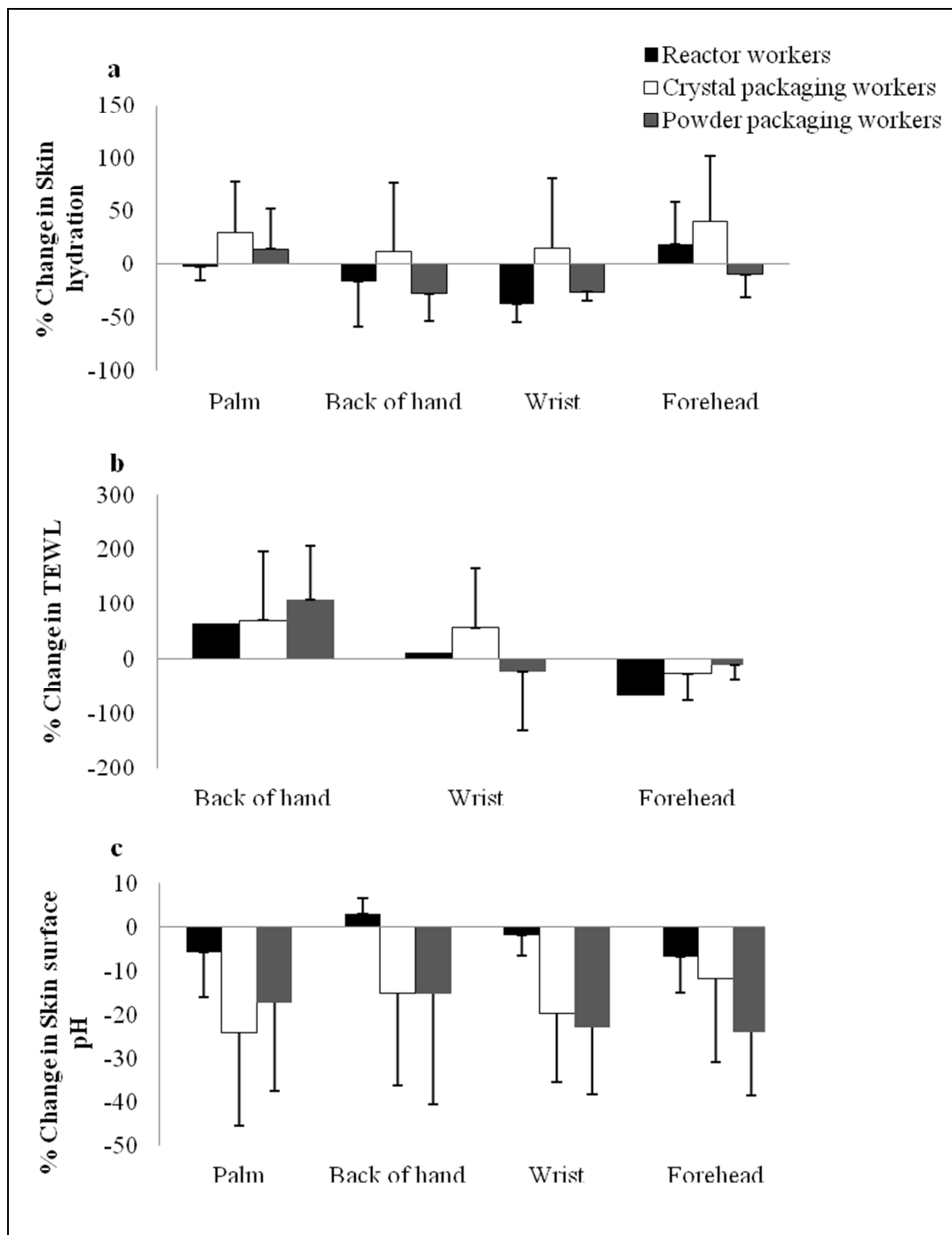


Fig. 1. Percentage change in a) skin hydration, b) TEWL and c) skin surface pH from before to the end of the work shift (mean \pm SD) as measured on the palm (not TEWL), back of hand, wrist and forehead of reactor workers (n=3 for skin hydration and skin surface pH and n=1 for TEWL), crystal packaging workers (n=5) and powder packaging workers (n=5).

With the exception of the back of hand of reactor workers, skin surface pH of all the work categories and anatomical areas decreased by the end of the work shift (Fig.1c). Decreases in skin surface pH ranged from 1.75% (wrist) to 6.72% (forehead) for the reactor workers, 11.76% (forehead) to 24.04% (palm) for crystal packaging workers, and from 15.21% (back of hand) to 23.90% (forehead) for the powder packaging workers.

All of the reactor (n=3) and powder packaging workers (n=5) as well as four of the crystal packaging workers reported that they experienced some or other skin problem, where in 61.54% of the cases the onset of the skin problem took place in the last six months.

The reactor workers, crystal and powder packaging workers' mean skin condition scores were 1.3 ± 0.1 , 1.5 ± 0.4 and 2.1 ± 0.4 respectively and ranged from 1 to 2.7. Only one each of the reactor and crystal packaging workers had a score below 1.3, thus indicating an increased risk for the development of skin diseases amongst the majority of workers.

A practically visible negative correlation of $r = -0.398$ was found between the number of years the workers have been employed and their skin condition score. The correlation was, however, not statistically significant with $p > 0.05$ and when explored by means of a scatter plot, no correlation was found (Scatter plot not included in results). A practically significant positive correlation of $r = 0.747$ ($p = 0.01$) was found to exist between the workers' skin condition score and their mean dermal exposure to copper throughout the work shift.

As indicated in Table 1, traces of copper were detected on all four of the workers' anatomical areas before the shift commenced.

With the exception of the reactor workers' forehead exposure, it is evident that the workers were exposed to an increasing load of copper sulphate as the work shift progressed. The before and end of shift forehead exposures of all the work categories differed significantly ($p = 0.003$) as well as the before and end of shift wrist exposure of the crystal packaging workers' ($p = 0.011$).

Table 1. Copper as sampled from the skin surface of reactor workers (n=3), crystal packaging workers (n=5) and powder packaging workers (n=5), and analysed according to NIOSH method 9102.

			Skin surface loading ($\mu\text{g}/\text{cm}^2$)			
			Mean	SD	Minimum	Maximum
Reactor workers	Palm	Before shift	1.03	0.27	0.27	1.32
		Break 1	1.36	1.07	0.56	2.58
		Break 2	1.01	0.83	0.50	1.96
		End of shift	1.61	1.45	0	2.83
	Back of hand	Before shift	0.23	0.24	0	0.48
		Break 1	0.24	0.14	0.10	0.37
		Break 2	1.23	1.32	0.25	2.73
		End of shift	1.32	1.14	0.65	2.63
	Wrist	Before shift	0.94	0.27	0.27	1.17
		End of shift	0.72	0.75	0	1.49
	Forehead	Before shift	0.16 ^a	0.28	0	0.49
		End of shift	0.14 ^a	0.14	0	0.27
Crystal packaging workers	Palm	Before shift	0.82	0.95	0	1.87
		Break 1	8.06	8.60	1.08	22.88
		Break 2	11.59	5.41	2.40	16.38
		End of shift	18.11	23.97	1.93	60.55
	Back of hand	Before shift	0.29	0.34	0	0.72
		Break 1	1.03	0.74	0.37	2.20
		Break 2	1.91	0.97	1.05	3.24
		End of shift	3.00	1.31	1.27	4.07
	Wrist	Before shift	0.88 ^d	0.70	0.07	1.50
		End of shift	4.70 ^d	2.32	1.25	7.60
	Forehead	Before shift	0.40 ^b	0.68	0	1.60
		End of shift	2.06 ^b	1.38	0.63	4.35

Table 1 – continued.

			Skin surface loading ($\mu\text{g}/\text{cm}^2$)			
			Mean	SD	Minimum	Maximum
Powder packaging workers	Palm	Before shift	1.86	1.73	0.02	4.44
		Break 1	7.75	6.63	1.29	18.84
		Break 2	8.08	1.09	6.59	9.19
		End of shift	13.09	10.07	1.09	25.05
	Back of hand	Before shift	1.57	1.60	0.24	4.25
		Break 1	3.17	4.20	0.52	10.63
		Break 2	2.92	1.81	0.63	5.54
		End of shift	13.82	17.31	1.45	43.48
	Wrist	Before shift	2.76	2.59	0.36	6.29
		End of shift	11.54	9.97	1.74	24.18
	Forehead	Before shift	0.64 ^{c,e}	0.76	0	1.86
		End of shift	2.65 ^{c,e}	1.29	1.21	3.78

a-c indicate statistically significant differences as determined by repeated measures ANOVAs with Bonferroni post-hoc test. The d and e indicate statistical significance as determined by dependent sample *t*-tests.

The mean concentration of copper sampled from the hands and wrists of the reactor workers, crystal and powder packaging workers at the end of the work shift were $1.22 \pm 1.01 \mu\text{g}/\text{cm}^2$, $8.56 \pm 14.17 \mu\text{g}/\text{cm}^2$ and $12.82 \pm 11.59 \mu\text{g}/\text{cm}^2$ respectively, making the powder packaging workers the work category most exposed to copper and the reactor workers the least.

Reactor workers were subjected to mean copper concentrations ranging from $0.14 \mu\text{g}/\text{cm}^2$ (forehead, end of shift) to $1.61 \mu\text{g}/\text{cm}^2$ (palm, end of shift), crystal packaging workers from $0.29 \mu\text{g}/\text{cm}^2$ (back of hand, before shift) to $18.11 \mu\text{g}/\text{cm}^2$ (palm, end of shift) and powder packaging workers to concentrations between $0.64 \mu\text{g}/\text{cm}^2$ (forehead, before shift) and $13.82 \mu\text{g}/\text{cm}^2$ (back of hand, end of shift).

Copper was detected on all surfaces selected for wipe sampling. Samples were taken from the laboratory and product storage workbenches as well as a tea room table and the telephone bench.

An average of $5.52 \pm 0.10 \mu\text{g}/\text{cm}^2$ and $4.91 \pm 3.92 \mu\text{g}/\text{cm}^2$ were detected on these flat surfaces at the plant's working (n=2) and recreational (n=2) areas respectively. Copper sampled from irregular surfaces at working areas (n=6) ranged from 153.68 μg (control handle of centrifuge conveyor) to 825.68 μg (control handle at pressure loading area) per sample with an average of 519.77 μg per sample, where samples taken at the recreational areas (n=2) ranged from 175.75 μg (handle of urn) to 563.53 (door handle inside change room) μg per sample with an average of 369.64 μg per sample.

DISCUSSION

Various endogenous, exogenous, environmental and measurement related factors have an influence on the parameters used to assess skin barrier function (Du Plessis *et al.*, 2013a). These were summarised by Darlenski *et al.* (2009), Du Plessis *et al.* (2013b) and Stefaniak *et al.* (2013) as age, gender, race, anatomical site, skin temperature, sweating, circadian rhythms, skin disease, skin washing, wet work, the use of topical products, contact with solvents, detergents, and irritants, occlusion, mechanical skin damage, smoking and caffeine consumption, air convection, ambient temperature, relative humidity, season and the presence of direct sunlight.

In clinical studies it is easy to rule out the impact of some of these factors on skin barrier function by following manufacturers' instructions of measuring equipment and making use of exclusion criteria for subjects (Du Plessis *et al.*, 2013a). Workplace studies, however, pose numerous challenges for researchers as you have less control over the measurement environment, the age, gender and race of the study population, the behaviour of the subjects and have to deal with time constraints (Kütting *et al.*, 2010; Du Plessis *et al.*, 2013b; Stefaniak *et al.*, 2013;). As a result Du Plessis *et al.* (2013b) and Stefaniak *et al.* (2013) developed international guidelines for the *in vivo* assessment of transepidermal water loss, skin hydration and pH in non-clinical settings. These guidelines require the researchers to report skin barrier function measurements not as change in absolute values but as percentage change relative to baseline values. In accordance with the guidelines, the percentage change in skin hydration, TEWL and skin surface pH in conjunction with dermal copper exposure measurements are discussed.

In 2013a, Du Plessis *et al.* reported the change in skin barrier function of workers exposed to cobalt and nickel at a base metal refinery. Similar to Du Plessis *et al.* (2013a) the percentage change in skin hydration of the chemical industry workers was highly variable. The palm, back of hand and wrist of the reactor workers and the back of hand, wrist and forehead of the powder packaging workers decreased by the end of the work shift by as much as 37.62%. In contrast the skin hydration of all anatomical areas of the crystal packaging workers increased.

In a different study, Du Plessis *et al.* (2010) stated that the skin hydration of base metal refinery workers decreased during the work shift, but recovered to just above baseline values by the end of the shift. The increase in skin hydration of the crystal packaging workers was, however, much greater with increases ranging from 11.75% for the wrist to 39.93% for the forehead. In turn this increased skin hydration was lower than the 133.33% reported by Du Plessis *et al.* (2013a).

The incidence of the decreased skin hydration of certain workers can probably be attributed to the workers' dermal exposure to sulphuric acid. When asked the majority of the workers also did not make use of any moisturising agents, which coincides with the statement by Du Plessis *et al.* (2010) that from an economic perspective, skin care is not a high priority amongst African industrial workers. The lack of proper skin care could therefore have aggravated the situation even further. The increase in crystal packaging workers' skin hydration may be the result of occlusion of the skin by PPE, but given the fact that all work categories made use of the same basic PPE, this is unlikely.

An increase in TEWL generally indicates a disruption in skin barrier function. With the exception of the forehead, TEWL of the back of hand and wrist in general increased by the end of the working shift. This correlates with the increased TEWL values of base metal refinery workers reported by Du Plessis *et al.* (2010) and Du Plessis *et al.* (2013a). The increase in TEWL of the hands and wrist can be linked to chemical and mechanical damage caused by dermal exposure to acidic compounds and dermal friction due to hard physical labour. Occlusion of the skin by protective gloves, thus restricting evaporation of moisture from the skin surface, could also have added to the deterioration of the hands' skin barrier function (Todd and Carman, 2001; Cohen and Rice, 2003; Hostýnek and Maibach, 2004a; Kezic and Nielsen, 2009).

With the exception of the back of hand of the reactor workers, the pH of the workers' skin surface decreased by the end of the work shift. This is in accordance with the decrease in skin surface pH of all the base metal refinery workers previously reported (Du Plessis *et al.*, 2013a). Circadian rhythm is known to exercise an impact on skin surface pH levels during the course of the day (Darlenski *et al.*, 2009; Schreml *et al.*, 2010). Lambers *et al.* (2006) and Schreml *et al.* (2010) reported peak skin surface pH values in the afternoon and low pH values in the evening. Results of the survey were contradictory to the above and the change in the workers' skin surface pH can therefore not be attributed to circadian rhythm. The decrease in skin surface pH is most likely due to the low pH of sulphuric acid to which workers are exposed. Although a decreased skin surface pH is said to give resistance to irritant dermatitis (Lambers *et al.*, 2006), a drastic decline in skin surface pH might evoke it. In addition Stefaniak *et al.* (2013) stated that the dissolution and/or partitioning of contaminants in contact with the skin can be influenced by changes in skin surface pH, which may result in increased dermal contaminant permeation. Although the dissolution of copper at different skin surface pH levels is unknown, studies by Bastidas *et al.* (2000), Mora *et al.* (2002), Wang *et al.* (2010) and Demirkıran (2013) showed that copper dissolution increases with a decrease in the pH of the solution media. In addition it can be assumed that the dissolution of copper, which is readily oxidised by skin exudates (Hostýnek and Maibach, 2004b; Hostýnek and Maibach, 2006) and which in part regulates skin surface acidity (Stefaniak *et al.*, 2011), will be enhanced at a lower skin surface pH.

Results of the Dalgard questionnaire indicated that the majority of workers experienced some or other skin problem, of which dry and scaly skin was the foremost complaints. The mean average skin condition scores of the reactor workers, crystal and powder packaging workers were 1.3 ± 0.1 , 1.5 ± 0.4 and 2.1 ± 0.4 respectively which correlated in a significant manner with the workers' dermal exposure. This may indicate that in addition to sulphuric acid exposure, the poor skin condition of workers can partially be attributed to dermal copper sulphate exposure. According to Dalgard *et al.* (2003) individuals from a non-healthcare-seeking population with skin condition scores exceeding 1.3 are more likely to develop skin diseases. Although copper rarely causes allergic contact dermatitis (Wöhrl *et al.*, 2001), under favourable conditions, exposure can potentially lead to the sensitisation of individuals (Forte *et al.*, 2008). Thereafter exposures to only a small amount, can cause an allergic response (Stanton and Jeebhay, 2001; Cohen and Rice, 2003). The low account of skin irritation or reaction incidences amongst these

workers can be contributed to the workers' ethnicity as well as to the low sensitisation potential of copper. Black skin is said to display reduced sensitivity to chemical or mechanical irritation when compared with Caucasian and East Asian skin (Muizzuddin *et al.*, 2010) and is also less prone to allergic contact dermatitis (Dogliotti, 1970).

Commercial moist wipes were successfully used in the collection of skin surface samples by Hughson and Cherrie (2005), Hughson *et al.* (2010) and specifically GhostwipesTM by Du Plessis *et al.* (2010) and Du Plessis *et al.* (2013a).

Copper samples were taken from the palm, back of hand, wrist and forehead of workers' skin before the shift started and periodically thereafter. A significant amount of copper was detected before the shift started which in general increased as the work shift progressed.

Copper that was found before the shift work commenced could be due to airborne particulates that settled on the workers' skin surface whilst they travel through some production areas of the plant to reach the change room. Surface sampling on the interior handle of the change room yielded 563.59 μg of copper which demonstrates that contamination on this and probably other surfaces of the change room could also be a contributing factor to before shift exposure. Another possibility is that workers put on unwashed, contaminated overalls, as an onsite laundry service is not provided.

The increased amount of copper sampled from the workers' skin during the shift is amongst others a result of inadequate chemical protection provided by their PVC protective gloves. The usage thereof is also only enforced when workers are said to be "in direct" contact with the product. Secondary exposure can therefore contribute significantly to dermal loading as averages of 5.52 $\mu\text{g}/\text{cm}^2$ and 519.77 μg of copper were detected on respectively flat and irregular surfaces inside the plant working areas.

The intermitted use of protective gloves by the workers can also elevate dermal penetration (Cohen and Rice, 2003; Winder, 2004), as the gloves trap copper sulphate against the skin and prevents the evaporation of sweat from the skin. In addition to a resulting decreased skin barrier function, copper can more readily be oxidised in the presence of a significant amount of skin

exudates, enabling the resulting ions to penetrate the skin's SC (Hostýnek and Maibach, 2004b; Hostýnek and Maibach, 2006).

During the survey, it was observed that once tea and lunch breaks occurred, the majority of workers moved directly from the plant working areas to the tea room, neglecting to wash their hands. The 175.75 µg of copper sampled from the urn's handle and 2.17 µg/cm² from the surface of a table inside the tea room point to possible worker-to-tea room contamination. The latter is also disquieting as it can lead to unwanted exposure in the tea room which is perceived to be a clean area where workers consume their food. Although copper has a low systemic toxicity, it along with other more toxic chemicals, can be ingested via the hand to mouth shunt when workers eat food with contaminated hands.

In general the highest concentrations of copper were sampled from the workers' skin at the end of the shift. Although shower facilities are available to the workers, only a few individuals made use of it. The remaining ones, therefore, would leave the workplace with a significant amount of dermal copper loading, which unknowingly can lead to the exposure of other and more sensitive individuals. In addition, this can also be an explanation for the detection of copper on the skin of workers before commencement of the shift. The afore mentioned is also a cause of concern as an *in vivo* study by Hostýnek *et al.* (2006) found the concentration of copper penetration through the SC to increase in a time dependent fashion.

In general dermal loading were the greatest for the powder packaging workers while the reactor workers were the least exposed. Reactor workers are mainly required to sample copper sulphate during the reactor phase, and conduct various tests inside a laboratory. They are therefore primarily exposed to small quantities of copper. Powder packaging workers are involved with the processing of copper sulphate from crystal to powder form as well as the bagging thereof. During processing the copper sulphate is dried and milled, leading to higher exposure than that of the crystal packaging workers that are required to work with a wetted product which is less likely to become airborne.

Skin surface loading results also showed the exposure of the workers' palm > back of hand > wrist > forehead, with the palm's exposure nearly twice that of the back of hand. Cohen and Rice (2003) found the SC of the foot sole and palm to be less permeable than the SC of other

anatomical areas such as the forehead. Todd and Carman (2001), however, stated that the thickened SC of the palm can act as a reservoir, increasing the chance of substance penetration, as it enables permeation of the absorbed substances long after initial exposure (Hostýnek *et al.*, 2006).

Some workers showed evidence of a disrupted skin barrier and this caused them to be more susceptible to both local and systemic toxicity, as an impaired skin barrier may not only lead to increased SC penetration, but also to the penetration of substances that could not have penetrated through intact skin (Nielsen, 2005; Nielsen *et al.*, 2007; Kezic and Nielsen, 2009). The extent of copper penetration through damaged skin is unknown, yet another heavy metal, nickel, showed an 84.87 fold increase in penetration through damaged skin when compared to intact skin (Larese Filon *et al.*, 2009)

CONCLUSION

In general the change in skin hydration of workers was highly variable, while TEWL increased by the end of the work shift and skin surface pH decreased. The increase in TEWL and decrease in skin surface pH can mainly be attributed to the workers' occupational exposure to sulphuric acid. As a result of intermitted use of inadequate chemical protective gloves, copper deposits were detected on the hands and wrists of workers. Surfaces inside the plant's working as well as recreational areas were found to be contaminated with copper sulphate, which in addition can be a contributing factor to the copper levels sampled from the workers' skin. The impaired barrier function and change in skin surface pH of certain workers' skin could lead to increased dermal penetration and subsequently to further skin barrier deterioration. The low account of skin irritation or reaction incidences is in part due to the ethnicity of the workers, but also bears testimony to the fact that copper rarely leads to allergic reactions of any significance.

In order to lower dermal exposure of workers and hence improve the condition and barrier function of their skin it was recommended that extraction ventilation at/above the chutes of the crystal and especially the powder bagging areas be installed. It was also recommended that attention be alerted to the improvement of cleanliness, *i.e.* at the plant's work and recreational

areas as well as with regard to personal hygiene amongst the workers. This could be supplemented by the provision of laundry facilities that would ensure workers put on clean overalls on a daily basis. Gloves with chemical and mechanical protective capabilities were recommended to minimise skin contact with the hands in particular, which in turn would reduce further contamination of other skin surfaces. Workers could furthermore be supplied with a suitable moisturising lotion to alleviate dry skin and improve and support the skin's protective properties against penetration and absorption of hazardous chemicals through the skin.

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CHAPTER 4

CONCLUDING CHAPTER

CONCLUDING CHAPTER

This chapter is written in conclusion to the mini-dissertation. Firstly, a conclusion to this study will be made, where amongst others the hypotheses as stated in Chapter 1.3 will be addressed. Thereafter recommendations will be made to the chemical industry, where the study was performed, on how to reduce dermal exposure and improve workers' skin barrier function. The limitations of this study as well as suggestions for future studies will then be discussed.

4.1 CONCLUSION

Todd and Carman (2001) stated that due to individual variability and sensitivity, virtually any substance can affect or permeate the skin, and therefore no substance should be excluded from a risk assessment. A study on available literature found copper to be a rare cause of skin irritation and allergic reactions (Wöhrl *et al.*, 2001; Hostýnek and Maibach, 2004; Forte *et al.*, 2008).

Of the many methods developed to assess dermal exposure (Soutar *et al.*, 2000; Du Plessis *et al.*, 2010), skin wipe sampling was proposed as the exclusive technique for the assessment of dermal exposures to metallic compounds (ICMM, 2007).

Making use of commercial GhostwipesTM, skin wipe sampling was conducted with relative ease in order to assess the dermal copper exposure of thirteen workers at a chemical industry that produces copper sulphate.

Copper was detected on the palm, back of hand, wrist and forehead of workers from all work categories before the commencement of the shift and in general such deposits were found to increase progressively throughout the day. Of the work categories the powder packaging workers proved to suffer the worst exposure and of the anatomical areas the palm revealed the most extensive dermal loading. A mean amount of 12.37 $\mu\text{g}/\text{cm}^2$ copper was sampled from the palm of workers at the end of the shift while approximately 50% lower copper levels were collected from the back of hand and wrist, and even lower levels from the forehead. *Hypothesis 1*, postulating that workers at the chemical industry are exposed to copper sulphate via the dermal exposure route, can therefore be accepted. The dermal exposure of workers was mainly

attributed to the improper usage of and protection provided by the workers' chemical protective gloves, as well as to secondary exposure via contact with contaminated surfaces and clothing.

The barrier function of the skin are amongst the many factors that can greatly influence the permeation of chemical substances through the skin (Cohen and Rice, 2003; Hostýnek, 2003; Winder, 2004; Kezic and Nielsen, 2009). As a result Du Plessis *et al.*, (2013) and Stefaniak *et al.* (2013) developed international guidelines for the *in vivo* assessment of skin barrier function parameters (transepidermal water loss, skin hydration and pH) in non-clinical settings and the assessment thereof in conjunction with dermal exposure measurements is becoming the norm (Du Plessis *et al.*, 2013).

The change in skin hydration of the workers was highly variable, as the skin hydration of reactor and powder packaging workers had mostly decreased and skin hydration of crystal packaging workers had increased by the end of the work shift. With the exception of a decrease in forehead transepidermal water loss (TEWL), TEWL of the back of hand and wrist generally increased, indicating a disruption in their skin barrier function. Skin surface pH for all work categories and on all anatomical areas tested, except the back of hand of the reactor workers, had decreased by the end of the work shift. *Hypothesis 2* carries the assumption that skin barrier function of workers at the chemical industry shows impairment by the end of the work shift, with a decrease in skin hydration and pH levels and an increase in TEWL. According to the results this can be accepted in part only as the changes in skin hydration were inconsistent and there were a few exceptions in the change of TEWL and skin surface pH. Apart from decreasing the skin surface pH, the workers' occupational dermal exposure to sulphuric acid had been, amongst other factors, responsible for the impairment of certain workers' hand and wrist skin barrier function.

Although copper penetration through the skin was described as poor (ATSDR, 2004), studies by Nielsen (2005), Nielsen *et al.* (2007), Kezic and Nielsen (2009) and Larese Filon *et al.* (2009) showed that dermal penetration of chemical substances may significantly increase through an impaired skin barrier. An increase in penetration of chemical substances would not only subsequently lead to further deterioration of the skin barrier (local damage), but would also increase the risk of allergic reactions and systemic toxicity (Todd and Carman, 2001; Du Plessis *et al.*, 2010).

The low incidence report of skin irritation or reactions amongst these workers bears testimony to the fact that copper rarely leads to allergic reactions (Wöhrl *et al.*, 2001) but can also in part be contributed to the workers' ethnicity as Africans are less prone to allergic contact dermatitis (Dogliotti, 1970) and black skin is said to display reduced sensitivity to chemical or mechanical irritation (Muizzuddin *et al.*, 2010).

4.2 RECOMMENDATIONS

The following recommendation are made to the South African based chemical industry on how to lower dermal exposure to copper sulphate and to improve the skin barrier function of workers:

Recommendation 1: Dermal as well as inhalation exposure to copper sulphate can primarily be decreased by means of installing extraction ventilation at/above the chutes where crystal and powder product bagging takes place. The extraction ventilation will prevent dust created by the bagging process from spilling into the working environment, and thereby reduce airborne copper concentrations that can either be inhaled, settle on the skin or on work surfaces potentially resulting in secondary dermal exposure.

Recommendation 2: On arrival at work, workers need to travel through certain production areas to the change room that is located inside the chemical industry. This may lead to dermal copper exposure before the commencement of the shift, as well as thereafter on returning home. Although it might not seem practicable from an economical point of view, it is none the less recommended that the change room be relocated in an area outside the production areas of the industry.

Recommendation 3: Copper was detected on the door handle inside the change room, which workers are required to open manually. It is recommended that an automatic door system be installed, or that this area certainly be cleaned on a daily basis.

Recommendation 4: In addition to the copper detected on the door handle of the change room, copper was detected on various other surfaces inside production as well as recreational areas of the industry. It is recommended that a cleaning schedule be implemented to ensure that plant

areas get cleaned at least weekly and recreational areas, such as the tea room and change room, on a daily basis. After implementation, the effectiveness of the cleaning schedule should be evaluated by means of surface sampling. If detectable amounts of copper are still present on surfaces, the cleaning schedule should be revised to be more frequent.

Recommendation 5: It was observed that when tea and lunch breaks occur, the majority of workers go directly to the tea room, neglecting to wash their hands. It is recommended that adequate hand wash facilities, with soap dispensers, be placed at the workers' disposal directly outside the tea room. In addition signage with instructions on how to wash hands properly should be posted at this area. As mentioned in Recommendation 9, workers should also undergo regular training on the correct manner to wash hands.

Recommendation 6: It was noted that numerous workers enter the tea room wearing their overall jackets, hard hats and other possibly contaminated personal protective equipment (PPE). To prevent contamination of this area, it is recommended that hooks be attached outside the tea room, which is located outside production areas, for the workers to place their PPE.

Recommendation 7: There is no provision for an onsite laundry service, which implies that numerous workers put on contaminated work clothing, which in turn might result in dermal exposure. It is recommended that onsite laundry services be established in order to provide workers with clean overalls on a regular basis. If such facilities are not reasonably practicable, the cleaning of contaminated overalls should be outsourced to a different company.

Recommendation 8: Shower facilities are available to the workers, yet the utilisation thereof is optional. It is recommended to enforce the use thereof by all workers at the end of the work shift. Care should be taken to ensure that there would be an adequate number of showers and that soap is readily available. Contact with contaminated work clothes, and or surfaces should be avoided after the workers have taken a shower.

Recommendation 9: Steps should be taken to ensure that workers receive training on and understand the importance of each of the following aspects:

- The relevance of dermal exposure, and the hazards associated with it in the workplace.

- The correct usage of PPE, especially gloves, and the proper donning and doffing thereof.
- Personal hygiene, for example to wash hands before consuming a meal, and proper housekeeping.
- The proper manner in which to wash hands.

Refresher training should be performed at regular intervals for example during health and safety meetings. In addition signage can be posed at conspicuous places to help stress the importance of the above aspects.

Recommendation 10: Adequate protective gloves, with chemical and mechanical protective capabilities, should be issued to the workers, and the usage thereof enforced at all times. Polyvinyl chloride (PVC), nitrile, neoprene and natural rubber latex gloves would be adequate to provide the proper chemical and to a degree mechanical protection. The usage thereof with an additional cotton liner, to absorb perspiration and add to worker comfort, is recommended. Chemical and mechanical protective gloves with attached cotton flock linings are also readily available. Protective gloves should also be re-issued before chemical breakthrough, as determined by the manufactures, occur or when gloves are damaged, whichever occurs first. Cotton liners should be replaced on a daily basis. Proper donning and doffing, and ensuring that hands are clean when putting on gloves, will prevent the contamination of the insides of gloves and therefore prevent the unnecessary trapping of chemicals against the skin.

Recommendation 11: It is recommended that the workers be supplied with oil, petroleum or oatmeal based moisturising lotions and be forced to use such products after work shift showers. Studies by McCormick *et al.* (2000), Miller *et al.* (2011) and Nebus *et al.* (2011) showed that these moisturising lotions are certainly as effective, and drastically more cost-effective, than prescription skin barrier repair lotions in lowering TEWL and improving skin hydration. Care should however be taken to ensure that the lotion is used on properly cleaned skin only, as it might otherwise trap contaminants against the skin.

4.3 LIMITATIONS

In retrospect, a number of shortcomings in the dermal exposure assessment of workers to copper sulphate and the concurrent assessment of the change in their skin barrier function have been identified:

- The study was performed in a non-climate controlled area and under time constraints due to the demanding working schedule of the workers. These are factors readily encountered during *in vivo* assessment of skin barrier function in non-clinical settings. It was for these reasons, *inter alia*, that the development of the international guidelines was undertaken (Du Plessis *et al.*, 2013; Stefaniak *et al.*, 2013).
- Dermal exposure and the change in skin barrier function assessment were not carried out on a control group. The inclusion of a control group could have led to the subtraction of consumer background exposure levels of the control group from the occupational copper exposure of the workers at the chemical industry. Comparisons and additional insight in the change of skin barrier function parameters between the exposed workers and the non-exposed control group could also have been obtained.
- There is no *in vivo* validation of the method used to remove copper sulphate from the workers' skin. The only method validation available is that for the removal of copper from glass surfaces. This had to be taken into consideration for the interpretation of and dermal exposure and surface sampling results.
- In addition to skin barrier function measurements, the workers were asked to complete a questionnaire developed by Dalgard *et al.* (2003) in order to assess the condition of their skin barrier function. The international guidelines for the *in vivo* assessment of TEWL, skin hydration and pH in non-clinical settings (Du Plessis *et al.*, 2013b; Stefaniak *et al.*, 2013) however, suggest that the health of the skin of the workers be assessed by a qualified person and for example by a validated teledermatology toolkit for standardised hand photographs in non-clinical settings.

- A short version of another validated questionnaire, developed by Susitaival *et al.* (2003), the Nordic Occupational skin questionnaire (NOSQ – 2002) was also given to the workers to complete. The purpose of the questionnaire was to screen and monitor skin diseases in the workplace. Due to the illiteracy of the participants and their lack of knowledge regarding medical terminology, this questionnaire had to be withdrawn from the study.
- Although there were budget constraints, the study was planned and executed in a manner to ensure that a representative amount of samples were taken from the skin of workers as well as surfaces at the chemical industry.

4.4 FUTURE STUDIES

The following aspects could be added to future studies to substantiate certain assumptions made in Chapter 3 of this mini-dissertation:

- Dermal exposure sampling of workers before they enter the premises. This would confirm whether the workers are already subject to signs of contamination on their skin even before shift exposures and which might have resulted from secondary exposure to contaminated surfaces and clothing or simply background copper exposure levels, and as such also a difference that calls for investigation.
- Sampling on the inside of PPE and especially protective gloves to confirm whether they are contaminated with copper sulphate, thus posing an additional source of the dermal exposure.

Future studies might include the following:

- Reassessment of the dermal exposure and changes in skin barrier function of the workers at the chemical industry after the implementation of the recommendations made in Section 4.2.

- The concurrent assessment of inhalation exposure with dermal exposure to copper sulphate.
- The assessment of dermal exposure to sulphuric acid, and the effect thereof on the skin barrier function.

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CHAPTER 5

ANNEXURE A-D

DALGARD SKIN CONDITION QUESTIONNAIRE

The Dalgard skin condition questionnaire as presented to the workers in English and Setswana:

During the last week, have you had any of the following complaints?

Mo bekeng e e fetileng, a o kile wa nna le nngwe ya dingongorego tse?

	No Nnyaa.	Yes, a little Ee. Go le gonnye.	Yes, quite a lot Ee.Go le gontsi	Yes, very much Ee. Thata.
1. Itchy skin Go babelwa ga letlalo				
2. Dry/sore rash Boswata bo bo omeletseng/botlhoko				
3. Scaly skin Letlalo le le obogang				
4. Itchy rash on your hands Boswata bo bo babelang mo diatleng				
5. Pimples Dipeisi/ Diso				
6. Other rashes on your face Boswata mo sefatlhegong				
7. Warts Diso/dokgoto				
8. Troublesome sweating Go fufulelwa thata				
9. Loss of hair Go wa ga moriri				
10. Other skin problems Mathata a mangwe a letlalo				

If yes, when did the skin problem start? Mark one answer.

Fa karabo ya gago e le ee, bothata jwa letlalo bo simolotse leng? Ka kopo, tshwaya karabo e le nngwe.

During the last week Mo bekeng e e fetileng	
During the last month Mo kgweding e e fetileng	
1-6 months ago Kgwedi e le ngwe go tse thataro (1-6)	
More than 6 months ago Go feta dikgwedi di le thataro tse di fetileng	

NORDIC OCCUPATIONAL SKIN QUESTIONNAIRE (NOSQ-2002/SHORT)

The short version of the Nordic Occupational Skin Questionnaire (NOSQ-2002) which was presented to the workers in both English and Setswana, but withdrawn due to the illiteracy and lack of knowledge regarding medical terminology of the population group.

G1. Workplace:	_____
Felotiro	
Department:	_____
Lefapha	

G2. Are you
A o
a man <input type="checkbox"/> 1
monna
a woman <input type="checkbox"/> 2
mosadi
G3. Year of birth: 19__
Selemo se o tsetsweng ka sona

G5. What is your present occupation? _____
O dira tiro ya mofuta ofeng jaanong?
Since when? _____(year)
Go simolola leng? (ngwaga)

G6. What is your major activity at work? _____
Tiro ya gago e kgolo ke efeng kwa tirong?
Since when? _____(year)
Go simolola leng? (ngwaga)

G7. How many hours per week do you work in your main job (on average)? _____
(hours/week)
 O dira diura di le kae ka beke (*ka gale/ka tlwaelo*)?
 (ura/beke)

G8. Do you perform any other paid work regularly?
 A go na le tiro e nngwe gape e o e dirang e e go dueleng?
 no 1
 nnyaa
 yes 2 **What kind of work?** _____
 ee mofuta mang wa tiro?
How many hours per week (on average)? _____ (hours/week)
 O dira diura di le kae ka beke (*ka gale/ka tlwaelo*) (ura/beke)

D1. Have you ever had hand eczema ?
 A o kile wa itemogela bolwetswe jwa letlalo mo matsogong?
 no 1
 nnyaa
 yes 2
 ee

D2. Have you ever had eczema on your wrists or forearms (excluding fronts of elbows)?
 A o kile wa itemogela bolwetsi jwa letlalo mo matsogong gare ga diatla le sekgono (kwa ntle ga bontle ba sekgono)?
 no 1 **(if you also answered "no" to question D1 move to question A1)**
 nnyaa (ga o arabile "nnyaa" mo potsong D1 fetela kwa potsong A1)
 yes 2
 ee

D5. When did you last have eczema on your hands, wrists or forearms? (one answer in each column if applicable)

O feleleditse leng go itemogela bolwetse jwa letlalo mo matsogong, lengenane la letsogo kgotsa diphaka? (karabo e le nngwe mo lebokosong fa go tlhokega)

	Hand eczema	Wrist/Forearm eczema
	Bolwetsi ba letlalo mo letsogong	Bolwetsi ba letlalo mo lengenane
I have it just now Ke e bona jaanong	<input type="checkbox"/> 3	<input type="checkbox"/> 3
not just now but within the past 3 months E seng jaanong fela ke ne ke na le yone dikgwedi tse tharo tse di fetileng	<input type="checkbox"/> 4	<input type="checkbox"/> 4
between 3-12 months ago magareng a dikgwedi tse tharo go ya go tse bosome le bobedi tse di fetileng	<input type="checkbox"/> 5	<input type="checkbox"/> 5
more than 12 months ago go feta dikgwedi di le bosome le bobedi	<input type="checkbox"/> 6	<input type="checkbox"/> 6

In which year was the last time? _____ (year) _____ (year)
(make your best estimate) (ngwaga) (ngwaga)
Ke ngwaga ofeng o o feleleditseng go nna le bolwetse ba letlalo?
(inaganele)

F1. Have you noticed that contact with certain materials, chemicals or anything else in your work makes your eczema worse? (one answer in each column if applicable)

A o kile wa lemoga gore fa o tshelwa ke marothodi a dichemicala mo letlalong la gago, o dira kwa tirong, go a gakala?

	Hand eczema	Wrist/Forearm eczema
	Bolwetsi ba letlalo mo letsogong	Bolwetsi ba letlalo mo lengenaneng
no nnyaa	<input type="checkbox"/> 1	<input type="checkbox"/> 1
yes ee	<input type="checkbox"/> 2	<input type="checkbox"/> 2
What? Eng?	_____	_____
	_____	_____
don't know Ga ke itse	<input type="checkbox"/> 0	<input type="checkbox"/> 0

F2. Have you noticed that contact with certain materials, chemicals or anything else outside your work makes your eczema worse? (one answer in each column if applicable)

A o kile wa lemoga gore fa o tshelwa ke marothodi a dichemicale mo letlalong la gago o se kwa tirong go a gakala?

	Hand eczema	Wrist/Forearm eczema
	Bolwetsi ba letlalo mo letsogong	Bolwetsi ba letlalo mo lengenaneng
no nnyaa	<input type="checkbox"/> 1	<input type="checkbox"/> 1
yes ee	<input type="checkbox"/> 2	<input type="checkbox"/> 2
What? Eng?	_____	_____
	_____	_____
don't know Ga ke itse	<input type="checkbox"/> 0	<input type="checkbox"/> 0

F4. Does your eczema improve when you are away from your normal work (for example weekends or longer periods)? (one answer in each column if applicable)

A bolwetse ba letlalo bo nna botoka mo go wena fa o sa dire (seka: ka mafelo a beke kgotsa ka malatsi a boikhutso)?

	Hand eczema	Wrist/Forearm eczema
	Bolwetsi ba letlalo mo letsogong	Bolwetsi ba letlalo mo lengenaneng
No Nnyaa	<input type="checkbox"/> 1	<input type="checkbox"/> 1
yes, sometimes ee, ka dinako tse dingwe	<input type="checkbox"/> 2	<input type="checkbox"/> 2
yes, usually ee, ka metlha	<input type="checkbox"/> 3	<input type="checkbox"/> 3
don't know ga ke itse	<input type="checkbox"/> 0	<input type="checkbox"/> 0

A1. Have you ever had an itchy rash that has been coming and going for at least 6 months, and at some time has affected skin creases? (*by skin creases we mean folds of elbows, behind the knees, fronts of ankles, under buttocks, around the neck, ears, or eyes*)

A o kile wa itemogela diso tse di babang tse di tlhagang di timela mo nakong ya dikgwedi tse thataro, mme tsa ama letlalo mo le leng boruma? (*ka boruma re raya mo letsogo le kobegang, fa sekgonong, fa moraga ga mangole, fa pele mo mangananeng a maoto, fa tlase ga marago, mo molaleng, fa ditsebeng le mo matlhong*)

no 1

nnyaa

yes 2

ee

don't know 0

Ga ke itse

PERSONAL FACTORS AND BASIC HYGIENE QUESTIONNAIRE

The following questions in regards to personal factors and basic hygiene, which could possibly be related to the occurrence of dermatitis and other skin problems, were presented to the workers in both English and Setswana. Van Wendel De Joode *et al.* (2007) previously used these questions in a study to ascertain dermatitis among metal workers and its relation with exposure to metal working fluids.

V1. Do you smoke?

A o a tshuba/goga?

Yes

ee

No

nnyaa

V2. How often do you wash your hands at work?

O tlhapa matsogo a gago ga kae kwa tirong?

2-4 times a day

Gabedi go ya go ganne ka letsatsi

5-10 times a day

Gatlhano go ya go gasome ka letsatsi

V3. Do u wear gloves at work?

A o rwala di kausu tsa matsogo kwa tirong?

Yes

Ee

No

Nnyaa

V4. Do you use moisturizing cream on your hands and wrists?

A o dirisa setshaso mo matsogong le mo mangenaneng?

Yes

Ee

No

Nyaa

V5. How often do you apply the moisturizing cream?

O itshasa ga kae ka setshaso?

After every hand wash

Morago ga go tlhapa matsago

2 + times a day

Go feta gabedi ka letsatsi

Once a day

Gangwe ka letsatsi

You don't use it at all

Ga o se dirise gotlhe gotlhe

V6. Do you use moisturizing cream on you face?

A o dirisa setshaso mo sefatlhegong sa gago?

After every hand wash

Morago ga go tlhapa matsago nako e nngwe le e nngwe

2 + times a day

Go feta gabedi ka letsatsi

Once a day

Gangwe ka letsatsi

You don't use it at all

Ga o se dirise gotlhe gotlhe

V7. Do you perform one or more of these activities a week?

A o dira nngwe kgotsa mmalwa wa ditiro tse di latelang ka beke?

Car repairing

Go baakanya koloji

Gardening

Tiro ya tshingwana

Odd jobs around the house

Tiro nngwe le nngwe mo legaeng

Welding

Go fiseletsa/welta

V8. Do you suffer from hay fever?

A o tshwarwa ke (mofikela) / letshoroma la kethimolo?

Yes

ee

No

nnyaa

Don't know

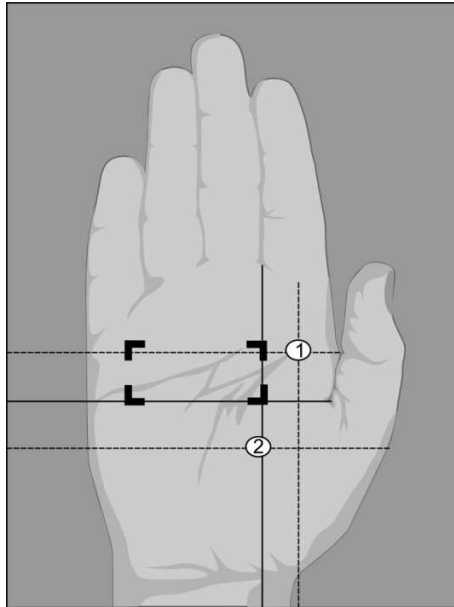
Ga ke itse

DESIGNATED SAMPLING AREAS

The anatomical mapping (indicated and described below) were primarily used to assure consistency in measurement and sampling areas, and also to make provision that skin barrier function measurement areas do not overlap dermal sampling areas.

The tables below indicate illustrations of the anatomical areas where skin barrier function measurements and dermal exposure samples were taken from. In addition explanations on the procedures followed during anatomical mapping are provided.

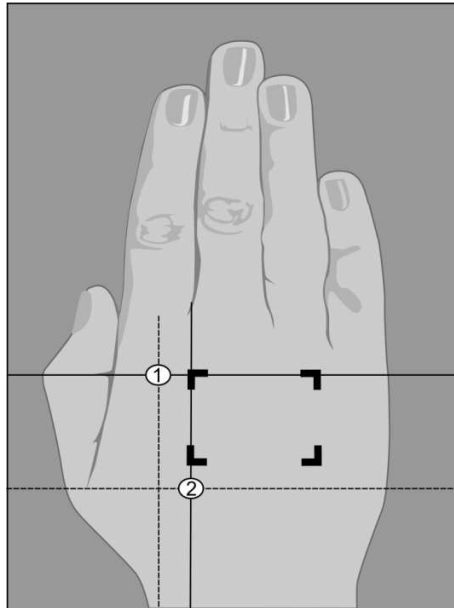
Specific anatomical markers were used to draw both vertical and horizontal imaginary lines from. The points at which these lines met either indicated the designated skin barrier function measurement areas (labelled 1 and 2), or the marker at which the acetate template for dermal sampling was placed. The dotted lines indicate the lines drawn for the measurement of skin barrier function and the solid lines indicate the lines for the placement of the acetate template.

Measurement area: The palm**Skin barrier function measurement areas**

Sampling point	Horizontal line	Vertical line
1	Articulate of the thumb's distal and proximal phalanges.	Middle of the index finger.
2	Articulate of the thumb's metacarpal and the proximal phalanges.	Area between the index and middle finger.

Dermal sampling area

	Horizontal line	Vertical line
Right hand	The lower right hand corner of the inner template area was placed in line with the lowest point of the skin-fold between the thumb's and the palm of the hand.	The lower right hand corner of the inner template area was placed downwards from the area between the middle and index finger.
Left hand	The lower left hand corner of the inner template area was placed in line with the lowest point of the skin-fold between the thumb's and the palm of the hand.	The lower left hand corner of the inner template area was placed downwards from the area between the middle and index finger.

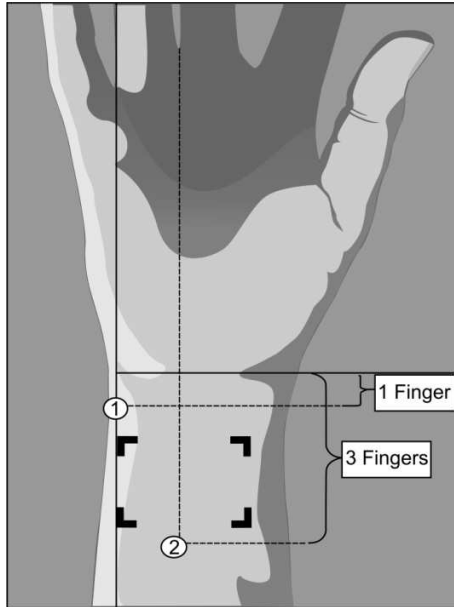
Measurement area: The Back of hand**Skin barrier function measurement areas**

Sampling point	Horizontal line	Vertical line
1	Articulate of the thumb's distal and proximal phalanges.	The middle of your index finger.
2	Articulate of the thumb's metacarpal and proximal phalanges.	The area between the index finger and the middle finger.

Dermal sampling area

	Horizontal line	Vertical line
Right hand	The upper left hand corner of the inner template area was placed in line with the articulation of the thumb's distal and proximal phalanges.	The upper left hand corner of the inner template area was placed downwards from the area between the middle and index finger.
Left hand	The upper right hand corner of the inner template area was placed in line with the articulation of the thumb's distal and proximal phalanges.	The upper right hand corner of the inner template area was placed downwards from the area between the middle and index finger.

Measurement area: The Wrist

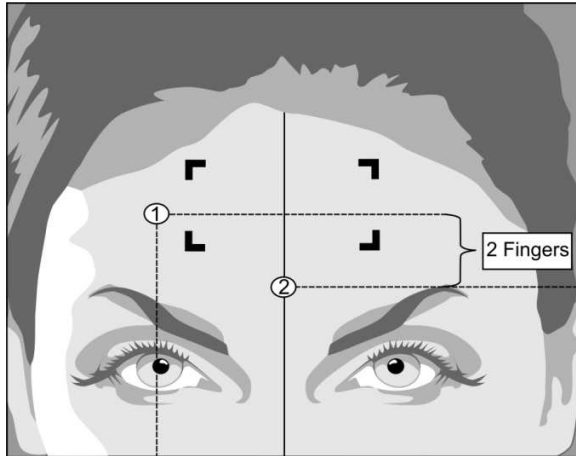


Skin barrier function measurement areas

Sampling point	Horizontal line	Vertical line
1	The width of one finger below the lowest part of the pisiform hand bone.	The area between the pinkie and ring finger.
2	The width of three fingers below the lowest part of the pisiform hand bone.	The area between the ring finger and the middle finger.

Dermal sampling area

	Horizontal line	Vertical line
Right hand	The outside of the template was placed at the bottom edge of the pisiform hand bone.	The upper left hand corner of the inner template area was placed downwards from the area between the pinkie and ring finger.
Left hand	The outside of the template was placed at the bottom edge of the pisiform hand bone.	The upper right hand corner of the inner template area was placed downwards from the area between the pinkie and ring finger.

Measurement area: The Forehead**Skin barrier function measurement areas**

Sampling point	Horizontal line	Vertical line
1	The width of 2 fingers above the highest point of the eyebrow.	The outside of right pupil.
2	The highest point of eyebrow.	The middle of the face.

Dermal sampling area

Horizontal line	Vertical line
The outside of the template was placed at the top edge of the eyebrow.	The middle of the template was placed in the middle of the forehead.