

**Measuring DNA damage and repair
as possible biological markers for long-term,
low-dose chemical exposure in hairdressers.**

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Table 1 below outlines the contribution of each of the researchers who participated in the planning and execution of this study.

Figure 1: List of contributors and their respective roles

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Prof. P. Pretorius: Study adviser	Assisting with the methodology and interpretation of Comet Assay and its results.
Prof. F. van der Westhuizen: Consultant	Assisting with the methodology and interpretation of the ORAC tests.

The declaration below confirms each of the contributors' individual role in this study:

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

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LIST OF ABBREVIATIONS

ACGIH	American Conference of Governmental Industrial Hygienist
OHSA	Occupational Health and Safety Act
MSDS	Material Safety Data Sheet
ppm	Parts per million
OEL-STEL	Occupational Exposure Limit - Short Term Exposure Limit
TWA	Time Weighted Average
VOC	Volatile Organic Compound
ACD	Allergic Contact Dermatitis
ICD	Irritant Contact Dermatitis
Endo III	Endonuclease III
FPG	Formamidopyrimidine DNA Glycosylase
CASP	Comet Assay Software Project Program
ORAC	Oxygen Radical Absorbance Capacity Assay
CTR	Control
STE	Short-term exposure
LTE	Long-term exposure
HCS	Hazardous Chemical Substance
BEI	Biological Exposure Indices
BAT	Biologische Arbeitsstofftoleranzwerte
RS	Reactive Species
BM	Biological Monitoring

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PREFACE

An important trend in Occupational Hygiene is the addition of biological monitoring as part of the monitoring plan. This is necessary because environmental monitoring simply doesn't give us information about how the individual experiences his/her environment. Personal differences in how deep or shallow we breathe, skin absorption, level of fitness, the fit and wear of personal protective equipment, etc. are all factors that could cause great differences in the level of exposure of individuals working in the same environment.

The aim of this study was to investigate the use of DNA damage and repair capacity in blood lymphocytes, as possible biomarkers to check for, and quantitatively measure, the effect of chemical exposure among hairdressers who have worked in the industry for long periods of time compared to individuals who have less than five years exposure and compare it to results of control subjects who don't have any similar chemical exposure.

It was decided to use the article format for this project. Thus, for sake of uniformity, the whole mini-dissertation is done according to the guidelines of the chosen magazine for possible publication, i.e. Occupational Health Southern Africa. This magazine requires that the references be inserted into the text as superscript numbers and that the list of references should be set out in Vancouver style.

ABSTRACT

Hairdressers are exposed to combinations of chemicals, some of which are known or suspected allergens, carcinogens or organic solvents. The objective of this study was firstly to compare DNA damage and repair capacity between individuals with short-term exposure (STE) to those with long-term exposure (LTE) and secondly to that of control subjects (CTR). In addition, the study also wanted to show that the Comet Assay can be used in biological monitoring to measure occupational exposure in hairdressing.

From information gathered in questionnaires, 20 test subjects and 11 controls were chosen, matched in gender, age and smoking habits. A heparinised blood sample was taken and lymphocytes isolated. One set of Comet Assays were performed with lesion-specific enzymes FPG and Endo III to test oxidative DNA damage. In another version of the Comet Assay, hydrogen peroxide was used to test individual DNA repair capacity. Serum from each sample was frozen immediately and later used for testing antioxidant capacity (ORAC). Levels of VOCs were measured inside salons using a photo-ionization detector.

DNA damage test showed significantly increased inherent DNA damage among the LTE group compared to the controls ($p=0.033$), while the DNA repair capacity test showed significant inherent DNA damage among the STE group ($p=0.0028$) compared to the control group. The controls had least damage after T60 ($p=0.05$ compared to STE), least DNA damage after treatment with H_2O_2 and had greater DNA repair capacity than the test groups. Additionally, the controls show an increased antioxidant capacity, especially compared to the LTE group ($p=0.042$). Levels of VOCs, measured well below occupational exposure standards.

It is concluded that hairdressers are exposed to a combination of low levels of harmful chemicals over the prolonged period of their working careers and that this could impact negatively on their health. This study also finds that the Comet Assay is in fact an effective tool for biological monitoring to measure the effects of chemical exposure in the hairdressing industry. This study recommends effective ventilation for every occupation that involves the use of chemicals, as well as proper training regarding the use and handling of chemicals and the importance of using personal protective equipment.

OPSOMMING

Haarkappers word blootgestel aan 'n kombinasie van chemikalieë waarvan sommige erkende of vermeende allergene, karsinogene of organiese oplosmiddels is. Hierdie studie het ten doel om eerstens die DNA-skade en DNA-herstel vermoë van individue met korttermyn blootstelling (STE) te vergelyk met diegene wat langtermyn blootstelling (LTE) gehad het, en tweedens om hierdie resultate te vergelyk met die van 'n kontrolegroep (CTR). Verder wou die studie ook bewys dat die Komeet Analise 'n geskikte metode is vir biologiese monitering om die effek van chemiese blootstelling in die haarsalonbedryf te bepaal.

Inligting vanuit vraelyste is gebruik om 20 proefpersone en 11 kontrole persone te kies. Die keuse van die persone het ouderdom, geslag en rookgewoontes in ag geneem. Heparien bloedmonsters is geneem en limfosiete geïsoleer. In een variasie van die Komeet Analise is skade-spesifieke ensieme FPG en Endo III gebruik om oksidatiewe skade aan te dui. In 'n ander weergawe is waterstofperoksied gebruik om DNA herstel vermoë te meet. Die plasma van elke monster is dadelik gevries vir latere gebruik in die antioksidant (ORAC) toets. Algemene lugkwaliteit binne die haarsalonne is gemeet met 'n foto-ionisasie detektor.

Die DNA skadetoets het betekenisvolle, verhoogde basislyn DNA skade onder die LTE groep getoon in vergelyking met die kontrolegroep ($p=0.033$), terwyl die DNA hersteltoets betekenisvolle, verhoogde basislyn DNA skade toon in die STE groep wanneer dit vergelyk word met die kontrolegroep ($p=0.0028$). Die kontrolegroep toon ook die minste skade na T60 ($p=0.05$ vergelyk met die STE groep), minste skade na behandeling met H_2O_2 , en het 'n beter DNA herstelvermoë as die toetsgroepe. Verder wys die kontrolegroep ook verhoogde antioksidant kapasiteit, veral in vergelyking met die LTE groep ($p=0.042$). Die gemete vlakke van vlugtige organiese verbindings (VOC) was ver onder die toegelate standarde vir beroepsblootstelling.

Die studie bevind dat haarkappers blootgestel word aan lae vlakke van 'n kombinasie van skadelike chemikalieë oor die verlengde tydperk van hul loopbane en dat dit negatief inwerk op hul gesondheid. Die studie wys ook dat die Komeet Analise wel geskik is vir biologiese monitering om die effek van chemiese blootstelling in die haarsalonbedryf aan te dui. Die studie bevind dat behoorlike ventilasie uiters belangrik is in enige beroep waar chemikalieë gebruik word, en benadruk die belangrikheid van voldoende opleiding in verband met die gebruik en hantering van chemikalieë en veral die effektiewe aanwending van persoonlike beskermingstoerusting.

CHAPTER 1: GENERAL INTRODUCTION

1.1 INTRODUCTION

As the field of Occupational Hygiene evolves as a science, it is becoming more apparent that there are shortcomings in regular environmental monitoring. Environmental monitoring doesn't give information on how the individual experiences and reacts to his/her environment. Personal differences in how deep or shallow an individual breathes, skin absorption, level of fitness, the fit and wear of personal protective equipment, personal hygiene, etc. are all factors that could cause great differences in the level of exposure and physiological reaction of individuals working in the same environment.

Biological monitoring could therefore play an important role in identifying susceptible individuals, particularly those suffering from a combination of high risk factors, such as high levels of exposure to chemicals, inherited cancer predisposing genes and a deficiency of protective factors such as inadequate or incorrect diet.¹

Biological monitoring measures the actual exposure of an individual, by

- a) measuring the amount of chemical absorbed,
- b) the chemical's metabolite in the blood or urine or breath, or
- c) its effect on an organ or system.

This type of monitoring measures exposure via all exposure routes i.e. inhalation, skin absorption and/or oral ingestion. Biological monitoring can become a valuable tool to evaluate the efficiency of personal protective equipment and engineering controls, which are already in place.

Analytical methods for existing biomarkers are sufficiently developed to allow routine human monitoring, but only for a relatively small number of chemicals.¹ Measurements of adducts formed by the reaction of electrophilic metabolites of genotoxic compounds with macromolecules are especially useful because it represents the dose that has escaped the detoxification process and that has reached the macromolecule, e.g. protein or DNA.¹ DNA damage is already associated with mutation and cancer and so it is an obvious target molecule, since the extent of adduct formation would bear a relationship to the subsequent stages of mutation in the carcinogenic process.¹

The present study investigated the use of DNA damage and repair capacity in blood lymphocytes, as possible biomarkers to check for, and quantitatively measure, the effects of chemical exposure

among hairdressers who have worked in the industry for long periods of time, compared to individuals who have less than five years exposure.

Studies measuring air quality inside hair salons found the levels of chemicals to be well below TWA exposure levels.^{2, 3} This could lead to the false belief that hairdressing is a safe occupation with no real health risks. However, the chemical exposure hairdressers face is a rather complex issue, since these workers are exposed to a variety of chemicals, including hair dyes, shampoos, hair conditioner, hair relaxers, permanent wave solutions, detergents, hairspray and perfumes.² Many studies already link the chemicals found in hairdressing products to cancers or classify them as allergens or sensitisers.² Only one study was found that investigated DNA damage among hairdressers.⁴ This Italian study showed significant increase in DNA damage among hairdressers already diagnosed with Irritant Contact Dermatitis (ICD). This led to the question if there could possibly be negative effects on the health of hairdressers with low-dose exposure to combinations of chemicals over prolonged periods of time.

1.2 PRESENT STUDY

1.2.1 Problem statement

Hairdressers and their assistants are exposed to low levels of chemicals on a daily basis. These chemicals are classified as allergens, sensitisers, carcinogens and organic solvents.² Even though hairdressers' exposure could be considered *low-dose* exposure, as it is measured to be far below TWA exposure levels, it is still important to investigate the effect of the combination of all these chemicals as this exposure could stretch over 30 or 40, or even 50, years.

1.2.2 Aims of the study

The aims of the study were threefold: namely

- a) to compare the level of DNA damage and repair capacity of people who have worked in the hairdressing industry for more than 15 years, to people who have worked in this industry for less than five years;
- b) to compare the DNA damage and repair capacity of both above-mentioned groups to control subjects, who, apart from the occasional hair dye, don't have similar chemical exposure; and
- c) to investigate the use of DNA damage and repair capacity as possible biomarkers for longterm, low-dose exposure to chemicals used in the hairdressing industry.

1.2.3 Hypothesis

It is proposed that

- a) pro-longed exposure to hairdressing chemicals causes greater levels of DNA damage and a lesser ability for DNA repair;
- b) the level of DNA damage and decreased DNA repair capacity would correlate with the number of years of exposure; and
- c) DNA damage and repair capacity can be used as biomarkers for longterm, low-dose exposure to chemicals in the hairdressing industry.

1.2.4 Planning of the proposed research

The following steps were used to test the validity of the hypothesis:

1. A questionnaire was drawn up to hand out to women's hairdressers in the Potchefstroom area.
2. From the information in the questionnaires, 10 hairdressers who have worked in the industry for over 15 years were chosen to represent the long-term exposure group (LTE).
3. Three trainees and seven hairdressers were chosen to represent the short-term exposure group (STE). Thus a total of 10 subjects for this group. Members of this group have less than five years experience/ exposure each.
4. 11 control subjects (CTR) were chosen to match the test groups on age, gender and smoking habits.
5. From each of these test subjects a 5 ml blood sample was taken and leucocytes extracted.
6. The leucocytes were then used in the Comet Assay to detect DNA damage and repair capacity.
7. The plasma from each subject was frozen immediately and later used in the ORAC test to determine antioxidant status of the test and control subjects.
8. A photo-ionization detector (ppbRAE) was used to measure levels of VOCs, CO and H₂S to assess the general air quality inside the hair salons.

The results will be analysed and discussed in chapters three and four.

1.3 REFERENCES

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CHAPTER 2: LITERATURE REVIEW

This chapter reviews existing material relevant to this study. It discusses the various chemicals used in the hairdressing field, and reviews the literature regarding biological monitoring and using DNA damage and repair mechanisms as biological markers. One of the main focus areas of this study was the use of the Comet Assay as a tool in biological monitoring, and therefore literature regarding the Comet Assay method is discussed, along with information on antioxidant testing and air monitoring which provides additional information for interpreting the Comet Assay results.

2.1 CHEMICALS USED IN THE HAIRDRESSING INDUSTRY AND THEIR KNOWN HEALTH EFFECTS

As far as occupations go, hairdressing is certainly not considered dangerous and yet it involves the use of a variety of chemicals. Table 1 lists the chemicals commonly found in hairdressing products.^{1,2}

Table 1: Chemicals found in hairdressing products

Hairdressing products	Chemical component
Permanent wave solutions	Thioglycolates, hydrogen peroxide, ammonia, glycols
Bleaching products	Hydrogen peroxide, potassium and ammonium and sodium persulphates
Hairspray	Propellant: Propane, pentane, butane, Hand pump: ethanol, iso propanol
Setting liquids	Hydrogen peroxide, organic acids, phosphoric acid
Dye products	Aromatic hydroxy compounds: Resorcinol, Hydroquinone, a-naphtol Amines: diaminobenzene, diaminotoluene, phenylenediamine
Shampoo	Formaldehyde
General cleaners	Isopropanol, ethanol

Table 2 below gives an overview of the various chemicals, their route of entry, affect on the human body as well as the associated possible disabilities or health risks they pose. Information was compiled using the Industrial Handbook of Toxicology.³ The summary below focuses more on those chemicals specifically found in permanent wave solutions, bleaching products and dyes as these products seem to pose the highest health risks.

Table 2: Summary of the health effects of some hairdressing chemicals

Route of entry	Mode of action	Signs and symptoms	Disability
AMMONIA - Threshold Limit Value: 25 ppm or 18 mg/m³			
Inhalation.	• Irritant. • Corrosive.	• Corneal burns and ulceration. • Irritate and burn skin and mucous membranes. • Inhalation may cause chemical pneumonia and later persistent obstructive respiratory failure. • Chronic effects: bronchiectasis and small airway obliteration.	• Eye damage may be permanent. • Burns may scar. • Possible permanent pulmonary impairment.
RESORCINOL - Threshold Limit Value: 10 ppm or 45 mg/m³			
Inhalation. Ingestion. Skin absorbtion.	• Irritant. • Sensitiser. • Liver and kidney damage. Possible Methemoglobinemia.	• Conjunctivitis and corneal damage. • Dermatitis with erythema and oedema. • Systemic symptoms like that of phenol intoxication.	No permanent effects.
HYDROQUINONE - Threshold Limit Value: 2 mg/m³			
Inhalation.	• Irritant. • Sensitiser.	• Conjunctivitis and keratitis. • Dermatitis. • Vitiligo.	• Corneal changes and vitiligo are permanent.
PHOSPHORIC ACID - Threshold Limit Value: 1mg/m³			
Inhalation.	• Irritant.	• Irritation of eyes, skin and respiratory tract. Acid burns possible.	No permanent effects.

Route of entry	Mode of action	Signs and symptoms	Disability
HYDROGEN SULPHIDE - Threshold Limit Value: 10 ppm or 14 mg/m³			
Inhalation.	• Irritant. • Inhibits cytochrome oxidase.	<i>High exposure may cause:</i> • Syncope, apnoea and cyanosis. • Unconsciousness, convulsions, coma and death. <i>Lower exposure may cause:</i> • Conjunctivitis and keratitis with photophobia or "gas eye". • Headache, dizziness, mental confusion, and weakness and pain in extremities and legs. • Nausea, vomiting, diarrhoea, chest pain, dyspnoea, coughing, rhinitis, bronchitis and pulmonary oedema.	• Coma. • Death. Those who survive the immediate effects recovery fully.
PENTANE - Threshold Limit Value: 600 ppm or 1800 mg/m³			
Inhalation.	• Irritant. • Central nervous system depressant.	• Irritation of skin and eyes.	No permanent effects.
THIOGLYCOLIC ACID - Threshold Limit Value: 1 ppm or 1 mg/m³			
Skin absorbtion.	• Irritant. • Sensitiser.	• Conjunctivitis. • Papulo-vesicular eruptions. • Dermal burns possible. • Anorexia.	No permanent effects.

Route of entry	Mode of action	Signs and symptoms	Disability
NAPHTHOL - Threshold Limit Value: None established			
Ingestion. Skin absorbtion.	• Irritant. • Haemolytic anaemia. • Kidney and liver damage.	• Conjunctivitis and corneal burns. • Dermatitis leading to hyper-pigmentation. • Nausea, vomiting and abdominal pain. • Headache, unconsciousness and convulsions. • Hepatomegaly and jaundice. • Haemolytic anaemia and hematuria.	No permanent effects.
ETHYL ALCOHOL- Threshold Limit Value: 100 ppm or 1900 mg/m³			
Ingestion. Inhalation.	• Irritant. • Central nervous system depressant. • Possible liver damage.	• Irritation of eyes and respiratory tract. • Dermatitis. • Headaches, drowsiness, dizziness and mental confusion. • Anorexia and nausea. • Narcosis.	No permanent effects.
FORMALDEHYDE – Threshold Limit Value: 1 ppm or 1.5 mg/m³ (Suspected carcinogen)			
Inhalation.	• Irritant. • Sensitiser.	• Irritation of eyes, conjunctivitis and corneal damage. • Burning of nose and throat, coughing, dyspnoea, chest tightness, pulmonary oedema an allergic asthma. • Headache and fatigue.	• Permanent sensitization. • Corneal damage.

Route of entry	Mode of action	Signs and symptoms	Disability
BUTANE - Threshold Limit Value: 800 ppm or 1900 mg/m³			
Inhalation.	Asphyxiant	Narcosis.	No permanent effects.
ISOPROPYL ALCOHOL - Threshold Limit Value: 400 ppm or 980 mg/m³			
Inhalation. Skin absorbtion.	- Irritant. - Metabolise to acetone. - Central nervous system depressant.	- Conjunctivitis and corneal oedema. - Irritation of upper respiratory tract. - Headache, dizziness, drowsiness with narcosis. - Dermatitis.	No permanent effects.
HYDROGEN PEROXIDE - Threshold Limit Value: 1 ppm or 1.5 mg/m³			
Inhalation.	Irritant.	- Conjunctivitis and corneal burns. - Irritation of nose and throat that may lead to bronchitis and pulmonary oedema. - Skin blanching and blistering. - Bleaching of body hair.	Permanent damage from burns.

From the table above it is clear that particularly the eyes, skin and lungs of users are most at risk.

2.2 RELATED STUDIES AND ARTICLES

Two separate studies in Norway and the Netherlands looked at levels of chemicals present in hair salons, specifically during winter time when doors and windows are kept shut and ventilation could be poor. However, both these studies showed levels of chemicals to be well below the Occupational Exposure Limits (OEL) set by their respective countries.^{1,2} Despite the low levels of chemicals measured, there are many studies that link the hairdressing industry to disease. Two epidemiological

studies in Italy and one in Germany looked at different occupations such as the metalwork industry, building industry, health care and hairdressing industry, and their typical health problems, and were able to link hairdressing to contact dermatitis^{4,5} and occupational cancer.⁶

The most common disorder in the hairdressing industry is contact dermatitis (CD).⁷ Contact Dermatitis can have an irritant (ICD) or allergic origin (ACD). ICD is mainly caused by frequent use of water and shampoo, while ACD is mostly attributed to nickel, formaldehyde, fragrances, phenylenediamine, thioglycolates, persulphates and ammonium compounds.⁸ There seems to be a particularly high exposure to nickel because some chemicals like thioglycolate, used in permanent wave solutions, can leach nickel out of stainless steel utensils.⁹ A previous study that used Patch test results also focused on the role of metals such as nickel as a cause of ACD as well as chromium and cobalt.¹⁰ Apart from allergic skin conditions, hairdressers also suffer from asthma. Curie and Ayres¹¹ was able to link persulphates used in hairdressing to occupational asthma, while Coppieters and Piette¹² showed that a fair percentage of hairdressers believed they suffer from asthma and show asthmatic symptoms. Even a natural product like Henna is thought to be the cause of immediate type hypersensitivity.¹³

Two separate studies have found that *p*-phenylenediamine caused beard and hand dermatitis.^{14,15} An article was featured on BBC News that warned people against using hair colour as these chemicals (*para*-phenylenediamine and tetrahydro-6-nitroquinoxaline) were shown to damage genetic material and to cause cancer in animals.¹⁶ Murata *et al.* studied oxidative damage induced by *o*-phenylenediamines and found that the carcinogenicity of this chemical is associated with oxidative DNA damage rather than bacterial mutagenicity.¹⁷

Thioglycolate is the active ingredient in permanent wave solutions. It has been linked to Hairdresser's Koilonychia¹⁸ (toxicity to the Vestibuloocular Reflex System) when used in combination with potassium bromate, which could lead to hearing loss¹⁹, and even to chromosomal damage that could affect hairdresser's reproductive function.²⁰ Galiotte *et al.* studied occupational genotoxic risk among Brazilian hairdressers and found an increase in the number of spontaneous abortions in hairdressers compared to controls.²¹ This study also concluded that DNA damage observed in the hairdresser test group was significantly higher than in the control group.²¹

Formaldehyde is found in shampoos. Emri *et al.* researched the effect of Formaldehyde on DNA damage and repair²² and concluded that Formaldehyde slowed the DNA repair process which might increase the photo-carcinogenic risk to humans who are exposed to this chemical. La Vecchia as quoted by Murata *et al.* also looked at several cohort and case control studies that have shown

increased risk of bladder cancer among hairdressers and barbers who are occupationally exposed to hair dyes.¹⁷

Another hair dye product known to cause DNA damage is 2,4-Diaminotoluene.²³ DNA damage and increased TNF α production was found in hairdressers with contact dermatitis, suggesting a possible relationship between allergen exposure, cytokine induction and allergic disease development.²⁴ A case reference study by Swanson and Burns²⁵ was done to assess the occurrence of salivary gland cancers associated with diverse occupations and industries. It was found that salivary cancer is elevated among women employed as hairdressers.²⁵

From the above-mentioned studies it is clear that hairdressers are exposed to a number of chemicals, which could have a negative impact on their health, including DNA damage. When this study was conducted, there were no previous studies on DNA damage being done in the hairdressing community in South Africa. It was therefore the aim of the study to assess the levels of DNA damage and repair capacity of hairdressers in South Africa by means of the Comet Assay, and to determine if this Assay could be used as possible biological marker for measuring long-term, low-dose chemical exposure in the hairdressing industry.

2.3 BIOLOGICAL MONITORING

Biological monitoring (BM) measures exposure to hazardous chemical substances (HCS) in 2 ways:

- a) by measuring the biomedical concentration of HCSs and/or their metabolites in biological samples of exposed individuals, e.g. blood lead for inorganic lead exposure, or urinary arsenic for inorganic arsenic exposure. The aim is to measure the degree of absorption into the body by measuring indicators in representative biological samples, typically urine or blood; and
- b) by measuring the biological effects of HCSs through determining the intensity of biochemical or physiological change due to exposure, such as red cell cholinesterase for exposure to organophosphate pesticides, or zinc protoporphyrin (ZPP) for exposure to inorganic lead.²⁶

The standard practice of environmental monitoring gives an indication of the amount of HCS in the air, but biological monitoring gives an indication of personal exposure of an individual. BM results are measured against ACGIH BEI (Biological Exposure Indices) or against DFG BAT (Biologische Arbeitstoftoleranzwerte) values.²⁸ BEI values represent the levels of analytes that are most likely to be observed in specimens collected from a healthy worker who has been exposed to chemicals to the same extent as a worker with inhalation exposure at the Threshold Limit Value (TLV) and is

regarded as advisory levels.²⁷ BAT values are defined as the “maximum permissible quantity of a chemical substance or its metabolites, or the maximum possible deviation from the norm for biological parameters, induced by these substances in exposed humans”. BAT values are considered ceiling values with the intent to protect workers from work related health impairments.²⁷ Existing BEIs are also listed in Table 3 in the Occupational Health and Safety Act, as well as in the Lead and Asbestos regulations.²⁶

There are critical factors that need to be taken into consideration for biological monitoring to be meaningful.

- i.) The timing of the sample collection is critical as different chemicals or their metabolites have different toxico-kinetics and different half-lives in the various sampling media (blood, urine and breath). If sampling is done too early after exposure, the chemical or its metabolites might not have reached its peak yet, or if taken too late most of the chemical or metabolite might have been excreted already.²⁸
- ii.) It is difficult to interpret the results of biological monitoring, because individuals respond differently to exposure. Personal factors, lifestyle choices (smoking, eating habits, etc.) and environmental exposures outside the workplace can effect BM results. Individual exposure is influenced by the standard of personal hygiene, work practice, genetic predisposition, individual differences in breathing rate and depth, physical exertion, pre-exposure burden, etc.²⁸ In some cases BEI values can be exceeded without it causing health effects in that individual, whilst other individuals may experience ill effects even before BEI values are reached.²⁷
- iii.) Another critical issue is quality assurance systems with regard to sampling, handling of samples and analysis of specimens.²⁷ Because of low analyte levels sampling collection and analysis should be carried out with high precision: giving very detailed information about the persons’ working time and exposure conditions, using only standardised procedures for collection, handling and storage of samples and strictly following validated methods of analysis.²⁸

The most widely excepted forms of biological monitoring include exhaled breath, urine and blood testing.²⁸

Biological monitoring has great application in measuring the efficacy of personal protective equipment, engineering controls and other human factors. Biomarkers are becoming increasingly important in toxicology and human health. Major research goals are to develop and validate

biomarkers that reflect specific exposures and permit the prediction of the risk of disease in individuals and groups.²⁹

New laboratory techniques and quality assurance systems have sorted out problems previously experienced with analysis, so that the focus of research can now be placed on methods of interpretation of results.²⁷ Recent advances in biological monitoring include the determination of protein and DNA adducts, unchanged volatile organic compounds in urine, monitoring of exposure to pesticides, antineoplastic drugs, hard metals and polycyclic aromatic hydrocarbons (PAH).²⁷ During a symposium on BM in 2005, new and improved methods of BM were presented for more than 20 chemicals. The most used method was mass spectrometry, specifically MS/MS for organics and ICPMS for metals.³⁰ As it is already known that hairdressing chemicals can cause changes in DNA,^{16,20,22,23} DNA damage and repair was chosen as the obvious biomarkers to use in this study.

2.4 DNA DAMAGE

The importance of studying DNA damage and repair cannot be overstated, since chromosomal damage contributes to acquired disorders such as genetic mutations. This could lead to cancer and birth defects in offspring of those who are exposed to mutagenic agents. DNA damage may increase due to exposure to a variety of chemical agents.³¹ Kovtum and McMurray stresses that unstable repeats in DNA are associated with various types of cancer and have been implicated in more than 40 neurodegenerative disorders.³² Thus, there is a continuing need to develop methods for studying exposed populations.

According to Li *et al.* there are two classes of genome-maintenance systems; one responsible for accurately reproducing genetic information and the other dedicated to the proper processing of DNA damage.³³

Sancar *et al.* distinguishes the following types of DNA damage:³⁴

- *Replication, recombination and repair intermediates*

These include fork structures, bubbles, Holiday structures, and other non-duplex DNA forms.

- *DNA base damages*

Base damages include reduced, oxidised or fragmented bases in DNA that are produced by reactive oxygen species or ionising radiation. Chemicals form various base adducts, either bulky adducts by large polycyclic hydrocarbons or simple alkyl adducts by alkylating agents.

- *DNA backbone damages*

These are abasic sites and single or double-strand DNA breaks. While abasic sites are generated spontaneously by unstable base adducts or by base excision repair, single-strand breaks are caused directly by damaging agents. Double-strand breaks are the result of ionising radiation or other DNA-damaging agents.

- *Cross-links*

Bifunctional agents form interstrand cross-links and DNA-protein cross-links. Although these aren't considered DNA damage in itself, very tight cross-links can inhibit transcription and replication.

2.5 DNA REPAIR MECHANISMS

Cells possess a DNA damage recognition system to respond to all types of DNA damage. The function of this system is to help the cell determine whether to repair the damage or program it for apoptosis. Weterings and Chen remarks that failure of cells to repair DNA damage effectively can result in chromosome breakage, cell death, onset of cancer and defects in the immune system of higher vertebrates.³⁵

There are various types of DNA repair mechanisms. What follows below is a brief summary of these as discussed by Sancar *et al.*:³⁴

- *Direct repair*

A small protein is presumed to recognise DNA damage by three-dimensional diffusion. After forming a low-stability complex with the DNA backbone at the damage site, it is thought to flip out O⁶MeGua base into the active site cavity, wherein the methyl group is transferred to an active site cysteine. The protein disassociates from the repaired DNA, but the C-S bond of methylcysteine is stable and therefore after one catalytic event, even the enzyme becomes inactivated and is accordingly referred to as a suicide enzyme.

- *Basic excision repair*

This mechanism is initiated by DNA glycosylase that releases the target base to form an abasic site in the DNA. Li *et al.* adds that cells use different base excision repair enzymes to remove DNA base lesions and repair single-strand breaks.³³

- *Nucleotide excision repair*

This mechanism is considered the main DNA repair system for removing bulky DNA lesions formed by exposure to radiation or chemicals or by protein addition to DNA. The damaged bases are removed by the excision nuclease, which is a multi-subunit enzyme system that makes dual incisions bracketing deletion in the damaged strand.

- *Double-strand break repair and recombination repair*

Three types of double strand-break repair mechanisms are distinguished, namely homologous recombination, single-strand annealing and non-homologous end-joining.

The homologous recombination takes place in three steps: strand-invasion, branch migration and the Holiday junction. In this type of repair the information that is lost from the broken duplex is retrieved from a homologous duplex. Where the two duplexes aren't exactly the same, gene conversion may take place.

In the single-strand annealing mechanism the ends of the duplex are digested by an exonuclease until regions of some homology on the two sides of the break are exposed. These regions are then paired and the non-homologous tails trimmed off, so that the two duplex ends may be ligated.

The most complex repair mechanism is the non-homologous end-joining and it is still poorly understood.³⁵ This process is initiated by the binding of a protein complex to both ends of the broken DNA molecule. Once the two DNA ends have been captured and tethered in a protein complex, non-ligatable DNA termini must be processed so that final repair can take place. Finally the ligase complex catalyses ligation of the processed DNA ends.³⁵

- *Cross-link repair*

Starting at the double-strand break, induced by replication, nuclease degrades one of the cross-linked strands in a 3' to 5' direction past the cross-link and thus converts the interstrand cross-link into an intrastrand dinucleotide adduct adjacent to a double-strand break.

Li *et al.* points out that the various repair mechanisms are interconnected and collaborative.³³

2.6 ASSAYS USED IN THIS STUDY

2.6.1 Comet Assay

- *What is the Comet Assay?*

It is a simple method for measuring DNA strand breaks in eukaryotic cells.³⁶ The Comet Assay is based on the ability of negatively charged loops or fragments of DNA to be drawn through an agarose gel in response to an electric field. The extent of DNA migration depends directly on the damage present in the cells.³⁷ Thus, the more DNA damage, the longer the migrating tail away from the nucleus, hence the name Comet Assay.

- *Critical evaluation of the Comet Assay*

The Comet Assay has become a very popular method for measuring DNA damage as it only requires a small sample and is relatively easy and cost effective, while being reliable for detecting DNA damage. By adding lesion-specific enzymes, this assay can be made more specific and sensitive as these enzymes recognise specific target sites, i.e. sites of oxidative damage to purines or pyrimidines.³⁶

Galiotte *et al.* points out that although the Comet Assay is not predictive of an individual's cancer risk, it is a very useful tool to evaluate early and still repairable genotoxic effects due to occupational or environmental exposure.²¹

However, the scientific community is divided as to the influence of confounding factors, such as diet, exercise, smoking and age on the results of the Comet Assay. Moller *et al.* recommends that age, gender and smoking status be used as criteria for the selection of populations and that data on exercise, diet and recent infections be registered before blood sampling.³⁸

Given these concerns, special care was taken with the preparation of the participant questionnaire for this study to ensure that details as to diet, smoking, age, medical conditions, as well as activities that could expose participants to chemicals outside of the working environment were noted. This would help the researcher to explain any deviances in averages of test subjects.

Another shortcoming of the Comet Assay as a tool for biomonitoring is the lack of standardised procedures, most notably the absence of an agreed-upon parameter for interpreting results.³⁷

After reviewing 45 different reports that compared results of different cytogenetic assays such as CA, MN and SCE with the Comet Assay, Faust *et al.* concludes that the results of the Comet

Assays show concordance with above-mentioned cytogenetic tests, indicating that the findings in the Comet Assay were truly positive or negative.³⁹

This researcher is thus convinced of the reliability of the Comet Assay as a tool for measuring occupational exposure in the hairdressing industry.

2.6.2 ORAC

Free radicals are reactive oxygen species derived from either normal metabolic processes and from external sources like X-rays, ozone, cigarette smoke, air pollutants and industrial chemicals.⁴⁰ Free radicals are very unstable and react quickly as they try to capture the electron they need to gain stability.

Should free radicals not be inactivated, their chemical reactivity can damage all cellular macromolecules including proteins, carbohydrates, lipids and nucleic acids. Their destructive effects on proteins may play a role in different diseases for example, cataracts, cancer, and heart disease.⁴⁰

The human body has several mechanisms to counteract the effects of free radicals. One important line of defence is a system of enzymes for example glutathione peroxidases. The second line of defence is the presence of antioxidants. An antioxidant can be defined as a molecule stable enough to donate an electron to a free radical, neutralising it, thus reducing its capacity for damage. Bagchi and Puri discuss various antioxidants including vitamin E, C and the carotenoids.⁴⁰

There are several methods of measuring antioxidant status of a biological sample. Some of the older methods include FRAP-, TEAC-, TRAP assays. This study utilizes the ORAC (Oxygen Radical Absorbance Capacity) Assay. According to Prior and Cao, the ORAC method is an improvement on previous methods as it takes the reaction between substrate and free radicals to completion and by using an area-under-curve technique for quantitation compared to the assays that only measure a lag phase.⁴¹

The principal of the ORAC method depends on the detection of chemical damage to phycoerythrin through the decrease of its fluorescence emission.⁴² The fluorescence of PE is highly sensitive to the conformation and chemical integrity of the protein. Under appropriate conditions the loss of PE fluorescence in the presence of reactive species (RS) is an indication of oxidative damage to the protein. The inhibition of the RS action by an antioxidant, which is reflected in the protection against the loss of PE fluorescence, is a measure of its antioxidant capacity against the reactive species.

Cao and Prior points out that two elements need to be considered in measuring the inhibition of the RS action by an added antioxidant sample, namely the time that the inhibition lasts and the percentage that the inhibition displays at different times.⁴²

Chemicals such as those used in hairdressing can function as free radicals that cause damage to proteins like DNA. Seeing that antioxidants is a natural mechanism of the body that fights the effects of free radicals, the antioxidant status of an individual is an indication of the measure of oxidative stress experienced by that individual. Thus, the antioxidant status measured by the ORAC method would complement the findings of the Comet Assay.

2.6.3 Air quality monitoring with a photo-ionization detector (PID)

- *What is a PID?*

For this study, it was decided to measure the levels of Volatile Organic Compounds (VOCs) inside hair salons using a photo-ionization detector (PID) or ppbRAE to give insight into the general air quality inside hair salons. What follows below is a short description of how the PID works as it pertains to its application in obtaining the ppbRAE readings used in this study.

A photo-ionization detector is a very sensitive broad-spectrum monitor like a low-level LEL monitor. It is used to measure VOCs and other toxic gases in low concentrations from ppb (parts per billion) up to 10 000 ppm (parts per million or 1% by volume).⁴³

- *How does a PID work?*

The photo-ionization detector uses an ultraviolet lamp to break down chemicals to positive and negative ions, which are then counted by the detector. Ionization occurs when a molecule absorbs the high energy UV light which excites the molecule, resulting in the negatively charged electron temporarily becoming a positively charged ion. The energy required to ionise an electron is called its Ionization Potential (IP) and expresses the bond strength of a gas or vapour. IP, just like the light from a UV lamp is measured in electron volts (eV). Thus, the gas becomes electrically charged.⁴³ In the detector these particles produce a current that is amplified and displayed on the meter as ppm (parts per million) or even ppb (parts per billion).

- *What does a PID measure?*

The PID mostly measures organic compounds containing carbon (C) atoms. Table 3 below summarises the commonly ionised chemicals and vapours.

Table 3: Ionisable chemicals and vapours by PID

Carbon compounds	Gases and vapours
Aromatics: compounds containing a benzene ring.	Benzene, toluene, ethyl benzene and xylene.
Ketones & aldehydes: compounds with a C=O bond.	Acetone, methyl ethyl ketone (MEK) and acetaldehyde.
Amines & amides: compounds containing nitrogen.	Diethylamine.
Chlorinated hydrocarbons:	Trichloroethylene (TCE) and perchloroethylene (PERC).
Sulphur compounds:	Mercaptants and sulphides.
Unsaturated hydrocarbons:	Butadiene and isobutylene.
Alcohols:	Isopropanol (IPA) and ethanol.
Saturated hydrocarbons:	Butane and octane.

In addition to the above-mentioned organic compounds PIDs can also measure some inorganic chemicals that aren't carbon-based. These are:

- Ammonia;
- Semi-conductor gases, arsine and phosphine;
- Hydrogen sulphide;
- Nitric oxide; and
- Bromine and iodine.

- *Selectivity and correction factor of a PID*

Although a PID is very sensitive and can accurately measure gases and vapours even to ppb, it isn't a selective monitor. In other words, it has very little ability to differentiate between chemicals. When encountering an unknown chemical release, the PID is set to its calibration gas of isobutylene. Once the chemical is identified, the PID sensitivity can be adjusted by using a correction factor (CF).

According to the application note of RAE systems on PID training, correction factors (also known as response factors) are scaling factors used to adjust the sensitivity for the PID to measure a particular gas directly compared to the calibration gas. The lower the CF, the more sensitive the PID is for a gas or vapour.⁴³ In this study individual gasses were not measured.

It is extremely important to calibrate the PID before and after measuring VOCs.

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CHAPTER 3: ARTICLE

Measuring DNA damage and repair as possible biological markers for long-term, low-dose chemical exposure in hairdressers.

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3.1 ABSTRACT

Hairdressers are exposed to combinations of chemicals, some of which are known or suspected allergens, carcinogens or organic solvents. The objective of this study was firstly to compare DNA damage and repair capacity between individuals with short-term exposure (STE) to those with long-term exposure (LTE) and secondly to that of control subjects. The study also wanted to show that the Comet Assay can be used in biological monitoring to measure occupational exposure in hairdressing. DNA damage test showed significantly increased baseline DNA damage among the LTE group compared to the controls ($p=0.033$), while the DNA repair capacity test showed significantly increased damage among the STE group ($p=0.0028$). The controls had least damage after T60 ($p=0.05$ compared to STE), least DNA damage after treatment with H_2O_2 and had greater DNA repair capacity than the test groups. Additionally, the controls show an increased antioxidant capacity, especially compared to the LTE group ($p=0.042$). These findings indicate possible harmful health effects and mutagenic changes hairdressers face because of constant exposure to chemicals and prove that these tests can be used as possible biological markers in this industry.

3.2 INTRODUCTION

As far as occupations go, hairdressing is certainly not considered dangerous and yet it involves the use of a variety of chemicals. Every day, clients request to have their hair coloured or bleached, relaxed or permanently waved, washed, styled and set with hairspray. This involves the use of chemicals that are classified as either known or suspected allergens, carcinogens or organic solvents.¹ Table 1 lists the chemicals commonly found in hairdressing products.^{1,2}

Table 1: Chemicals found in hairdressing products

Hairdressing products	Chemical components
Permanent wave solutions	Thioglycolates, hydrogen peroxide, ammonia, glycols
Bleaching products	Hydrogen peroxide, potassium and ammonium and sodium persulphates
Hairspray	Propellant: Propane, pentane, butane, Hand pump: ethanol, isopropanol
Setting liquids	Hydrogen peroxide, organic acids, phosphoric acid
Dye products	Aromatic hydroxy compounds: Resorcinol, a-naphtol, Hydroquinone Amines: diaminobenzene, diaminotoluene, phenylenediamine, diaminotoluene
Shampoo	Formaldehyde
General cleaners	Isopropanol, ethanol

In two separate studies there were concerns about the levels of chemicals present in hair salons especially during winter when doors and windows are kept shut and ventilation is poor. Both these studies, however, reported levels of chemicals to be well below Occupational Exposure Limits (OELs) set by their respective countries.^{1,2} Despite the low levels of chemical exposure, several studies link the hairdressing industry to disease. Furthermore, general studies about different occupations and their typical health problems, link hairdressing to Contact Dermatitis^{3,4} and occupational cancer.⁵

The most common disorder in the hairdressing industry is Contact Dermatitis (CD).⁶ Contact Dermatitis can have an irritant (ICD) or allergic origin (ACD). ICD is mainly caused by frequent use of water and shampoo, while ACD is mostly attributed to nickel, formaldehyde, fragrances, phenylenediamine, thioglycolates, persulphates and ammonium compounds.^{6,7} There seems to be a particularly high exposure to nickel because other chemicals like thioglycolate, used in permanent wave solutions, can leach nickel out of stainless steel utensils.⁶ Two studies by Kanerva *et al.*⁸ and Conde-Salazar *et al.*⁹ confirm the role of metals such as nickel, chromium and cobalt as causes of ACD, while two other studies link hairdressing to occupational asthma^{10,11} and even immediate type hypersensitivity.¹² In two separate studies done by Hsu *et al.*¹³ and Shapiro *et al.*¹⁴ phenylenediamine was found to be the cause of beard and hand dermatitis. In 2002 there was also an article on BBC News that warned people against the use of hair colour as these chemicals (*para*-phenylenediamine and tetrahydro-6-nitroquinoxaline) have been shown to damage genetic material and to cause cancer in animals.¹⁵

Thioglycolate is the active ingredient found in permanent wave solutions. It has been linked to Hairdresser's Koilonychia,¹⁶ toxifying of the vestibuloocular reflex system, which may cause hearing loss,¹⁷ and even lead to chromosomal damage that could affect the reproductive function of hairdressers.¹⁸ DNA damage was also shown to be caused by formaldehyde used in shampoos, and is thought to slow down the DNA repair process which in turn might increase the photocarcinogenic risk to humans who are exposed to this chemical.¹⁹ Another hair dye product that was shown to cause DNA damage was 2,4-diaminotoluene.²⁰ DNA damage and increased alpha tumour necrosis factor (TNF α) production was found in hairdressers with Contact Dermatitis, suggesting a possible relationship between allergen exposure, cytokine induction and allergic disease development.²¹ A case reference study was done to assess the occurrence of salivary gland cancers associated with diverse occupations and industries. The results show that salivary cancer is elevated among females working as hairdressers.²²

From the above-mentioned studies it is clear that hairdressers are exposed to a number of chemicals which could impact negatively on their health, including causing DNA damage. As far as could be determined, no studies on DNA damage in the hairdressing community in South Africa have been done before. It was therefore the aim of this study to assess the levels of DNA damage and repair capacity in a selected group of hairdressers in South Africa.

The Comet Assay (or Single Cell Gel Electrophoresis abbreviated as SCGE) is based on the ability of the negatively charged loop/fragments of DNA to be drawn through an agarose gel in response to an electric field. The extent of DNA migration depends directly on the DNA damage present in the cells.²³ The name *Comet Assay* is fitting as the migrating DNA drawn from the nucleus resembles a comet.

The ORAC test measures antioxidant capacity of different biological samples. In this case plasma was used. It is well-known that free radicals cause damage to DNA and other cellular macromolecules, and that the antioxidant status gives an indication of the body's ability to combat this damage.²⁴ It was decided to include this test to see if there is any significant difference in the antioxidant status between the different test groups, which could perhaps be linked to oxidative stress caused by constant chemical exposure.

3.3 METHODOLOGY

3.3.1 Sampling strategy and group selection

Hairdressers and control subjects were recruited from the Potchefstroom area. The hairdressers were divided into two groups, namely those who have been in this occupation for longer than ten years (LTE group) and those with less than five years hairdressing experience (STE group). The age, gender and smoking habits of these groups matched that of the control subjects (CTR group) who were also people working and living in the Potchefstroom area, but who were not exposed to hairdressing chemicals, other than the occasional hair dye or highlight. This study was carried out with the written approval of the ethics committee of the North-West University (Ethical committee approval number: 05M17) and the written consent of the participants.

3.3.2 Air Sampling

Levels of Volatile Organic Compounds (VOC) were measured inside selected hair salons using a ppbRAE with a photo-ionisation detector to give insight into the chemical exposure of hairdressers and their assistants. Measurements were taken while the hairdressers or assistants were applying

colour to hair, or were drying and styling hair with hairspray. CO and H₂S measurements were taken using an EntryRAE at the same time the ppbRAE was used for VOC measurements. Both these instruments were calibrated before and after monitoring.

3.3.3 Comet Assay

A heparinised blood sample was taken from each subject and were kept cool and brought back to the lab within 2 hours. No more than 6 samples were taken at a time to expedite their processing. Lymphocytes were isolated by centrifugation at 1600 x g for 20 minutes at room temperature on a Histopaque (Sigma) density gradient. The buffy coat was washed in cold PBS and cell suspensions of 2 000 viable cells per μ l were prepared. The viability was determine with Trypan-Blue exclusion technique. The Comet Assay was performed as previously described by Kumaravel and Jha²³ with the inclusion of treatment of the nucleoids with the lesion-specific enzymes FPG (formamidopyrimidine DNA glycolylase) and Endo III (Endonuclease III).

In short, 30 μ l of the cell suspension was mixed with 90 μ l of 0.5% low melting point agarose and spread evenly on a slide pre-coated with 1% high melting point agarose. Three slides were used per sample: one as control (untreated), one for treatment with FPG and one for treatment with Endo III. All the slides were left overnight in lysing solution (containing 5M NaCl, 0.4 M EDTA, 1% Triton X-100 and 10% DMSO) at 4°C. One slide per sample was left as control and the other two slides were washed and treated with 50 μ l each of the lesion-specific enzymes FPG and Endo III. The treated slides were left to incubate in a damp container for 30 minutes at 37°C. Thereafter the slides treated with lesion-specific enzymes as well as the control slides were washed in ddH₂O and left for 30 minutes to alkaline unwind in the electrophoresis buffer (0.3M NaOH and 0.05M EDTA made up with ddH₂O). After 40 minutes of electrophoresis at 30V and 290mA, the slides were neutralised in Tris buffer and stained with ethidium bromide. For each sample a minimum of 50 comets were photographed using an Olympus IX 70 fluorescent microscope and analysed with the CASP programme. The percentage tail DNA (%DNA migrated form the head) and Tail Moment (%DNA X Tail length) were used as parameters and these sets of data were analysed in Excel.

To measure DNA repair capacity 360 μ l of each cell suspension was mixed with 40 μ l H₂O₂ (final concentration of 60 μ M H₂O₂) and incubated for 10 minutes at 37°C. The treated cell suspensions were centrifuged immediately and washed at least twice with cold PBS. For each treated sample 30 μ l was mixed with 90 μ l of 0.5% low melting point agarose, spread on a slide and marked as H₂O₂ treated cells. The rest of the cells were washed again with PBS, re-suspended in HAMS medium and then incubated at 37°C, to aid the repair process. After 30 minutes, 30 μ l of this cell

suspension was mixed with 90 µl of 0.5% low melting point agarose and spread on a slide. The rest of the cell suspension was left to incubate another 30 minutes. Again 30 µl of this cell suspension was mixed with 90 µl of 0.5% low melting point agarose, and spread on a slide. The cells were then subjected to the Comet Assay. The DNA repair capacity was calculated as follows:

$$RC = 1 - \left\{ \frac{\% \text{Tail DNA after 60 minutes}}{\% \text{Tail DNA after H}_2\text{O}_2 \text{ treatment}} \right\}$$

3.3.4 Antioxidant Status

3.3.4.1 The ORAC method

During isolation of lymphocytes, plasma was extracted in eppendorph tubes, mixed with perchloric acid (1:1, v/v) and frozen. Treating serum/plasma with perchloric acid preserved ascorbic acid and ensured ORAC was measured in the non-protein fraction of plasma. The ORAC method used was previously described by Prior and Cao²⁸ and required that serum samples be thawed and centrifuged for 10 minutes at 16 000g and 4°C. Supernatant was recovered and diluted 1:10 with phosphate buffer of 75mM and pH 7.4 (containing K₂HPO₄ and NaH₂OP₄ in H₂O). 20 µl of this dilution was used for analysis. For the reaction to take place, Trolox standards (Trolox, 250 µM – prepared fresh every 3 months) were prepared with six different end concentrations namely, 0 µM, 2.5 µM, 5 µM, 10 µM, 15 µM and 20 µM. Some 20 µl of each of the different concentrations was loaded into a microtitre plate in duplicate. Then 20 µl of the diluted test samples was loaded into the rest of the microtitre plate in triplicate. 80 µl of fluorescein solution (Fluorescein (Sigma) made to stock solution of 265 mM and working solution of 112 nM) was added to each well. The microtitre plate was then loaded in a Biotec FL600 plate reader (fluorescence spectrophotometer) which was set to 25°C to check fluorescence.

The reaction was started by adding 100 µL AAPH (working solution at 48 mM) with 1000 µl stepper. The above-mentioned stock solution (2,2 Azobis (2-amidinopropane) dihydrochloride) was made to 72 mM. AAPH needs to be prepared fresh before use and kept on ice. The reader was initialised to record fluorescence (excitation 485nm, emission 520nm, static mode) every 5 minutes for 3 hours. Readings were taken until the final reading declined to +/- 5% of the initial reading. Results were calculated using the principal described by Prior and Cao.²⁸

3.3.5 Statistical analysis

Comet Assay data were analysed using one-way ANCOVA adjusted for age, smoking and gender. Non-normally distributed data were transformed using logarithmic transformations. Logarithmic

transformations were analysed using Tuckey HSD and Kruskal-Wallis tests with multiple comparisons. Statistical significance was taken at $p=0.05$ and less.

3.4 RESULTS

3.4.1 Subject information

The information about the test and control subjects gathered from the questionnaires is given in Table 2. The STE group consists of trainee hairdressers or those who have just started out in their careers and are therefore very young compared to the LTE group. The LTE group is on average about 24 years older than the STE group, with many more years of occupational exposure.

Table 2: Subject information

	CONTROL GROUP (CTR)	SHORT-TERM EXPOSURE GROUP (STE)	LONG-TERM EXPOSURE GROUP (LTE)
Total no of subjects	11	10	10
Male or Female	2M + 9F	10F	2M + 8F
Average age	35.8	20.7	44.6
Hairdressing experience	None	1 – 4 Years 2.6 (average)	10 – 42 Years 24.3 (average)
Smoking	6	4	5
Non-smoking	4	5	4
Ex-smokers	1	1	1

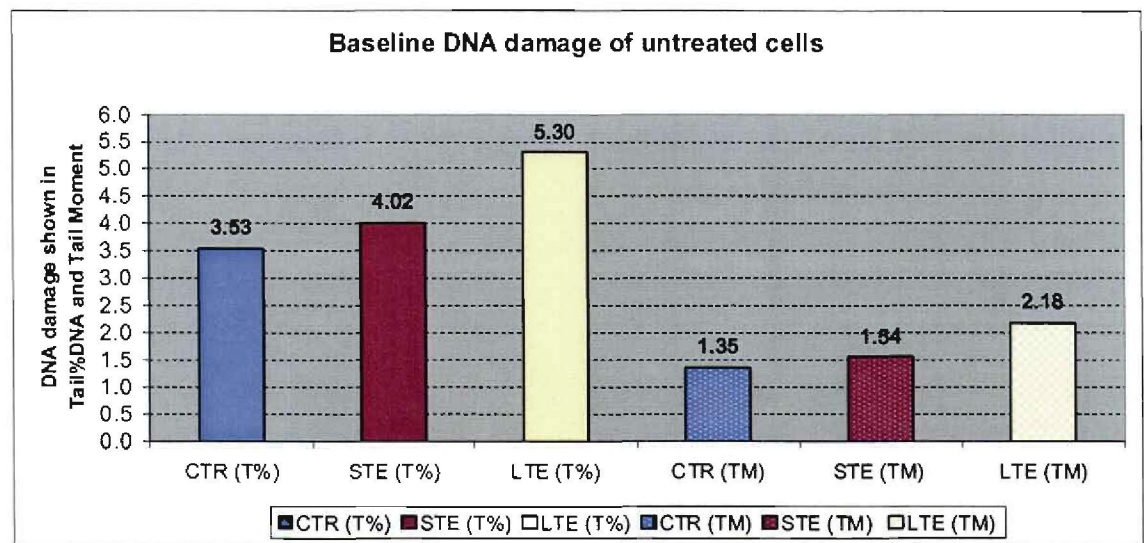
In choosing the control group, both very young and much older control subjects were chosen to try to minimise the effect of age. The subjects in the control group are on average about 15 years older than the STE group, and only about 9 years younger than the LTE group. The distribution of smoking and non-smoking individuals was fairly equal in the different groups.

3.4.2 Comet Assay

- *DNA damage test results*

Figure 1 shows results of the baseline DNA damage of untreated cells in this test in both Tail%DNA and Tail Moment respectively. Finding the best set of data to interpret Comet Assay results is recognised as one of the biggest problems when doing Comet Assays. In a recent article by Kumaravel and Jha²³, a study was done to evaluate the given parameters of Comet software programs. It was found that Tail%DNA gave the best correlation and could be considered most meaningful to interpret results. In this study good correlation was seen between Tail%DNA and Tail Moment, with Tail Moment giving better definition between the groups for some sets of results. It was therefore decided to use both sets of data.

Figure 1: Baseline DNA damage given in both Tail%DNA (T%) and Tail Moment (TM)



As can be seen from Figure 1, the untreated cells of the CTR group showed the least baseline damage before any treatment of the cells. There seems to be a small trend of DNA damage increasing from the CTR group to the STE group to the LTE group, with a significant difference of $p=0.033$ in Tail%DNA between the CTR and LTE groups and $p=0.047$ in Tail Moment.

Figure 2 shows the DNA damage averages after treatment with lesion-specific enzymes FPG and Endo III with Tail%DNA used as parameter.

Figure 2: DNA damage averages of FPG and Endo III given in Tail%DNA

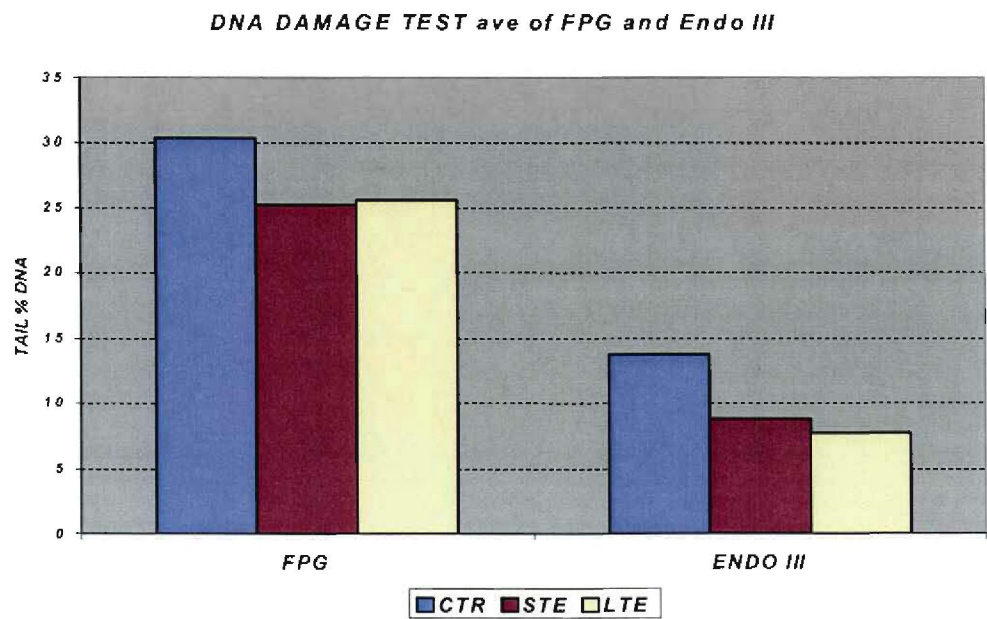


Figure 3 shows DNA damage averages after treatment with lesion-specific enzymes FPG and Endo III with Tail Moment used as parameter.

Figure 3: DNA damage averages of FPG and Endo III given in Tail Moment

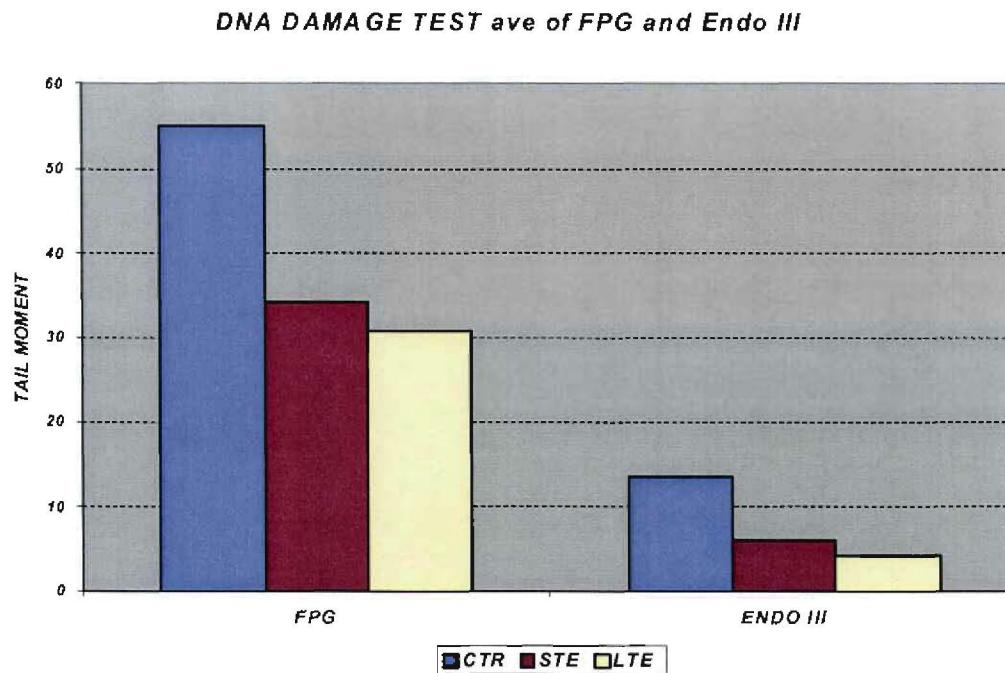


Figure 2 shows that after treatment with FPG and Endo III, the DNA damage is greater in the control group than in the test groups, with significant differences of $p=0.002$ between CTR and LTE and $p=0.010$ between CTR and STE in Tail%DNA. Figure 3 shows that in Tail Moment these differences are $p=0.0002$ and $p=0.002$ respectively. Also, in all three the groups it's clear that FPG has more target sites than Endo III.

- *DNA repair results*

Figures 4 and 5 summarise the results of the DNA repair tests. Figure 4 gives the results in Tail%DNA while figure 5 shows the results in Tail Moment..

Figure 4: DNA repair (Averages as given in Tail%DNA)

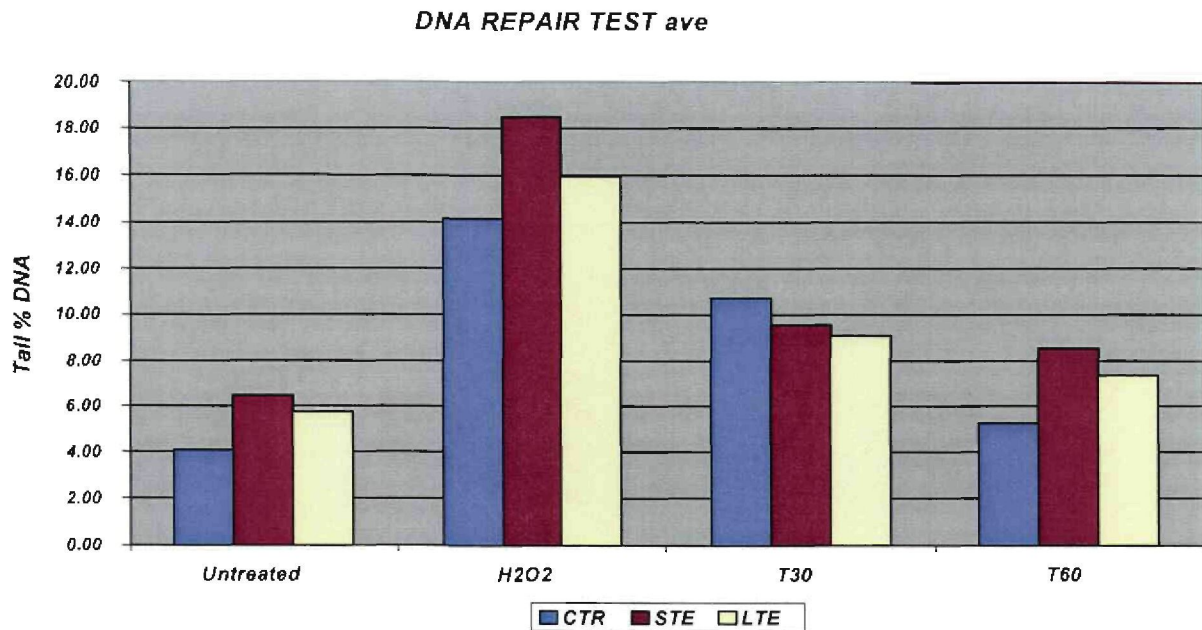
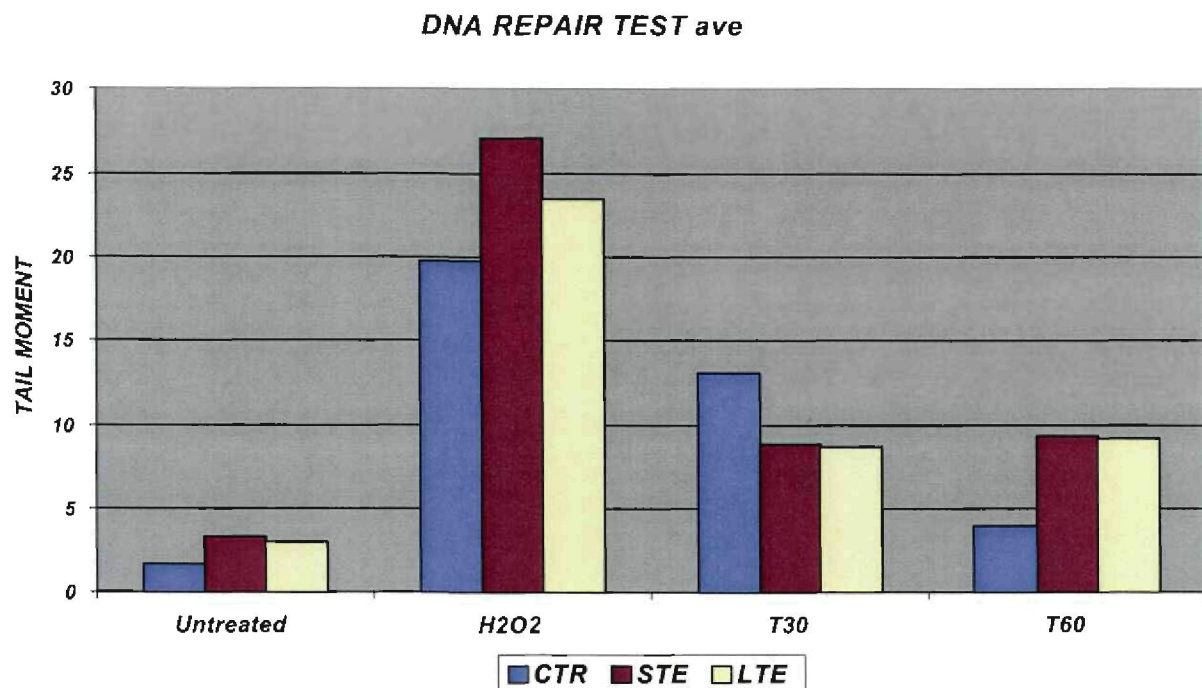


Figure 5: DNA repair (Averages as given in Tail Moment)



For all 3 of these test groups, a control slide was made using freshly isolated lymphocyte that hadn't had any treatment. The untreated cells of the control group show the least amount of baseline damage compared to the untreated cells of the test groups, with a significant difference

between the CTR and STE groups of $p=0.0028$ in Tail%DNA and $p=0.037$ in Tail Moment, as seen in Table 3. This serves as confirmation of the results given in figures 1 - 3.

Table 3: Baseline damage in untreated cells

Baseline damage in untreated cells			
	CTR	STE	LTE
Tail%DNA	4.05	6.46	5.75
Tail Moment	1.69	3.25	2.98

Furthermore, and especially defined by Tail Moment, the control group shows much less DNA damage after treatment with hydrogen peroxide than the test groups. An interesting observation is the fact that, when comparing only the STE and LTE groups, baseline DNA damage and the amount of damage after hydrogen peroxide treatment are greater in the STE group than the LTE group. Damage after hydrogen peroxide treatment can be seen in Table 4.

Table 4: Damage after treatment with hydrogen peroxide

Damage after treatment with hydrogen peroxide			
	CTR	STE	LTE
Tail%DNA	14.13	18.53	15.97
Tail Moment	19.74	27.12	23.44

Table 5 shows DNA damage after 60 minutes of repair time. The control group again shows least DNA damage, with a significant difference being found between the control group and STE groups of $p=0.05$ in Tail%DNA and $p=0.037$ in Tail Moment.

Table 5: Damage after 60 minutes repair time

Damage after 60 minutes repair time			
	CTR	STE	LTE
Tail%DNA	5.26	8.54	7.31
Tail Moment	3.97	9.32	9.16

DNA repair capacity is the ability of DNA to repair itself after damage occurs. Inability or poor DNA repair capacity is linked to an increased risk of cancer.^{29,30} DNA repair capacity is calculated according to the formula below:

$$RC = 1 - \left\{ \frac{\% \text{Tail DNA after 60 minutes}}{\% \text{Tail DNA after H}_2\text{O}_2 \text{ treatment}} \right\}$$

The DNA repair capacity of the three groups is shown in Table 6 below.

Table 6: DNA repair capacity of the different groups

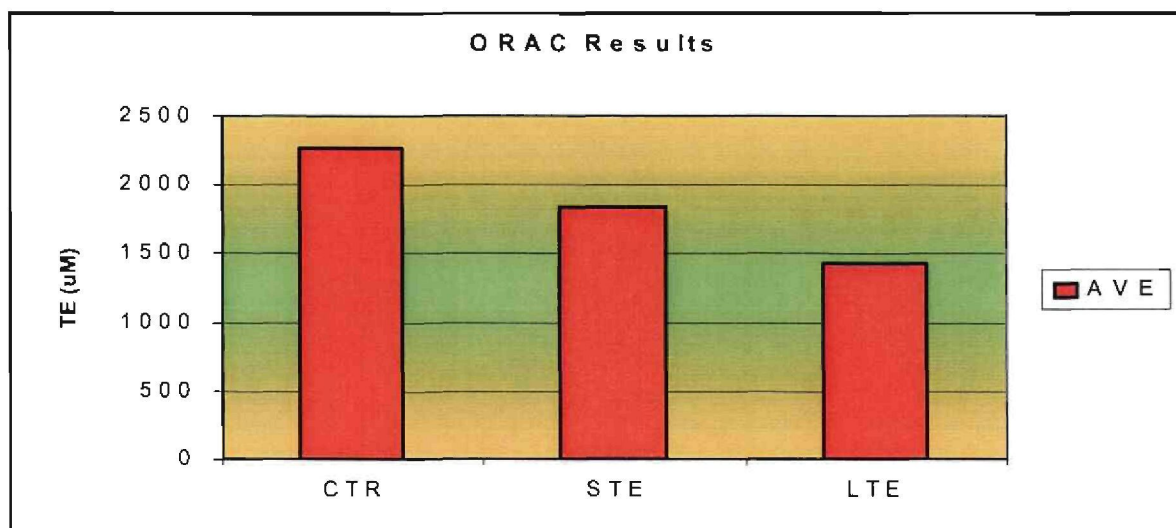
Repair capacity values			
	CTR	STE	LTE
Tail%DNA	0.63	0.54	0.54
Tail Moment	0.80	0.66	0.61

Both Tail%DNA and Tail Moment show greater DNA repair capacity among the control group than the test groups, with better definition in Tail Moment. In addition, Tail Moment also shows a slightly better DNA repair capacity for the STE group when compared to the LTE group.

3.4.3 ORAC results

The ORAC test measures antioxidant capacity of different biological samples. In this case plasma was used. Figure 6 shows the results of the ORAC test for the different groups. Antioxidant capacity is measured in TE (μM).

Figure 6: ORAC results of the different test groups



From Figure 6 the following conclusions can be made:

- the Control Group (CTR) shows the best antioxidant capacity with a significant difference of $p=0.042$ between the CTR and LTE group.
- employees who have worked in the hairdressing industry for less than 5 years (STE) show better antioxidant capacity than employees who have worked in the hairdressing industry for longer than 10 years (LTE), although this difference is not statistically significant.

3.4.4 Direct reading results

Levels of VOCs, CO and H₂S were well below the standards given in OSHA and ACGIH. Only in two salons, did the average levels of VOCs exceed 2ppm. In the first salon average levels, taken at two different points, measured 5.6 ppm and 4.7 ppm respectively, with maximum levels measuring 10.2 ppm and 5.5 ppm. In the second salon averages measured were 4.3 ppm and 1.6 ppm, with maximum levels measured at 16.5ppm and 3.5ppm.

3.5 DISCUSSION

3.5.1 Comet Assay findings

From the results it is clear that the control group (CTR) showed the least baseline damage, when compared to the test groups. In the DNA damage test there is significant difference between the control group and LTE group in both Tail%DNA and Tail Moment. In the DNA repair test there are significant differences between the control group and STE group, in both Tail%DNA and Tail Moment. This means that the baseline DNA damage of the test groups are higher than that of the control group before any treatment of the cells started. This could be an indication of genomic instability due to occupational exposure.

In the DNA damage test, treatment with FPG caused much more DNA damage than treatment with Endo III. The use of lesion-specific enzymes is a useful modification of the Comet Assay because it increases both its sensitivity and specificity.²⁶ FPG, according to the manufacturer (New England Biolabs), act both as a N-glycosylase and as AP-lyase. The N-glycosylase activity releases damaged purines from double-stranded DNA, generating an apurinic site (AP-site). The AP-lyase activity recognises damaged purines especially 8-oxyguanine. Endo III, releases damaged pyrimidines from double stranded DNA.²⁶ This shows that FPG enzymes found more target sites and that there are more oxidative damage to purines than to pyrimidines.

However, whether the FPG or Endo III were used, the control group, surprisingly, showed the most DNA damage after treatment with lesion-specific enzymes. Andersson and Hellman²⁷ researched the different roles of FPG and Endo III on catechol-induced DNA damage in extended-term cultures of human lymphocytes. Their study showed that low concentrations of catechol lessened the damage caused by FPG and they speculated that low concentrations of catechol could have an antioxidant effect, actually helping protect DNA from damage. Therefore one explanation for the control group showing more DNA damage *after* treatment with lesion-specific enzymes could be that certain hairdressing chemicals show an antioxidant effect that makes the DNA damage of the

test groups, picked up by the FPG enzyme, appear lower than that of the control group. However, this low-dose chemical exposure does not protect against all oxidative damage and that is why the ORAC test still shows the control group to have the best antioxidant status.

Another possible explanation could be the adaptive response of the human body. When strenuous exercise is performed, oxidative damage occurs to the DNA as was found in both human and animal studies. This theory is supported by three different studies^{31,32,33} that found that constant, regular exercise caused an adaptive response, whereby the body up-regulates DNA repair enzymes so that oxidative damage to the DNA normalises. It is plausible to argue that a similar adaptation occurs here, although chemical and not physiological, with the body of the hairdresser adapting to the constant exposure to chemicals that cause oxidative damage, by increasing the efficiency of DNA repair mechanisms and thereby normalising levels of damage. Thus, because of the up-regulated DNA repair mechanisms, there is less DNA damage in the test groups (STE and LTE) than in the control group (CTR) after treatment with lesion-specific enzymes.

The DNA repair capacity test shows an overall better capacity of the CTR group to deal with the DNA damage inflicted by hydrogen peroxide and then to repair that damage. This is remarkable when the age difference between the control group and the test groups is taken into account, seeing that the average age for the control group is 35.8 years compared to the 20.7 years of the STE group.

Even though the control group was carefully selected to take into account age and smoking habits, it is easy to expect that the younger STE group should have the least baseline damage and be able to repair DNA damage better than the older groups. In a review article³⁴ that investigated the effect of age on the Comet Assay, 20 articles were reviewed and found that only four of these showed significant correlation between age and level of DNA damage. They attributed this to the fact that young subjects are more physically active which could increase the level of baseline DNA damage and mask the age-related variation in DNA damage between the old and the young. It is the opinion of the researcher that the poor DNA repair capacity of the young STE group, when compared to the older control group, is yet another indication of the severity of DNA damage due to the occupational exposure that the STE group endures.

3.5.2 Antioxidant status findings

The ORAC test measures antioxidant status and, as expected, the CTR group shows the best antioxidant status. Antioxidants are used to combat free radicals, which could harm structures in

the body such as proteins (DNA). A high antioxidant status would be an indication of low levels of oxidative stress. This shows the control group to have the least oxidative stress. It is the opinion of the researcher that the control group shows the least oxidative stress, because they do not have the same constant chemical exposure that causes oxidative stress in the two test groups.

3.5.3 Air quality findings

Measurements were taken in hair salons while hairdressers or their assistants were applying colour, or drying and styling hair using hairspray. These measurements can be compared to the STEL values given in OHSA³⁵ and ACGIH³⁶. OHSA standards refer to the South African standards as given in the Occupational Health and Safety Act, Act 85 of 1993, while ACGIH refers to the international standards set by the American Conference of Governmental Industrial Hygienists. According to the definition of the OEL-STEL in ACGIH, exposure above the TWA up to the STEL value (but not exceeding this value) should be less than 15 minutes, should occur less than four times per day, and there should be at least 60 minutes between successive exposures in this range.

Similar to the findings by Hollund and Moen¹ and van der Wal *et al.*², the levels of VOCs, CO and H₂S in the hair salons in Potchefstroom were well below the standards given in OHSA and ACGIH. The two salons where the average levels of VOCs exceeded 2ppm and maximum levels exceeded 10ppm and 16ppm, are worrisome. The STEL values for formaldehyde are set quite low 2ppm in OHSA, with a ceiling limit of only 0.3ppm according to ACGIH. The ceiling limit according to ACGIH should not be exceeded at *any* time. It is quite plausible to argue that in some instances, in especially these two salons, the STEL and ceiling limits for formaldehyde are exceeded and that it certainly could happen for longer than 15 minutes at a time and occur more than four times per day, depending on how busy the salons are at any given time. A peak value of 16.5ppm could even exceed the limits for hydrogen sulphide. This possibility needs to be investigated further.

3.6 CONCLUSIONS AND RECOMMENDATIONS

In this study two versions of the Comet Assay showed significantly greater baseline DNA damage in the untreated cells of the test groups compared to the control group. The DNA repair capacity test shows significantly greater DNA damage after 60 minutes of repair time in the test groups compared to the control group, as well as decreased DNA repair capacity. Therefore, this study finds that the Comet Assay is a reliable method that could be used in biological monitoring to test the effects of chemical exposure in the workplace. Additionally, the antioxidant capacities of the test

groups are significantly less than that of the control group. It is concluded that these results indicate a very real possibility of change in genetic material due to occupational exposure in the workplace.

It is important to note that although individual VOC levels could not be measured, and although all the measurements were found to be relatively low, these results underline the importance of this study: people working in the hairdressing community are exposed to low levels of combinations of chemicals during their entire careers which could be 40–50 years. The real concern is that DNA damage and decreased DNA repair capacity could lead to changes in genetic material and this in turn could lead to mutagenic changes and possibly cancer.

It is crucial to acknowledge that hairdressing personnel could be overexposed to certain chemicals on a daily basis throughout their careers. It is recommended that screening should be done for students in this industry as Contact Dermatitis and allergies are the most common health problems associated with the hairdressing industry.

The training of hairdressers should address the following:

- the hazards of working with chemicals on a daily basis,
- the importance of proper ventilation in the chosen work space, whether the salon is located in a shopping mall, house or separate shop,
- the necessity and proper use of personal protective equipment e.g. gloves, safety glasses and respirators.

Finally this study recommends more detailed studies be done to investigate the occupational risks in the hairdressing industry.

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CHAPTER 4: RECOMMENDATIONS

4.1 Recommendations

This study set out to show that long-term, low-level exposure in the hairdressing industry could impact negatively on the health of those working in this environment. The biological markers chosen were DNA damage and repair, and indeed there were clear differences between the test and control subjects. The results from this study certainly indicate possible genetic changes due to occupational exposure of hairdressers. It is therefore concluded, that this study proved all three the hypothesis proposed, to be correct. However, this should be regarded as a pilot study and a follow-up study with larger test and control groups would be needed to comprehensively research the extent and effects of exposure in the hairdressing industry. In this study samples were taken during summer months when salons without extraction ventilation would have their doors and windows open. The exposure during winter periods could be much higher. All sampling was done during the beginning of the week as this was more convenient for the hairdressers, but business picks up by the end of the week and there is a possibility that if sampling was done on busy Fridays and Saturdays, the DNA damage might have been more due to chemical build up during the week.

Allergic Contact Dermatitis is the most common disorder¹ among hairdressers. Apprentices represent more than 70% of the total number of effected hairdressers.¹ It is therefore recommended that trainee hairdressers should first be screened for skin sensitivities and susceptibility for allergies before commencing with their training as allergies to metals and rubber gloves are common and this condition will only worsen over the duration of their careers.^{1,2}

During the training of new apprentices, it is important that training colleges should include the following components in their training program;

- The dangers of working with chemicals on a constant long-term basis.
- The health effects of chemicals and selection of safe products rather than those that are perhaps less safe, but more economical. The decision-making process needs to change.
- Importance of proper ventilation especially in small businesses that don't have extraction ventilation, but rely instead on natural dilution ventilation. Where natural dilution ventilation is used, there should be an awareness that seasonal differences could lead to increased exposure when doors and windows are kept closed during winter periods.
- Choosing appropriate PPE and the proper use thereof.

Lind *et al.*³ studied the permeability of different chemicals used in hairdressing products through natural rubber, Polyvinylchloride (PVC), Nitrile rubber (NR) and Polyethene gloves. Their study

found that natural rubber gloves have the lowest breakthrough time, making this the safer option. Unfortunately, many people are allergic to natural rubber and since allergic reactions can be life-threatening, Lind *et al.*³ recommend NR or PVC gloves as the best choice in hair dressing. Gloves should not be reused or turned inside out. Gloves should always be used when working with chemicals, not only when working with dyes. Additionally, safety glasses and respirators should be considered when mixing chemicals even though these might not necessarily be specified by the manufacturer.

Although hairdressing involves the use of combinations of different chemicals, this industry is not formally regulated. According to the Occupational Health and Safety Act (OHS act) and Hazardous Chemical Substance regulations it is required for employers to:

- Provide a safe environment to work in;
- Ensure entry en exit medical examinations for employees;
- Keep all medical records for at least 40 years; and
- Provide PPE to employees free of charge.

There are currently no formal guidelines or regulations specifically regulating occupational hygiene in the hairdressing industry in South Africa. The Government Gazette⁴ published in 2001 refers to the qualifications of hairdressers and the content of their training modules, but makes no mention of any health and safety requirements for this specific industry. At least in Europe, the European Agency for Safety and Health at work⁵, formulated a questionnaire to help the hairdresser do a basic risk assessment. A basic occupational guideline also exists in Australia (New South Wales) that broadly describes and advises hairdressers on health and safety issues.⁶

It is the opinion of this researcher that a basic guideline on health and safety in the workplace should be compiled for hairdressers in South Africa to use as a reference, and that some occupational health regulations from the OHS Act should be made applicable to this industry and be enforced.

From a product stewardship and responsible care perspective, it is recommended that manufacturers of hairdressing chemicals should try to produce the safest possible products considering the possible mutagenic effect of some of these chemicals. It is also recommended that all products must be supplied with safe handling instructions (Safety Data Sheets) that explain the health and safety risks of the product and underline the correct handling procedures.

4.2 REFERENCES

1. Ferrari M, Moscato G, Imbriani M. Allergic cutaneous diseases in hairdressers, Med. Lav. 2005;96(2):102-118.
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3. Lind M-L, Johnsson S, Meding B, Boman A. Permeability of Hair Dye Compounds *p*-Phenylenediamine, Toluene-2,5-Diaminesulfate and Resorcinol through protective gloves in hairdressing. Annal of Occupational Hygiene. 2007;51(5):479-485.
4. South Africa. South African Qualifications Authority: Hairdressing, Cosmetology and Beatty. Government Gazette (429) no 22126, general notice notice 559 of 2001. March 2001.
5. European Agency for Safety and Health at work. Available from: <http://osha.europa.eu>
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CHAPTER 5 - ADDENDUM

5.1 HEALTH AND EMPLOYMENT QUESTIONNAIRE

Thank you for your willingness to participate. Your cooperation is very important to the success of the study. All information will be treated as confidential and will not be released to another party without your consent. Your name will also not be linked to results stated in the study. NB! Please note that should you agree to give a 5 ml blood sample at a later stage, it will only be used for Comet Assay and antioxidant tests.

Date:		Subject number:	
1. Subject information:			
Age:		Gender:	
City where you live:			
2. Occupation:			
Job Title:			
Which tasks do you perform?		How often (e.g. 3/week or 1/month)	
Shampoo/condition:			
Cut:			
Blow drying:			
Perms:			
Straighten/relax:			
Colouring:			
Bleach/Highlight:			
Set/Hairspray:			
Other:			
Other:			
For how many years have you been doing this work?			
Working Hours?			
3. Health:			
Do you take any medicine?			
List current medication (like for flu):			
List chronic medicines (like for arthritis, diabetes, high blood pressure, high cholesterol, etc.)			
Do you have a family history of allergies? (like sinusitis, rhinitis, asthma, skin) Name these if any.			
4. Supplements:			
Do you take any health supplements e.g. multivitamins (especially Vit. C and E, Selenium) or antioxidants e.g. Procydin, Pro-oxydin, Vitaforce Ant-oxidant, Rooibos Figurets or Herbal teas like Rooibos, Honeybush, Chinese Green tea.			
Please list:			
5. Alcohol:			
Do you use alcohol?			
Beer	Wine	Hard Liquor	
How much and how often?			

6. Smoking:	
Do you smoke?	
How many per day?	
For how long have you been smoking?	
If no, have you smoked in the past?	
How many and for how long?	
How many people in your household smoke?	
7. Hypersensitivity reactions	
A. Do you suffer from any of the following?	
Cough:	Shortness of breath:
Tightness in the chest:	Wheezing:
Decreased ability to do heavy exercise:	
How long ago did your symptoms start?	
Have you ever been diagnosed with allergic asthma?	
B. Sinusitis:	
Headache:	
How long have you been suffering from these symptoms?	
C. Do you have any of the following on your hands or arms...	
redness:	swelling:
Itch/pain:	crust formation:
flakiness:	
How long ago did your symptoms start?	
Have you ever been diagnosed with allergic/Contact Dermatitis?	
8. Physical Exercise:	
Social – list:	
How often:	
Competitive (like Argus, Comrades, Two Oceans, etc.)	
List:	
How often:	
9. Hobbies:	
Do you do any of the following on a regular basis as a hobby or for an additional income?	
Spray-painting	Glass staining
Do you regularly come in contact with solvents or volatile chemical substances outside of work?	
Benzene	Petrol (degreasing e.g. car parts)
Thinners	Glue or glue products
10. What do you use for heating or cooking? (e.g.. electricity, gas, fire, paraffin)	

5.2 Afrikaans copy of consent from

TOESTEMMINGS VORM

Aan die ondertekenaar van die toestemming:

U word uitgenooi om deel te neem aan die navorsingsprojek. Dit is belangrik dat u die volgende algemene beginsels, wat op alle deelnemers aan ons navorsingsprojekte van toepassing is, sal lees en verstaan:

1. Deelname aan die projek is heeltemal vrywillig en geen druk, hoe subtiel ookal, mag op u geplaas word om deel te neem nie.
2. Dit is moontlik dat u persoonlik nie enige voordeel uit u deelname aan die projek sal trek nie, alhoewel die kennis wat deur middel van die navorsing opgedoen mag word andere tot voordeel kan strek.
3. Deur toe te stem tot deelname aan die projek, gee u ook toestemming dat die data wat gegenereer sal word, deur die navorsers vir wetenskaplike doeleindes na goeddunke gebruik kan word met die voorbehoud dat dit vertroulik sal wees en dat u naam nie sonder u toestemming met enige van die data verbind sal word nie.
4. Dit staan u vry om uself te enige tyd sonder opgawe van redes aan die projek te onttrek en u sal op geen wyse daardeur benadeel word nie. U word egter vriendelik versoek om nie sonder deeglike besinning aan die projek te onttrek nie, aangesien dit o.a. die statistiese betroubaarheid van die navorsingsresultate nadelig mag beïnvloed.
5. 'n Samevatting van die aard van die projek, die vermeende risikofaktore, faktore wat moontlik ongerief of ongemak vir u kan veroorsaak, die voordele wat verwag kan word en die bekende en/of waarskynlike permanente nagevolge wat u deelname aan die projek op u as deelnemer mag hê, word in Deel 1 van hierdie dokument vir u uiteengesit.
6. U word aangemoedig om op enige stadium enige vrae wat u in verband met die projek en die prosedures in verband daarmee mag hê aan die projekteier of sy personeel te stel, wat u navrae graag sal beantwoord. Hulle sal ook die navorsing volledig met u bespreek.
7. Indien u minderjarig is, is die skriftelike toestemming van u wettige voog (biologiese ouers of wetlike voogde) nodig alvorens u aan hierdie projek mag deelneem asook u (skriftelik indien moontlik) instemming om deel te neem – geen dwang mag op u geplaas word nie.
8. U word daarop gewys dat van u vereis word om die Universiteit te vrywaar teen aanspreeklikheid weens skade, besering of dood tensy nalatigheid aan die kant van die Universiteit of enige van sy werknemers of studente voorgekom het. Indien u getroud is, word van u eggenoot/e vereis om afstand te doen van enige eise wat hy/sy andersins teen die Universiteit sou kon hê as gevolg van enige benadeling of die dood van u weens die projek/proef in Deel 1 uiteengesit tensy dit aan die nalatigheid van die Universiteit, sy werknemers of studente te wyte is.

Toestemming

Titel van projek: Comet Assay analysis of DNA damage and repair in hairdressers exposed to occupational chemical substances.

Ek, die ondergetekende (volle name) het die voorafgaande gegewens in verband met die projek/proef hierbo genoem gelees en ook die mondelinge weergawe daarvan aangehoor en ek verklaar dat ek dit verstaan. Ek was die geleentheid gegun om tersaaklike aspekte van die projek met die projekteier te bespreek en ek verklaar hiermee dat ek vrywillig aan die projek/proef deelneem. Ek gee hiermee my toestemming om as proefpersoon/deelnemer in bogenoemde projek op te tree.

Ek vrywaar hiermee die Universiteit asook enige werknemer of student van die Universiteit, teen enige aanspreeklikheid wat teenoor my, in die loop van die projek/proef mag ontstaan tensy nalatigheid aan die kant van die universiteit voorgekom het.

Ek onderneem verder om geen eise teen die Universiteit in te stel weens skade of persoonlikheidsnadeel wat ek weens die projek/proef of die toedoen van ander proefpersone mag ly nie, tensy dit aan die nalatigheid van die Universiteit, sy werknemers of studente te wyte is.

.....
(Handtekening van proefpersoon/deelnemer)

Onderteken te op

GETUIES

1. 2.

Onderteken te op

Vir alle navorsing en intervensies op proefpersone onder die ouderdom van 21 jaar is die skriftelike toestemming van die wettige voog nodig.

Hiermee gee ek (volle name)
wettige voog van die proefpersoon/deelnemer hierbo genoem toestemming dat hy/sy aan hierdie projek mag deelneem en ek vrywaar hiermee die Universiteit asook enige werknemer of student van die Universiteit, teen enige aanspreeklikheid wat teenoor my in die loop van die projek mag ontstaan tensy sodanige besering, skade of dood veroorsaak is deur die nalatigheid van die Universiteit, sy personeel of sy studente.

Handtekening: Datum:

Verwantskap:

Vir navorsing op getroude proefpersone/deelnemers is die onderstaande vrywaring deur die eggenoot/-e nodig:

Hiermee onderneem ek, ... (volle name), die eggenoot/-e van die deelnemer in hierdie aansoek, om geen eis teen die Universiteit in te stel vir behandeling weens besering, skade of die dood van die gemelde persoon as gevolg van die projek in hierdie aansoek uiteengesit of die toedoen van 'n ander proefpersoon/deelnemer nie, tensy sodanige besering, skade of dood veroorsaak is deur die nalatigheid van die Universiteit, sy personeel of sy studente.

Handtekening: Datum:

Verwantskap:

5.3 GUIDELINES FOR AUTHORS

Researchers wishing to submit their studies for publication in **Occupational Health Southern Africa** should follow the guidelines below:

Guidelines for articles:

The article should follow the format of: Introduction, Methodology, Results, Discussion and References. The length should be between 2 000 and 2 500 words. The article must include a short abstract of less than 150 words.

The manuscript should be typed in 1.5 spacing, using only one side of the paper. Pages should be numbered consecutively and leave wide margins. Illustrations, tables and graphs should be clearly identified and should be submitted separate to text in electronic format.

A separate title page should contain the title, the author(s)' full names and contact details for correspondence. A word count should be included on this page.

References should be inserted in the text as superscript numbers and listed at the end of the article in numerical order. References should be set out in the Vancouver style.