DNA damage and repair in nail technicians caused by occupational exposure to volatile organic compounds.

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Preface

The mini- dissertation was written in article format. The study was conducted on the exposure of nail technicians to hazardous chemical substances (HCS) in their everyday work environment. The genotoxicity of various HCS were also investigated, using the comet assay method to determine DNA damage, as well as the DNA repair capacity. A control group was chosen, with the same confounding factors, which were smoking and age, as the experimental group.

Chapter 1 reflects a general introduction with the relevant literature of the various compounds found in nail salon products, as well as a description of nail salons in general. The aims and hypothesis of this study is also included in this chapter. Chapter 2 consists of an in depth discussion on the health effects of the volatile organic compounds found in nail salons. DNA repair mechanisms and DNA damage are discussed in this chapter of the manuscript. Chapter 3 includes tables and figures which will be included as part of the text to ensure that the article is presented in a readable and understandable format. A brief description of the method used will be given in the article. The article entitled DNA damage and repair in nail technicians caused by occupational exposure to volatile organic compounds will be submitted to the Annals of Occupational Hygiene for peer reviewing and publication. Chapter 4 includes a final summary and conclusion, as well as recommendations for future studies.

The dissertation is written according to the guidelines of the chosen journal for potential publications which is the Annals of Occupational Hygiene. References should be listed in alphabetical order by name of first author, using the Vancouver Style of abbreviation and punctuation.

Author's Contribution

The study was planned and executed by a team of researchers. The contribution of each researcher is depicted in the Table below.

Name	Contribution
Ms. N. van der Merwe	• Literature searches, interpretation of data and writing of article; • Recruiting subjects; • Personal exposure measurements; • Sampling of personal data; • Comet assay experimentation.
Mr. P.J. Laubscher	• Co-Supervisor; • Assisted with approval of protocol, interpretation of results and documentation of the study; • Giving guidance with scientific aspects of the study.
Ms. A. Franken	• Supervisor; • Assisted with designing and planning of the study, approval of protocol, interpretation of results and documentation of the study.
Ms. C. Spoelstra	• Assisted with the personal exposure measurements; • Recruiting subjects.
Ms. E. van Dyk	• Guidance in the execution of the comet assay technique; • Assisted with the interpretation of DNA damage and repair data.

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

I declare that I have approved the above mentioned article and that my role in the study as indicated above is representative of my actual contribution and that I hereby give my consent that it may be published as part of Nicolene van der Merwe's M.Sc (Occupational Hygiene) dissertation.

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Ms. A. Franken
(Supervisor)

Mr. P.J Laubscher
(Co-Supervisor)

Ms. E. van Dyk

Ms. C. Spoelstra

List of abbreviations

AGD	anogenital distance
ALDH	aldehyde dehydrogenase
$ALDH_2$	aldehyde dehydrogenase 2
BER	Base Excision Repair
°C	Degrees Celsius
CA	Cyanoacrylate
CNS	Central Nervous System
CYP	Cytochrome P450
ddH_2O	double distilled water
DBP	Dibuthyl Phthalate
DEHP	Di-2-ethylhexyl phthalate
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic Acid
dsDNA	double stranded deoxyribonucleic acid
EA	Ethyl methacrylate
EDTA	Ethylene diamine tetra acetic acid
EEG	Electroencephalogram
Endo III	Endonuclease III
FDA	Food and Drug Administration
FPG	Formamidopyrimidine DNA glycosylase
HCS	Hazardous Chemical Substances
HEPES	4-(2-Hydroxethyl)-1-piperazineethanesulfonic acid
HMPA	High Melting Point Agarose
HVAC	Heating Ventilation and Air Conditioning
LC_{50}	Lethal Concentration
LD_{50}	Lethal Dosage
LMPA	Low Melting Point Agarose

List of abbreviations (continues)

µg	Microgram
µl/kg	Micriliter per kilogram
µl	Microliter
µM	Micromolar
M	Meter
mA	Milli ampere
mM	Millimolar
mg/kg	Milligrams per kilogram
mg/m^3	Milligrams per cubic meter
MMR	Mismatch Repair
m/s	Meters per second
NAD^+	Nicotinamide ademine dinucleotide
NER	Nucleotide Excision Repair
NHEJ	Non-Homologous end Joining
NIOSH	National Institute for Occupational Safety & Health
NaOH	Sodium Hydroxide
NaCl	Sodium Chloride
OEL	Occupational Exposure Limit
OH	Hydroxyl ion
OSHA	Occupational Safety & Health Administration
PBS	Phosphate Buffered Saline
ppm	parts per million
ROS	Reactive Oxygen Species
SAIOH	The Southern African Institute for Occupational Hygiene
STEL	Short Term Exposure Limit
TWA	Time Weighted Average
VOC	Volatile Organic Compounds

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Abstract

Objectives: The aim of this study was to determine if exposure to volatile organic compounds can lead to DNA damage and impaired DNA repair capacity. Nail cosmetics is a fast growing industry around the world where employees and clients are subjected to various chemical substances which may be harmful to their health: such as formaldehyde, toluene, acetone, xylene, ethylmethacrylate, methylmethacrylate and n-buthyl acetate. These chemicals have the potential to be harmful to their health and exposure to these chemicals should be actively controlled. Formaldehyde is classified as a human carcinogen by the IARC, whereas, toluene and xylene are group three carcinogens, classified in 1999 (not classified as carcinogenic to humans), and various studies have linked DNA damage and impaired DNA repair to the above mentioned substances. *Methods:* Fifteen nail technicians were monitored by means of personal air sampling, measuring formaldehyde, toluene, xylene, acetone and ethylmethacrylate exposure. Fifteen unexposed subjects were chosen and matched for age and smoking habits with the exposed group. Heparinised blood samples were obtained from each test subject with which the Comet Assay was performed on lymphocytes to determine DNA damage and repair ability. *Results:* Exposure to ethylmethacrylates and methylmethacrylates leads to DNA damage. Methylmethacrylate causes DNA damage by specifically targeting pyrimidine (fpg) bases. N-buthyl acetate, xylene and acetone exposure impaired DNA repair capacity. The exposed group showed signs of Class III and Class IV DNA damage, whereas the control group had little Class III damage and no indication of Class IV damage. The overall DNA repair ability of the nail technicians was slightly impaired when compared to that of the control group, which is in concurrence with previous studies. Smoking habits and age did not show significant influences on the level of DNA damage and repair when compared with the control group. *Conclusion*: Exposure to volatile organic compounds such as ethylmethacryale and methylmethacrylate may lead to DNA damage and altered DNA repair in some individuals, although further studies are recommended.

Keywords: Nail salon industry, volatile organic vapours, DNA damage, DNA repair, Fpg damage, Endo III damage, Comet assay

Opsomming

Doelstellings: Die doel van hierdie studie is om vas te stel of blootstelling aan vlugtige organiese verbindings lei tot DNA skade en verlaagde DNA herstel kapasiteit. Nael salonne is ‘n vinning groeiende industrie wȩreldwyd, waar werkers en klante blootgestel word aan verskeie chemise substanse, wat skadelik vir hul gesondheid kan wees, byvoorbeeld formaldehied, tolueen, asetoon, xileen, etielmetakrilate, metielakrilate en n-butiel asetaat. Blootstelling aan hierdie chemikalië kan skadelik vir hul gesondheid wees, en moet beheer word. Formaldehied is geklassifiseer as ‘n menslike karsinogeen deur die IARC in 2006, terwyl tolueen en xileen in groep drie geklassifiseer is (nie karsinogenies vir mense) deur die IARC (1999). Verskeie studies het oorkomste getoon tussen bogenoemde chemikalië en die vlak van DNA skade, asook velaagde DNA herstel vermoë. *Metode:* Vyftien nael tegnikuste was gemoniteer deur gebruik te maak van persoonlike lug monitering. Respiratoriese blootstelling aan formaldehied, tolueen, xileen, asetoon en etiel metakrilate is gemeet. Vyftien nie-blootgestelde persone is gekies en gepaar met die proefgroep ten opsigte van ouderdomme asook rookgewoontes. Gehepaniseerde bloed monsters van elke proefpersoon is verkry. Die Komeet analise is gebruik om die DNA skade en herstel kapasiteit in limfosiete te ondersoek. *Resultate:* Etielmetakrilate an metielmetakrilate lei to DNA skade. Metielmetakrilate veroorsaak DNA skade spesifieik die pirimidien basisse aanteval. N-butiel asetaat, xileen en asetoon lei tot verlaagde DNA herstel vermoë. Die proefgroep het tekens getoon van Klas III en Klas IV DNA skade, terwyl die kontrole groep min Klas III en geen Klas IV skade getoon het nie. Die algehele herstel vermoë van die nael tegnikuste was effens laer as die herstel vermoë van die kontrole groep. Rook gewoontes en ouderdom het nie noemenswaardige verskille getoon teenooor die hoeveelheid DNA skade en DNA herstel vermoë in vergelyking met die kontrole groep. *Gevolgtrekking*: Blootstelling aan vlugtige organiese verbindings soos etielmetakrilate an metielmetakrilate kan lei to DNA skade en verlaagde DNA herstel in sommige individue, alhoewel verdere studies aanbeveel word.

Sleutelwoorde: Nael salon industrie, vlugtighe organiese verbindings, DNA skade, DNA herstel, Fpg skade, Endo III skade, Komeet analise

CHAPTER 1

GENERAL INTRODUCTION

1. General Introduction

The beauty industry across the world is thriving, which puts cosmetologists at an increasing risk, as popularity of this occupation has increased with 327% over the last 10 years (Porter, 2009). Beauty salons are seen as a comfort zone for women all over the world, a place to relax and be indulged. Therefore, it is not seen as a hazardous environment by most people. Cosmetologists are exposed to various substances such as formaldehyde, acetone, toluene, xylene, n-buthyl acetate, ethylmethacrylate and methylmethacrylates, which may be harmful to their health. Many of these chemicals will not necessarily exceed the Occupational Exposure Limit (OEL), but because it affects the same organs and tissues it will have an adverse health effect on workers with long term exposure. Some chemicals will aggravate or cause asthma, and lead to allergic reactions (Roelofs *et al*., 2008)

Porter (2009) also suggests that 95% of nail technicians are female with an average age of 38. Women of childbearing age are especially vulnerable to even low levels of chemical exposure as many of the substances in nail caring products are teratogenic. Thus, the fetus is vulnerable (Porter, 2009).

Neither legislative, nor regulatory oversight of the cosmetic industry has been adequate to protect workers health and safety. In general, inspections are not initiated in beauty salons, except in response to a health complaint, as it is not seen as hazardous by most people (Gorman and O'Connor, 2007). This field has not been studied enough to enforce legislation. Workers are uninformed and therefore they are often unaware of the dangers that the above mentioned chemicals may have on their health. Due to the nature of the occupation it is not desirable to utilize the recommended personal protective equipment as it is seen as a hostile image. The levels of exposure to these harmful chemicals have not yet been established within South African nail salons and recommendations to ensure the health of these workers must be made.

Effects of environmental toxicology, cancer and aging are often tissue and cell specific, thus it became important to develop a technique which can detect DNA damage in individual cells (Singh *et al*., 1998). The Comet assay is a simple approach for sensitive detection of DNA damage, as well as the assessment of DNA repair in individual cells. It is a rapid, sensitive and inexpensive method for measuring DNA double strand breaks, single strand breaks and alkali-labile sites at a single cell level (Celic *et al*., 2003). Exposure to formaldehyde, which is a known carcinogen, as well as toluene and xylene

which are class three carcinogens classified by the IARC (1999a,b), may lead to increased cytogenetic damage of exposed workers.

The Comet Assay detects DNA strand breaks by measuring the migration of DNA from immobilized nuclear DNA. Tice (2000) describes that the advantages of the comet assay for assessing DNA damage in human lymphocytes include: (1) damage to the DNA in individual cells can be measured; (2) only a small number of cells are needed to carry out the assay (<10,000); (3) the assay can be performed on virtually any eukaryotic cell type; (4) and it is a very sensitive method for detecting DNA damage. Thus the comet assay will be used to study the occurrence of DNA damage in the peripheral lymphocytes of nail technicians.

In a study conducted by Hipakka and Samimi (1987) and Roelofs *et al* (2008), where organic vapours and metacrylic dusts were measured using personal air sampling, it was found that these chemicals were not potentially harmful to the nail technicians' health. It is noted however, that due to the weather, all windows and doors were open during the time of this study, creating a steady and natural draft. This study was conducted in the 1980's; many techniques, apparatus and chemicals have been changed and modernized. Therefore further studies should be conducted (Hipakka and Samimi, 1987).

2. Hypotheses

Exposure to organic vapours in the nail salon industry leads to DNA damage.

Exposure to organic vapours in the nail salon industry leads to impaired DNA repair capacity.

3. Aims and approach of this study

The general aims of this study were:

- To evaluate the respiratory exposure of nail technicians in the nail salon industry to different volatile organic compounds in their daily work environment,
- To determine the level of DNA damage which arise from exposure to these substances, as well as the level of DNA repair,
- To compare the level of DNA damage and DNA repair of nail technicians and a paired group of control subjects not exposed to these chemicals,

The results will be corrected for confounding factors such as smoking habits and age.

4. References

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CHAPTER 2

LITERATURE REVIEW

1. Introduction

This chapter consists of a discussion of the literature which is necessary to understand and interpret this article. It includes a discussion of the various organic vapours present in nail salons as well as health effects which may arise with exposure to these substances. This study also includes a discussion of DNA damage and repair caused by these substances, as well as the different methods to detect and assess the damage caused.

2. Volatile organic compounds and occupational exposure in nail salons

Beauty salons are seen as a comfort zone for women all over the world; a place to relax and be indulged. Therefore it is not seen as a hazardous environment by most people. Products commonly used in nail salons include nail polish, nail polish - and - glue remover, hardeners and fingernail glue. Acrylic, or sculptured nails have become increasingly popular. Hazardous chemicals used to construct these fingernails can have toxic effects on the health of workers. These products are composed of various substances such as acetone, ethylmethacrylate, methylmethacrylate, ethyl cyanoacrylate, formaldehyde, toluene, n-buthyl acetate and xylene. Many of these chemicals are known carcinogens that can cause DNA damage and have a wide range of health effects on exposed workers (Porter, 2009). Gorman and O'Connor (2007) suggest that nail technicians are exposed to these substances in their daily routine, which presents an additional threat due to the fact that most nail salons are small businesses with inadequate natural ventilation. According to Porter (2009) 95% of nail technicians are female, with an average age of 38. Women of childbearing age are especially vulnerable to even low levels of toxic exposure, as many of the substances are teratogenic. Gorman and O'Connor (2007) states that NIOSH estimates about half of the workplace indoor air quality complaints can be attributed to lack of adequate ventilation. Ventilation inspections are rarely performed, due to the fact that small businesses are unaware of this requirement and many building owners or managers do not provide this service. These vapours are mainly absorbed through inhalation, but also through the skin. Hipakka and Samimi (1987) found no considerable evidence that exposure to these chemicals cause DNA damage, or have significant health effects as it did not exceed the Occupational Exposure Limit (OEL). It was however recommended that further studies should be conducted which include confounding factors such as age,

smoking habits, number of years working as a nail technician, hours worked per day, as well as number of days worked per week. It is also noted that the study was conducted more than twenty years ago, and new methods, technology as well as exposure levels may have changed over the years. Many nail technicians work in beauty salons, where various services are provided such as massaging and facials, which do not expose workers to hazardous chemical substances. Therefore, the exact time-length of exposure should be considered (Porter, 2009; Gorman and O'Connor, 2007; Roelofs and Tuan, 2007).

Nail salon Environment

The nature of this profession requires the technician to sit on one end of the table not more than 0.6 meters away from the clients' hands where the chemicals are applied. Nail sculpturing entails very fine work (Gorman and O'Connor, 2007). Therefore, technicians' often lean forward, raising the concentration of vapours in the breathing zone of workers. Many of the chemicals in question, such as acetone, are located on top of the worktable, and are often not closed because of continuous use. To preserve the comfort zone image of a nail salon or a beauty parlour, the wearing of respirators cannot be allowed. Sophisticated looking local extraction ventilation may be the best method in controlling exposure.

3. Legislation

The Occupational Health and Safety Act, (Act No 85 of 1993) has established Regulations for Hazardous Chemical Substances (Table 1).

Table 1: Occupational Exposure Limits for volatile organic compounds. (Regulations for Hazardous Chemical Substances, 1995).

Substance	TWA OEL		STEL OEL	
	ppm	mg/m^3	ppm	mg/m^3
Acetone	750	1780	1500	3560
Ethanol	1000	1900		
Ethyl acetate	400	1400	150	650
Ethyl benzene	100	435	125	545
Ethylmethacrylate	100	-	-	-
Methylmethacrylate	100	410	125	510
n-buthyl acetate	-	-	-	-
Formaldehyde	* 2	* 2,5	* 2	* 2,5
Toluene	50	188	150	560
xylene	100	435	150	650

* - Indication of Control Limits, whereas the rest of the values are Recommended Limits

4. Health risks, toxicological information and metabolism of nail product components.

The main route of absorption of these chemicals is through inhalation. However, before a vapour reaches the lungs, it passes through the nose. A large part of the water soluble vapours like ethylmethacrylate, will be absorbed by the watery fluid of the nasal mucosa. Inhaled vapours that reach the lungs diffuse from the alveolar space into the blood, and dissolve. Absorption may also occur via the skin, though it is not very permeable, and is therefore a relatively good barrier against toxic substances (Klaassen and Watkins, 2003).

4.1 Acetone

Acetone is commonly used in the nail cosmetic industry as a nail polish-and-glue remover. It is the simplest example of ketones, and is a colourless, volatile and flammable liquid. It is harmful by inhalation, ingestion or skin absorption, and when oxidized, acetone forms peroxide which is a highly unstable compound (INCHEM, 1998).

4.1.1 Chemical and physical properties

Table 2: Information of the chemical and physical properties of acetone. (Mallinckrodt Chemicals, 2007a; NIOSH, 2007).

Structure	$(CH_3)2CO$
Appearance	Clear, colourless, volatile liquid.
Odour	Fragrant, mint-like
Solubility	Miscible in all proportions in water.
Vapour pressure (mmHg)	400 at 39.5 ˚C
Molecular weight	58.08

4.1.2 Acetone absorption, metabolism and excretion

Acetone is one of three ketone bodies that occur naturally throughout the body, and can be formed by oxidation of fatty acids. Under normal conditions, the production of ketone bodies occurs almost entirely in the liver and to a smaller extent in the lungs and kidneys. Exogenously supplied acetone enters many metabolic reactions, predominantly in the liver where it is metabolized into acetate and formate, which accounts for the entry of carbon from acetone into cholesterol, fatty acids, urea and amino acids, and formation of 3-carbon gluconeogenic compounds (INCHEM, 1998). INCHEM (1998) also states that acetone is rapidly absorbed via the respiratory and gastrointestinal tracts of humans and animals.

Following inhalation exposure, acetone is rapidly removed from the body with halflife-times in alveolary air and blood of 4 and 6 hours respectively. During inhalation exposure, humans absorb approximately 50 % of the amount inhaled. The nasal cavities appear to have a limited ability to absorb and excrete acetone vapours, compared with the rest of the respiratory tract. Endogenous acetone is eliminated from

the body by excretion in urine, exhalation or by enzymatic metabolism (INCHEM, 1998). Exhalation is the major route of acetone elimination.

4.1.3 Health effects

Acute exposure to acetone may lead to central nervous system (CNS) depression, which is the most common effect and can range from sedation to coma. With significant exposure, respiratory depression and death may also occur. Acetone causes irritation to the skin and eyes, and may lead to irritant dermatitis with chronic exposure. CCOHS (1997) states that upon inhalation exposure of 300-500 ppm, irritancy of the nose and throat may occur. As concentrations approach 1000 ppm symptoms like headaches, light-headedness and tiredness are noticeable. When inhalation exceeds 2000 ppm, dizziness, a feeling of drunkenness, drowsiness, nausea and vomiting are noted. Unconsciousness may result if exposure is higher than 10 000 ppm, as well as probable collapse, coma and death. There have been reports of an increased percentage of abnormal sperm shapes and miscarriages in exposed workers. It has also been reported that acetone exposure can affect pregnancy, due to the fact that acetone can act on the central nervous system of the developing fetus, producing abnormalities analogous to fetal alcohol syndrome, including craniofacial abnormalities and learning deficits. Tachycardia, hypotension, hyperglycemia and ketonemia have also been reported. Long term exposure to acetone may cause liver and kidney damage (CCOHS, 1997).

4.1.4 Toxicological information

Mallinckrodt Chemicals (2007a) give the LD_{50} of acetone, orally administered to rats as 5800 mg/kg. The LC_{50}, of acetone in the air is 100 mg/m^3 for rats.

4.2 Ethylmethacrylate

Ethylmethacrylate (EA) is an acrylic monomer used in the manufacturing of a variety of polymers and co-polymers as components of many commercial products (Williams and Iatropoulos, 2009). It is a component used in the making of acrylic - and liquid acrylic fingernails (Porter, 2009).

4.2.1 Chemical and physical properties

Table 3: Information of the chemical and physical properties of ethylmethacrylate. (Anachemia, 2010).

Structure	$C_6H_{10}O_2$
Appearance	Clear, colourless liquid
Odour	Unpleasant odour
Solubility	soluble in water
Vapour pressure (mm Hg)	26.2 at 23 ºC

4.2.2 Ethylmethacrylate absorption, metabolism and excretion

Exposure to ethylmethacrylate in nail salons usually occurs via inhalation or dermal contact. Two metabolites were identified in a study done by Ghanayem (1987) in the urine, namely, N-acetyl-s-(2-carboxyethyl)cysteine and *N*-acetyl-s-(2-carboxyethyl) cysteine ethyl ester. This suggests that an addition of sulfhydryls to acrylate is a pathway of ethylmethacrylate metabolism. Hydrolysis of the ethyl ester may occur before or after conjugation. Acrylic acid and gluthathione (GSH) is the easiest way to measure EA internal dosage. Further degradation of the GSH conjugates results in the formation of the mercapturic acids detected in the urine (Sweeney *et al*., 2004). The major route of EA excretion is via CO_2 exhalation (approximately 70 %) followed by urinary excretion.

4.2.3 Health effects

Animal studies have indicated that exposure to EA increases incidences of squamous cell papillomas and carcinomas of the forestomach. Ethylmethacrylate caused dose-related irritation and inflammation, hyperkeratosis and hyperplasia of the forestomach (Williams and Iatropoulos, 2009). According to McCarthy *et al*. (1994) ethylmethacrylate did not induce single strand breaks when incubated with DNA. Pozzani *et al.* (1949) found that inhalation of methylmethacrylate and ethylmethacrylate cause lung injury as

well as liver damage. Ethylmethacrylate also causes headaches and nausea and is extremely irritating to skin and eyes. An earlier study conducted by Spencer *et al.* (1997) indicated that insufficient amounts of ethylmethacrylate, and methyl acrylate were present during evaluation of nail technicians. A study conducted by Walker *et al*. (1991) indicated an increase in colon and rectum cancer in workers from a polymerizing acrylate monomers manufacturer. These mortalities were only evident after three years employment with intense exposure (Walker *et al.*, 1991).

4.2.4 Toxicological information

The LD_{50} of ethylmethacrylate is as follows, oral administration in rats, 14800 mg/kg, whereas the LC_{50} is 15000 mg/m^3 for 3 hours (Anachemia, 2010).

4.3 Methyl methacrylate

Methyl methacrylate is an acrylic monomer used in the nail salon industry as a compound contained in acrylic gel (Porter, 2009; Gorman and O'Connor, 2007).

4.3.1 Chemical and physical properties

Table 4: Information of the chemical and physical properties of methyl methacrylate (Mallinckrodt Chemicals, 2008).

Structure	CH_2:C(CH_3)$COOCH_3$
Appearance	Clear colourless liquid
Odour	Sweet sharp odour
Solubility	Soluble in water
Vapour pressure (mm Hg)	40 @ 25.5C

4.3.2 Methyl methacrylate absorption, metabolism and excretion

Exposure to methyl methacrylate is primarily dermal and is rapidly metabolized through hydrolysis due to serum non-specific carboxylesterase with fast uptake and degradation of metacrylic acid to less toxic compounds (Bereznowski, 1995). Metacrylic acid and gluthathione (GSH) is the easiest way to measure methyl methacrylate dosage. Further degradation of the GSH conjugates results in the formation of the mercapturic acids detected in the urine (Sweeney *et al*., 2004). The major route of methyl methacrylate excretion is via CO_2 exhalation

4.3.3 Health effects

According to Spencer *et al*. (1997) methyl methacrylate is known to cause dermatitis and is a potent skin sensitizer. Methyl methacrylate also causes mucous membrane and eye irritant, some cases have also been reported that methyl methacrylate may cause occupational asthma and aggravate existing asthma (Spencer *et al*., 1997). High concentrations have been linked to central nervous system depression and unconsciousness. Animal studies done on rats have indicated that methyl methacrylate may cause liver, kidney and cardiovascular damage with high inhalation exposure (Bereznowski, 1995; Spencer *et al*., 1997). In 1974 the use of methyl methacrylate in in nail products was banned due to the toxicity of the chemical (Gorman and O'Connor, 2007). Overexposure can include coughing, chest pain, headache, drowsiness, nausea,

anorexia, irritability and narcosis. Upon inhalation of very high concentrations this chemical has been linked to cardiac arrest because of its ability to cause low blood pressure (Mallinckrodt Chemicals, 2008). Chronic exposure in dental technicians using bare hands with methyl methacrylate molding putty developed changes in the nerve impulse transmission in the fingers and may cause tingling or prickling sensation of the skin.

4.3.4 Toxicological information

According to Mallinckrodt Chemicals, the LD_{50} values of methyl methacrylate is the following: oral administration in rats, 7872 mg/kg, and for inhalation the LC_{50} value is determined as 78 000 mg/m^3.

4.4 Formaldehyde

Formaldehyde is used in the cosmetic industry as a nail polish hardener, as well as a compound in nail polishes. An aldehyde is an organic compound containing a terminal carbonyl group. Oxidation of aldehydes yields acids and reduction of aldehydes produce alcohols. These substances participate in many chemical reactions and undergo polymerization into chains that contain monomer molecules (Franks, 2005).

4.4.1 Chemical and physical properties

Table 5: Information of the chemical and physical properties of formaldehyde (Mallinckrodt Chemicals, 2007b; NIOSH, 2003).

Structure	HCHO
Appearance	Clear, colourless liquid
Odour	Pungent odour
Solubility	Infinitely soluble, miscible solubility in water
Vapour pressure (mm Hg)	1.3 at 20 ^{o}C
Molecular weight	30

4.4.2 Formaldehyde absorption, metabolism and excretion

Formaldehyde in gas form, is highly water soluble which, when inhaled, acts rapidly at the site of contact and is quickly metabolized by enzymes in the respiratory tract and red blood cells. Inhaled formaldehyde is almost entirely absorbed in the respiratory tract (Franks, 2005). Aldehyde dehydrogenase (ALDH) oxidizes aldehydes to carboxylic acids with NAD^{+} as the cofactor (Klaassen and Watkins, 2003). $ALDH_2$ is a mitochondrial enzyme that is primarily responsible for oxidizing simple aldehydes, such as formaldehyde. As human erythrocytes contain formaldehyde dehydrogenase and aldehyde dehydrogenase, absorbed formaldehyde may be rapidly metabolized (IARC, 2006).

Formaldehyde is also converted to formic acid in the body, which may cause a rise in the acidity of the blood (acidosis) and which can also be contributed to the developing tissue necrosis and lactate production (Patnaik, 1999).

Formaldehyde is metabolized and excreted quickly, and therefore does not accumulate in the body. Kum *et al.* (2007) claims that formaldehyde is responsible for oxidative stress and the free radicals that it produce are the main cause of organ tissue necrosis.

4.4.3 Health effects

Kum *et al.* (2007) claims that formaldehyde is responsible for oxidative stress and the free radicals that it produce are the main cause of organ tissue necrosis, especially in the liver. Toxicity and carcinogenity of formaldehyde has also been attributed to its ability to form adducts with DNA and proteins. A marked decrease in mitochondrial membrane potential and inhibition of mitochondrial respiration, which was accompanied by reactive oxygen species formation, was noticeable when rat hepatocytes were incubated with formaldehyde (Teng *et al*., 2001).

Vaughan *et al*. (2000) state that occupational exposure to formaldehyde has been linked mostly with an increased risk of nasopharyngeal cancer. Formaldehyde, being an allergen and an irritant, can result in hypersensitivity allergies to multiple types of chemicals after prolonged and repeated exposure. Diluted formaldehyde can result in burn-like blisters and deadening of the skin due to coagulation necrosis (North, 2007). Exposure to formaldehyde may cause severe irritation to the skin and eyes, as well as the upper respiratory tract. Formaldehyde is a bronchospasm stimulant that causes burning and lacrimation of the mucosa upon inhalation of low concentrations. At a concentration of 10 ppm the lacrimate effect becomes intolerable. Inhalation of high concentrations causes mucosal burning and choking, coughing, sore throat, drooling and difficulty with swallowing (Dart *et al*., 2000). Respiratory effects are dependent on duration and concentration of exposure therefore; short term exposure can cause respiratory epithelial injury. Dart *et al.* (2000) also state that chronic inhalation causes headaches, nausea and flu like symptoms. Prolonged exposure will also cause parenchymal irritation with symptoms like cough, chest pain, dyspnea and wheezing. High concentrations can lead to death (Patnaik, 1999). Neurological effects of formaldehyde include light-headedness and direct central nervous system depression (Dart *et al*., 2000). Formaldehyde is thought to act through sensory nerve fibres that signal through the trigeminal nerve to reflexively induce bronchi constriction through the vagus nerve (Klaassen and Watkins, 2003). Other effects of significant formaldehyde exposure include anxiousness and tachycardia from pain. Hypovolemia and hypotension may occur during profound acidosis. Animal studies have shown that at concentrations of 40 ppm, formaldehyde is lethal to mice with symptoms such as

dispnea, listlessness, loss of body weight, inflammation of the nasal tissue and pathological changes in the nose, larynx, trachea and brongi (Patnaik, 1999). Formaldehyde also induces a variety of toxic effects in the liver. These effects include dose-related focal hepatic necrosis, hepatic enlargement, decreased liver weight, and hepatocellular fatty degeneration (Kum *et al*., 2007).

Formaldehyde-induced mutation causes large losses of DNA in human lymphoblasts. Animal studies indicated that formaldehyde cause cancer. It causes olfactory tumours and produces skin tumours at the site of subcutaneous application. Similar tumorigenic properties of formaldehyde are expected in humans, as formaldehyde was recognised as a human carcinogen by the IARC (2006). Monticello *et al.* (1996) state that formaldehyde reacts more readily with single stranded DNA, which is present in increased amounts during cell division with generation of DNA-protein cross-links providing a valuable indicator of site-specific DNA exposure. With exposure to high concentrations, the probability of inducing heritable DNA damage is greater than at low concentrations due to the saturation of epithelial protective mechanisms and the sustained stimulation of the proliferative response. Taatjes (1997) suggests that formaldehyde causes DNA-adducts with the presence of dioxygen. Formaldehyde is proposed to bind intercalated drugs to DNA via a methylene linkage. Formaldehyde production in the body is dependant on iron, as well as DNA adduct formation. Studies have shown that glutathione induces DNA adduct formation, but also inhibits adduct formation by trapping formaldehyde (Taatjes, 1997).

4.4.4 Toxicological information

Formaldehyde administered orally to rats resulted in a LD_{50} value of 100 mg/kg. Inhalation of formaldehyde revealed a LC_{50} of 203 mg/m^3 (Mallinckrodt Chemicals, 2007b).

Formaldehyde is associated with nasal and nasopharyngeal cancer and possibly leukaemia and brain cancer (NCL, 2004). In 2006, formaldehyde was classified as a confirmed human carcinogen by the IARC (2006).

4.5 Toluene

In the cosmetic industry, toluene is a compound of nail polish, as well as fingernail glue (Porter, 2009). Alkyl-benzenes are single ring aromatic compounds containing one or more saturated aliphatic side chains (Mattia, *et al*., 1991).

4.5.1 Chemical and physical properties

Table 6: Information of the chemical and physical properties of toluene (Mallinckrodt Chemicals, 2007c).

Structure	C_6H_5-CH_3
Appearance	Clear, colourless liquid
Odour	Aromatic, benzene-like
Solubility	0.05 g/100 g water @ 20 ºC
Vapour pressure (mm Hg)	22 at 20 ºC
Molecular weight	92.14

4.5.2 Toluene absorption, metabolism and excretion

Toluene is a highly volatile substance and is mainly absorbed via inhalation, but also through the skin (Sato and Nakajima, 1978). Toluene is well absorbed from the lungs and gastrointestinal tract. It accumulates rapidly in the brain and is subsequently deposited in other tissues according to their lipid content, thus toluene mainly accumulates in the adipose tissues (Woiwide *et al.,* 1979; Mattia *et al*., 1991). The primary route of toluene metabolism is by hydroxylation to benzyl alcohol by members of the cytochrome P450 (CYP) super-family. It is believed that in humans, benzyl alcohol is metabolized to benzaldehyde by CYP rather than alcohol dehydrogenase; however, this belief does not appear to be universal. Benzaldehyde is in turn metabolized to benzoic acid, primarily by mitochondrial aldehyde dehydrogenase-2. Benzoic acid is metabolized to either benzoyl glucuronide or hippuric acid. Benzoyl glucuronide is produced by the reaction of benzoic acid with glucuronic acid, which accounts for 10-20% of benzoic acid elimination. Studies have shown that benzaldehyde might be responsible for accelerating reactive oxygen production in the nervous system, liver, lungs and kidneys, and may contribute to the overall toxicity of toluene (Mattia *et al*., 1991,1993). Hippuric

acid is also known as benzoylglycine and is produced from benzoic acid and is also the primary urinary metabolite of toluene (Woiwode *et al.*, 1979). Many studies have shown that toluene may also be a carcinogen. However, because it has many of the same metabolites of benzene, results are often attributed to the latter. These metabolites, hidroquanine and methylhydroquanine are recognized as human carcinogens (Pitarque *et al*., 1999). A significant amount of toluene (25%-40%) is exhaled unchanged via the lungs, although the greater proportion is metabolized and excreted via other pathways.

4.5.3 Health effects

The central nervous system is the primary target organ of toluene, and symptoms may range from slight dizziness and headaches, to unconsciousness, respiratory depression and death. Clinical signs include abnormal EEG activity, tremors, nystagmus and cerebral atrophy, as well as impaired hearing, vision, and speech (Klaassen and Watkins, 2003). According to Donald (1991) many case studies have shown that woman who abuse toluene during pregnancy put their unborn children at great risk. Intrauterine growth retardation, premature delivery, congenital cranio-facial, limb, cardiac, renal, and central nervous system malformations have been reported. A number of women had renal tubular acidosis, a rare condition associated with toluene. Exposure to toluene may cause irritation to skin and eyes and possibly dermatitis with long term exposure. Toluene has also been linked to colour vision loss (CCOHS, 1997).

4.5.4 Toxicological information

Toluene administered orally to rats resulted in a LD_{50} value of 636 mg/kg. Inhalation of toluene revealed a LC_{50} (Lethal Concentration) of 49 mg/m^3 for four hours (Mallinckrodt Chemicals, 2007c). In 1999, toluene was classified as a group three carcinogen, thus not carcinogenic to humans (IARC, 1999).

4.6 Xylene

An aromatic hydrocarbon (or arene) is a hydrocarbon of which the molecular structure incorporates one or more planar sets of six carbon atoms, which is known as a benzene ring that is connected by delocalized electrons numbering the same as if they consisted of altering single or covalent bonds. One of the simplest aromatic hydrocarbons is xylene (Science Lab, 2008). In the nail salon industry xylene is an important component of nail polish (Porter, 2009).

4.6.1 Chemical and physical properties

Table 7: Information of the chemical and physical properties of the Xylene isomers. (NIOSH, 2007; Science Lab, 2008).

	meta-xylene	**ortho-xylene**	**para-xylene**
Structure	$C_6H_4(CH_3)_2$	$C_6H_4(CH_3)_2$	$C_6H_4(CH_3)_2$
Appearance	Colourless liquid	Colourless liquid	Colourless liquid
Odour	Aromatic	Aromatic	Aromatic
Solubility	Methanol & diethyl ether soluble. Cold or hot water insoluble.	Acetone & diethyl ether soluble. Cold or hot water insoluble.	Methanol & diethyl ether soluble. Cold or hot water insoluble.
Vapour pressure (mmHg)	6 at 20 ˚C	0.9 at 20 ˚C	0.9 at 20 ˚C
Molecular weight	106.2	106.2	106.2

4.6.2 Xylene absorption, metabolism and excretion

Patnaik (1999) states that over 90 % of absorbed xylenes are metabolized to o-, m-, and p-isomers of methyl benzoic acid and excreted in urine as methyl hippuric acids. Small amounts of xylenes may remain stored in adipose tissue; thus repeated exposures may cause accumulation in the blood. About 5 % of absorbed xylene is excreted unchanged in expired air within a few hours, while less than 2 % is hydroxylated to xylenols. Occupational exposure primarily occurs via inhalation (Klaassen and Watkins, 2003).

4.6.3 Heath effects

Inhalation of high concentrations may cause workers to be flushed, experience confusion, dizziness, tremors and other symptoms of CNS toxicity, upper airway irritation, and in severe cases, pulmonary edema. Studies have indicated that aromatic hydrocarbons do in fact induce ROS formation, although it was less potent than aliphatic and cyclic hydrocarbons (Dreiem *et al*., 2003).

Aromatic hydrocarbon solvents cause direct cellular toxicity to the lungs and gastrointestinal tract, and toxic metabolites cause injury in the central nervous system as well as in the renal, cardiac, hepatic, and hematopoietic systems (Dreiem *et al.,* 2002). Patnaik (1999) states that the toxic properties of the xylene isomers are similar to toluene or ethyl benzene; targeting organs like the CNS, eyes, gastrointestinal tract, kidneys, liver, blood, and skin. These organs are only affected at high levels of exposure. The primary symptoms, according to Dart *et al.* (2000) are CNS depression, which range from drowsiness, confusion to coma (following a mild euphoria), dyspnea, and mucous membrane irritation.

Acute exposure produces symptoms such as: headache, fatigue, irritability, lethargy, nausea, anorexia, flatulence, irritation of the eyes, nose and throat, as well as motor in coordination and impairment of equilibrium. Flushing, redness of the face, a sensation of increased body heat, increased salivation, tremors, dizziness, confusion, and cardiac irritability have also been reported. Sudden death following acute inhalation is believed to be related to sensitization of the myocardium and hypoxia which is induced by simple asphyxiation effects (Patnaik, 1999).

Prolonged skin exposure may cause dermatitis. When xylene comes into contact with the eyes, alterations in the rods and cones as well as functional alterations such as colour vision deficits may occur. The visual motor performance may also be altered (Klaassen and Watkins, 2003).

4.6.4 Toxicological information

The lethal concentration (LC_{50}) in the rat for a 4-hour xylene inhalation exposure is 5000 ppm, and the oral lethal dose (LD_{50}) in the same species is 4300 mg/kg (Science Lab, 2008). In 1999, xylene was classified as a group three carcinogen, thus not carcinogenic to humans (IARC, 1999).

5. Genotoxicity

Exposure to Volatile organic vapours (VOC's) in nail salons are possible carcinogens to humans, because it contains chemicals such as formaldehyde, a known carcinogen, as well as toluene and xylene, which are classified as class three carcinogens (not carcinogenic to humans) by the IARC (1999). These compounds can damage DNA of living cells and if not repaired, these DNA lesions can initiate a cascade of biological consequences at cellular and organ level and eventually the whole body (Lee and Steinert, 2003).

5.1 DNA damage

DNA damage can be divided into two groups, namely endogenous damage from ROS, produced from normal metabolic by-products; and exogenous damage caused by external agents such as ultra violet radiation, x-rays and chemicals (especially aromatic compounds) (Friedberg, 2004). xylene, toluene and formaldehyde have been found to induce reactive oxygen specie formation (Dreiem *et al*., 2002, 2003; Teng *et al.,* 2001; Mattia *et al*., 1991,1993; Wellejus *et al.,* 2002).

Human genetic material is constantly exposed to physical or chemical factors which may cause DNA damage. These factors may be intracellular or environmental. DNA damage includes chemical modification of the bases, this disrupts the normal helical structure of DNA by forming adducts or forming chemical bonds with DNA. Chemicals can cause DNA damage directly or by the metabolites of a certain substance (Keretetse *et al.*, 2008).

5.1.2 Types of DNA damage

According to Lindahl (1993) DNA is the carrier of genetic information and has limited chemical stability. Hydrolysis, oxidation and nonenzymatic methylation of DNA occur at significant rates *in vivo*, and are counteracted by specific DNA repair processes. The spontaneous decay of DNA is likely to be a major factor in mutagenesis, carcinogenesis and ageing. Different types of DNA damage occur due to endogenous cellular processes, namely, oxidation, alkylation, hydrolysis and mismatching of bases (Cooke *et al*., 2003).

Free radicals, depending on their reduction and oxidation potential, tend to extract electrons from nearby molecules to reach stability and this target molecule then becomes a new radical (Iorio, 2002). Free radicals that are formed in the body as a consequence of aerobic metabolism can produce oxidative damage to macromolecules in somatic cells (Cooke *et al*., 2003). Abstraction of hydrogen atoms from the sugar-phosphate backbone of DNA generates 2-deoxyribose radicals leads to strand damage via complex reaction cascades (Gates, 2009).

Cellular DNA damage also arises from hydrolytic reactions, endogenous reactive agents such as -OH, or exposure to various exogenous agents. This is the simplest reaction that may lead to DNA damage according to Lindahl (1993). Spontaneous depurination and depyrimidination occur because of hydrolysis, accompanied by additional damage to both purines and pyrimidines in DNA. Gates (2009) states that spontaneous hydrolysis of the phosphodiester linkages in DNA does not occur to a significant extent under ordinary biological conditions, although this reaction may be accelerated by a range of catalysts such as phosphodiesterases, lanthanide ions and transition metal ions. Abasic sites, which result from hydrolysis, lose their genetic encoding and can thus lead to mutations during replication (Cooke *et al*., 2003). The hydroxyl radical is probably the proximal species that causes DNA damage by oxidative mutagens and accounts for much of the cellular damage caused by ionizing radiation (Ramotar *et al*., 1991). Repair specific enzymes formamido pyrimidine glycosylase (Fpg) and endonuclease III (Endo III) may be used to assess oxidative DNA base damage, because Fpg detects 8-OH guanine and other oxidatively damaged purines, whereas Endo III detects damaged pyrimidines (Piperakis *et al*., 2000; Speit *et al*., 2004).

The preferred sites of DNA alkylation strongly depends on the nature of the alkylating agent. Hard alkylating agents, which are small in size, positively charged, and has low polarizability, displays increased reactivity with hard oxygen nucleophiles in DNA. Whereas soft alkylating agents (large, uncharged and polarizable), favour reactions with softer nitrogen centres of DNA (Gates, 2009). Alkylation damage includes a variety of base modifications, which can result in mutations and lead to carcinogenesis. Repair enzymes recognize these bases and produces sites of base loss (Drablos *et al*., 2004).

Chemicals can bind covalently to DNA and these form chemically stable products known as DNA adducts. This adducts range in size and complexity and plays a major role in action of mutagens and carcinogens (Peluso *et al*., 2004).

5.2 DNA repair mechanisms

DNA repair is vital to maintain the integrity of genetic information. Various repair mechanisms exists in the human body to repair different types of DNA damage. There are several mechanisms which will recognise, excise and replace these damaged areas through replication, recombination or mismatch repair (Garrett and Grisham, 2005; Mol *et al*., 1999).

There are also mechanisms in place which will reverse damaging chemical changes in DNA; this is known as excision repair.

5.2.1 Base Excision Repair (BER)

BER acts on single bases that have been damaged. This damage could have occurred because of oxidation or other chemical modification during normal cellular processes. The damaged base is excised by DNA glycolase, which cleaves to the glycosidic bond, creating an apuinic acid (AP) site. Here, the sugar-phosphate backbone is intact, but a purine or pyrimidine is missing. Endonuclease cuts the DNA and excision nuclease removes the damaged base. DNA polymerase repairs the gap. The complementary strands' information is used to repair DNA with the correct bases (Garrett and Grisham, 2005). In this study the base excision repair mechanism was used to identify purine and pyrimidine damage, using Fpg and Endo III respectively (Piperakis *et al*., 2000; Speit *et al*., 2004).

5.2.2 Mismatch Repair (MMR)

MMR is used to correct errors that may occur during DNA replication. Mismatch repair relies on methylation patterns in the DNA to determine which strand is the template. This occurs just after replication. Between the start of methylation and the end of replication, only the parental dsDNA (double stranded DNA) is methylated (Mol *et al*., 1999). During this time the repair system reviews dsDNA for mismatched bases. The strand methylized being the correct nucleotide sequence. Enzymes scan newly synthesized DNA for mismatched bases, excise the region and replace it (Garrett and Grisham, 2005).

5.2.3 Nucleotide Excision Repair (NER)

NER recognises larger areas of damaged DNA than BER. This is the main pathway for the removal of carcinogenic lesions. This system cuts the DNA on both sides of the

lesion and removes the entire section. DNA polymerase then fills the gap and is covalently closed with DNA ligase (Garrett and Grisham, 2005).

There are two mechanisms for the repair of Double Strand Breaks:

5.2.4 Non-homologous end joining (NHEJ)

In this mechanism of DNA repair, ligase joins the two ends directly. Short homologous sequences, known as micro-homologies, guide the accurate repair of DNA. Loss of damaged nucleotides at the break site can lead to deletions or joining of translocations that do not match. This repair mechanism is very important before DNA is replicated, since there is no template available for homologous recombination repair (Garrett and Grisham, 2005).

5.2.5 Homologous recombination

A homologous sequence is required, which is used for a template of repair. Using this mechanism, chromosomes can be repaired using the homologous sequence as a template. Homologous recombination is normally used to fix DNA, so that no information is lost (Garrett and Grisham, 2005).

6. Confounding factors that influence DNA repair

Several factors have been investigated which may have an effect of the level of DNA damage, as well as the degree to which DNA repair mechanisms are affected. These factors include age, diet, exercise, smoking habits and period of exposure. In this study the influence of age and smoking have been taken into account.

6.1 Age

The effect of age on the level of DNA damage is inconsistent. A study done by Piperakis (2000) showed a small increase in DNA damage with increased age, but overall, age seems to have little effect on the level of DNA damage (Moller *et al*., 2000).

6.2 Smoking

Studies done by Andreoli (1997) did not indicate a significant increase in DNA damage between control and exposed groups. However, studies done by Celik (2003) and Pitarque (1997) indicated an increased level of DNA damage in smokers, which are exposed to the same chemicals as the non-smokers.

6.3 Period of exposure

Due to the fact that many of the VOC's found in nail salons can accumulate in the body, period of exposure plays an important role when evaluating DNA damage, as there may be a correlation between period of exposure and the level of DNA damage, thus with increased exposure, DNA damage may be elevated (Hipakka and Samimi, 1987).

7. Measuring of DNA damage

7.1 Comet Assay

The comet assay has become more popular over the last decade as a method to measure the level of DNA damage in terms of strand breaks and alkaline labile sites. The comet assay is a simple, reliable and relatively fast method of measuring DNA damage (Moller *et al.,* 2000). The comet assay requires few steps. First, cells are isolated and embedded in agarose. These cells are then lysed and subjected to alkaline electrophoresis. Cells are stained and examined under a fluorescence microscope. Cells which are damaged, forms a comet consisting of a head and tail. The amount of DNA that has migrated is correlated with the degree of damage (Singh *et al.*, 1988; Keretetse *et al.*, 2008; Zhu *et al*., 1999). Damage increases from Class I to Class IV as indicated by the increasing tail migration of the DNA (class 0:0-6 %; class I:6,1-17 %; class II:17,1-35 %; class III:35,1-60 %; class IV:60,1-100 %) (Giovannelli *et al.,* 2002). Modifications of the different steps are present in a diversity of laboratories. For this study, the method described by Keretetse *et al*. (2008) was used. For example, an additional step has been added in which a variety of enzymes have been used. This includes Fpg, which recognizes 8-oxodG and formamidopyrimidine lesions and Endonuclease III, which recognizes cytosine hydrates (Moller *et al.,* 2000). These enzymes are therefore responsible for removing oxidized purine and pyrimidine damage (Eide *et al*., 1996). The level of DNA damage is determined by the length of the comet tail or by the 'tail moment' (this is the length of the comet tail multiplied by the intensity of fluorescence of the tail).

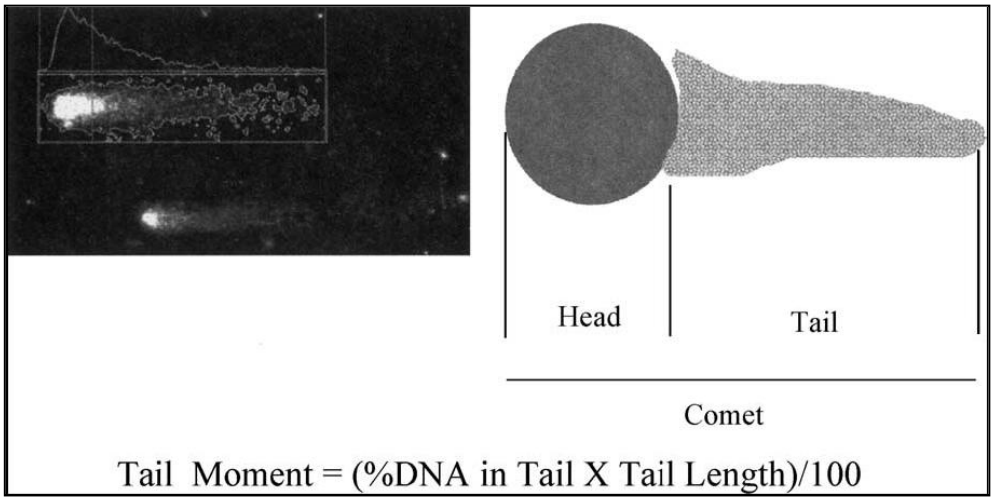

Figure 1: Diagram of a typical comet showing the distribution of DNA in tail and head (Lee and Steinert, 2003).

A study done by Moller *et al.* (2000) indicated that the comet assay is suitable for the evaluation of DNA damage. In this study, different confounding factors have been investigated and it was found that age, smoking and gender are criteria for the selection of populations when investigating occupational exposure.

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CHAPTER 3

ARTICLE

Instructions for Authors

Oxford Journals

- *Originality.* Only original work, not published elsewhere, should be submitted. If the findings have been published elsewhere in part, or if the submission is part of a closely-related series, this must be clearly stated and the submitted manuscript must be accompanied by a copy of the other publication(s) (or by a copy of the other manuscripts if they are still under consideration).

- *Authorship.* The corresponding author should be identified in the submission. Full postal addresses must be given for all co-authors. The preferred practice is that persons should only be named as authors if they have made significant identifiable intellectual contributions to the work, and other contributions may be recognised by acknowledgement at the end of the submission, in accordance with the guidance issued by the International Committee of Medical Journal Editors. A letter consenting to publication should be signed by all authors of a submission and sent to the Editorial Office.

- *Conflict of interest.* The source of financial support for the work must be stated in the Acknowledgements, unless it is clear from the author's affiliations. Other conflicts of interest must be declared to the Editor at the time of submission. These may include financial interest in products described, including stock or share ownership, and payment for consultancy or legal testimony using the material in the paper. These conflicts will not necessarily prevent publication, but the Editor may decide that the declaration should be included in the paper.

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- *Structure.* Papers should generally conform to the pattern: Introduction, Methods, Results, Discussion and Conclusions - consult a recent issue for style of headings. A paper must be prefaced by an abstract of the argument and findings, which may be arranged under the headings Objectives, Methods, Results, and Conclusions. Keywords should be given after the list of authors.

- *Survey design.* Sampling surveys should be planned using modern statistical principles so that the quality of the data is good enough to justify the inferences and conclusions drawn.

- *Units and symbols.* SI units should be used, though their equivalent in other systems may be given as well.

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Title: DNA damage and repair in nail technicians caused by occupational exposure to volatile organic compounds

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Abstract

Objectives: The aim of this study was to determine if exposure to volatile organic compounds can lead to DNA damage and impaired DNA repair capacity. Nail cosmetics is a fast growing industry around the world where employees and clients are subjected to various chemical substances, which may be harmful to their health: such as formaldehyde, toluene, acetone, xylene, n-buthyl acetate, ethylmethacrylate and methylmethacrylates. These chemicals have the potential to be harmful to their health and exposure to these chemicals should be actively controlled. Formaldehyde is classified as a human carcinogen by the IARC, whereas toluene and xylene are group three carcinogens, classified in 1999 (not classified as carcinogenic to humans). Various studies have linked DNA damage and impaired DNA repair to the above mentioned substances. *Methods:* Fifteen nail technicians were monitored by means of personal air sampling, measuring formaldehyde, toluene, xylene, acetone and ethylmethacrylate exposure. Fifteen unexposed subjects were chosen and matched for age and smoking habits with the exposed group. Heparinised blood samples were obtained from each test subject with whom the Comet Assay was performed on lymphocytes to determine DNA damage and repair ability. *Results:* Exposure to ethylmethacrylates and methylmethacrylates leads to DNA damage. Methylmethacrylate causes DNA damage by specifically targeting pyrimidine (fpg) bases. N-buthyl acetate, xylene and acetone exposure impaired DNA repair capacity. The exposed group showed signs of Class III and Class IV DNA damage, whereas the control group had little Class III damage and no indication of Class IV damage. The overall DNA repair ability of the nail technicians was slightly impaired when compared to that of the control group, which is in concurrence with previous studies. Smoking habits and age did not show significant influences on the

level of DNA damage and repair when compared with the control group. *Conclusion*: Exposure to volatile organic compounds such as ethylmethacryale and methylmethacrylate may lead to DNA damage, whereas xylene, acetone and n-buthyl acetate and altered DNA repair in some individuals, further studies are recommended.

Keywords: Nail salon industry, organic vapours, DNA damage, DNA repair, Fpg damage, Endo III damage, Comet assay

Introduction

In their everyday work routine nail technicians are exposed to various substances, such as formaldehyde, acetone, toluene, xylene, n-buthyl acetate, ethylmethacrylate and methylmethacrylates that are present in nail products. The aim of this study is to establish whether the volatile organic compounds used by nail technicians can lead to DNA damage and impaired DNA repair capacity. Porter (2009) suggest that 95% of nail technicians are female, with an average age of 38. Women of childbearing age are especially vulnerable to even low levels of chemical exposure, as many of the substances in nail care products are teratogenic, which may endanger the fetus (Gorman and O'Connor, 2007). Due to the nature of the occupation nail technicians are required to do very fine work, they are often no more than 0.6 meters away from where these chemicals are released (Porter, 2009). Many nail technicians work in beauty salons where various services are provided, such as massaging and facials which do not expose workers to hazardous chemical substances, therefore the exact length of exposure varies considerably and should be considered (Gorman and O'Connor, 2007; Porter, 2009; Roelofs *et al*., 2008).

Many of the chemicals to which they are exposed will not necessarily exceed the Occupational Exposure Limit (OEL), but because it affects the same organs and tissues, it can have an additive health effect on workers with long term exposure (Hipakka and Samimi,1987). Some chemicals will aggravate or cause asthma and lead to allergic reactions (Mallinckrodt Chemicals, 2007a, b, c; Anachemia, 2010; Science Lab 2008). Formaldehyde is a classified human carcinogen (IARC, 2006), whereas toluene and xylene are considered group 3 carcinogens, thus not carcinogenic to humans (IARC, 1999). Formaldehyde is water soluble, and is therefore easily absorbed in the respiratory tract. Certain health effects of these substances may lead to neurological symptoms, such as headache, dizziness, fatigue, tremors, in-coordination, anxiety and short-term memory loss to name a few (Mallinckrodt Chemicals, 2007).

Due to the fact that most nail salons are small businesses situated mostly in a retail complexes that does not make provision for extraction ventilation and has inadequate natural ventilation, nail technicians are put at an increasing toxicological risk with long term exposure to volatile organic substances (Porter, 2009; Roelofs and Taun, 2007).

Hipakka and Samimi (1987) found no considerable evidence that exposure to the above mentioned chemicals cause DNA damage or has significant health effects, as it did not

exceed the Occupational Exposure Limit (OEL). It was however recommended that further studies should be conducted, which include confounding factors such as age, smoking habits, number of years in the industry, hours worked per day, as well as the number of days worked per week (Hipakka and Samimi, 1987). It is also noted that the study was conducted more than twenty years ago and new methods have been developed for the evaluation of DNA damage. The chemicals nail technicians are exposed to and the level of exposure may have varied and changed over the years. A study done by Keretetse *et al*. (2008) on African petrol attendants which are exposed to xylene and toluene (also present in nail salons) found that their exposure was below the Occupational Exposure Limits of the individual chemicals. DNA damage can be divided into two groups, namely endogenous damage from ROS, produced from normal metabolic by-products; and exogenous damage caused by external agents such as ultra violet radiation, x-rays and chemicals (especially aromatic compounds) (Friedberg, 2004). Human genetic material is constantly exposed to physical or chemical factors which may be intracellular or environmental. Chemicals can cause DNA damage directly or by the metabolites of a certain substance (Keretetse *et al*, 2008). Hydrolysis, oxidation and nonenzymatic methylation of DNA occur at significant rates *in vivo*, and are counteracted by specific DNA repair processes. Different types of DNA damage occur due to endogenous cellular processes, namely oxidation, alkylation, hydrolysis and mismatching of bases (Cooke *et al*., 2003; Gates, 2009). Xylene, toluene, formaldehyde and di-buthyl phthalates have been found to induce reactive oxygen species formation (Dreiem *et al*., 2002, 2003; Teng *et al.,* 2001; Mattia *et al*., 1991,1993; Wellejus *et al.,* 2002).

Table 1: Occupational Exposure Limits for volatile organic compounds measured in nail salons (Regulations for Hazardous Chemical Substances, 1995).

Substance	TWA OEL		STEL OEL	
	ppm	mg/m^3	ppm	mg/m^3
Acetone	750	1780	1500	3560
Ethanol	1000	1900		
Ethyl acetate	400	1400	150	650
Ethyl benzene	100	435	125	545
Ethylmethacrylate	100	-	-	-
Methylmethacrylate	100	410	125	510
n-buthyl acetate	-	-	-	-
Formaldehyde	* 2	* 2,5	* 2	* 2,5
Toluene	50	188	150	560
Xylene	100	435	150	650

* Indication of Control Limits, whereas the rest of the values are Recommended Limits

Materials and Methods

Sampling strategy

Fifteen nail technicians in the Potchefstroom and Klerksdorp area were recruited for this study. The control subjects, women living in the Potchefstroom area, were matched to the nail technicians with regard to smoking habits and age.

The control group were not exposed to any of the chemicals found in nail salons. All participants signed a consent form. Results and information of participants were kept confidential. The study was carried out with the written approval of the ethics committee of the North West University, Potchefstroom Campus (Ethics Number 05M17), as an extension on a study done in 2007 on the risk of DNA damage in hair salons.

Air Sampling

Exposure to formaldehyde, acetone, toluene, Xylene, methylmethacrylate, n-buthyl acetate and ethylmethacrylate were measured using personal air sampling pumps, and the appropriate sorbent tubes. Monitoring took place over one work day for each nail technician for a duration of 8 hours which represents a full work shift. Due to the fact that formaldehyde could not be measured with the other volatile organic compounds (VOC), a double adjustable flow holder was used, which accommodated for this.

Formaldehyde was measured at 0.1 L/min, whereas the rest of the chemicals were measured at 0.2 L/min.

Formaldehyde was sampled by means of NIOSH 2541 analytical method, using solid sorbent XAD-2 tubes (2-hydroxymethyl piperdine, SKC 226-30-04). The personal sampling pumps were calibrated prior to investigation to accommodate both flow rates, as well as proceeding sampling to ensure that the flow rate was consistent whilst measuring.

Acetone, toluene, xylene and ethylmethacrylate were sampled by way of one solid sorbent tube (coconut shell charcoal, SKC 226-01) for all the chemicals, using analytical methods similar to those recommended by NIOSH 1501.

Proceeding measurements, the sorbent tubes were capped airtight, and securely packed for transport. The samples for formaldehyde were stored at a temperature of ± 25 °C and the samples for the additional vapours were stored at ± 5 °C. All sorbent tubes were sent to an accredited laboratory for gas chromatography / Mass Spectrometry analysis.

Blood samples

Heparinized blood samples were obtained on Wednesday afternoon by professional nurses,it was capped, labelled, and kept at 4 ˚C for no more than 2 hours, where after the comet assay analysis was performed. No more than 6 samples were taken at a time to expedite their processing.

Comet assay

Lymphocyte isolation

2 mL of the blood samples were transferred to Falcon tubes, containing 2 mL Histopaque. Samples were centrifuged at 5000 rpm for 30 minutes. The buffy coat was removed, washed twice in PBS (phosphate buffered saline), and re-suspended in 1000 µl of PBS. Each sample contained at least 1000 cells.

Comet assay

The comet assay was performed under alkaline conditions. Control slides were plated using 30 µl of the cell suspension mixed with 90 µl of LMPA (low melting point agarose) at 37 ˚C, and were spread evenly on a microscope slide which was previously coated with 300 µl HMPA (high melting point agarose). Slides were kept on ice to stop cell metabolism.

DNA damage

Three slides of each sample were prepared, one as control, one for treatment of Fpg, and one for treatment with Endo III, using 30 µl of untreated cells, and 90 µl of LMPA. These slides were also immersed in Lysing buffer containing 5 M NaCl 0.4 M EDTA, Triton X-100, and 10 % DMSO at 4 ˚C and kept overnight.

One slide was left as control and the others washed in ddH_2O, and treated with 50 µl of the lesion-specific enzymes. The slides were left to incubate for 30 minutes at 37 ˚C in a damp container.

Repair capacity

After control slides were plated, 40 µl H_2O_2 was added to 360 µl of the cell suspension, and left to incubate for 10 minutes. The mixture was centrifuged and re-suspended in 300 µl PBS. Control slides for repair capacity were prepared using 30µl of the cell suspension mixed with 90 µl of LMPA. After centrifuging, the cells were re-suspended in 280 µl HAMS F10 solution, which assist in the repair process of the cells. The cells were left to incubate for 30 and 60 minutes, where after slides were prepared using 30 µl of the cell suspension, and 90 µl LMPA. All slides were then left overnight in lysing buffer at 4 ˚C.

Electrophoresis

After all the slides were washed in ddH_2O, they were left in electrophoresis buffer (0.6 M NaOH. 0.05 M EDTA, ddH_2O) for 30 minutes. All steps onwards were done at 4 ˚C for all slides. Thereafter, slides were placed in an electrophoresis tank for 40 minutes at 30 V and 300 mA to allow DNA alkaline unwinding. Slides were rinsed and placed in neutralizing buffer (0.5 M NaCl pH 7.5) for 15 minutes. thereafter, slides were stained using ethidium bromide.

For each sample a minimum of 50 comets were photographed using an Olympus IX 70 Fluorescent microscope. The tail intensity were analysed using a Comet Assay IV programme and the data was processed in Microsoft Excel.

Cells which are damaged forms a comet consisting of a head and tail. The amount of DNA that has migrated is correlated with the degree of damage (Singh *et al.*, 1988; Keretetse *et al*, 2008). Damage increases from Class 0 to Class IV as indicated by the increasing tail migration of the DNA (class 0:0-6 %; class I:6,1-17 %; class II:17,1-35 %; class III:35,1-60 %; class IV:60,1-100 %) (Giovannelli *et al.,* 2002).

The repair capacity (RC) was determined using the following equation:

$$RC = 1 - \left\{ \frac{\%\text{Tail DNA after 60 minutes}}{\%\text{Tail DNA after } H_2O_2 \text{ treatment}} \right\}$$

Data was transferred to Excel and statistically analysed using Statistica 8. Comparisons were made using confounding factors. Statistically significant correlations were determined using the Spearman Rank Order. The T-Test and Mann Whitney U Test were used to identify statistically significant differences between control and exposed groups and the smoking habits. The ANOVA analysis was used to determine statistical significance between control and exposed groups regarding age. Statistical significance is taken at $p \leq 0.05$ or less.

Results

Table 2 indicates that control and the exposed groups were evenly matched in the confounding factors age and smoking. The nail technicians and the control group were evenly distributed in age groups of < 25 years, 25-35 years and >5 years. The number of smokers (27 %) was less than the number of non – smokers (73 %).

Table 2: Characteristics of the study groups.

	Control Group	**Exposed Group**
Number of Subjects	15	15
< 25	5	5
25 - 35	5	5
> 35	5	5
Smokers	4	4
Non-smokers	11	11

The descriptive statistics of personal exposure monitoring are given in Table 3, including average exposure, baseline damage, pyrimidine (Fpg) and purine (Endo III) damage. The correlation between the various chemicals and DNA damage was determined using the Spearman Rank Order Correlation. A statistically significant correlation (p=0.01) was found between the level of exposure to methylmethacrylate and ethylmethacrylate and the level of baseline damage. Exposure to methylmethacrylate also showed a significant correlation with the level of pyrimidine (Fpg) damage (p=0.02). Exposure to toluene and xylene indicated a negative correlation between the level of exposure and Endo III DNA damage. The remaining VOC's had an insignificant correlation (p>0.05) with any of the DNA damage parameters. None of the chemicals investigated exceeded their individual Occupational Exposure Limit, or the action level.

Table 3: Correlations of the average exposure, baseline damage as well as purine (Endo III) and pyrimidine (Fpg) damage.

Variable	**Mean Exposure ± SD (ppm)**	**Baseline Damage (r-values)**	**Pyrimidine damage (Fpg) (r-values)**	**Purine damage (Endo III) (r-values)**
Acetone	12.75 ± 9.97	0.28	0.04	-0.29
Ethanol	1.52 ± 1.37	-0.23	-0.10	-0.15
Ethyl acetate	0.37 ± 0.40	0.00	-0.19	0.15
Ethylmethacrylate	1.75 ± 1.65	**0.77***	0.59	-0.07
Methylmethacrylate	0.01 ± 0.01	**0.57***	**0.68***	0.02
Toluene	0.02 ± 0.01	0.25	0.32	**-0.66***
n-buthyl acetate	0.06 ± 0.08	-0.32	-0.41	-0.29
Xylene	0.01 ± 0.00	-0.17	0.28	**-0.52***
Formaldehyde	0.16 ± 0.09	-0.01	-0.09	-0.43
# Total chemical exposure	1.67 ± 1.10	0.35	0.10	-0.31

The sum of all exposure to chemicals measured

* Values indicated in bold represents statistical significant values with p≤0.05

Table 4 illustrates the correlation between exposure to various chemicals, DNA damage, DNA damage measured in the repair process and repair capacity. n-buthyl acetate and xylene showed a positive correlation between the level of exposure and DNA damage 30 minutes after maximum damage was caused and repair was initiated (p=0.02). Acetone and the total chemical exposure indicated a significant negative correlation with the repair capacity.

Table 4: Correlations between exposure to chemicals, baseline DNA damage, damage measured during the repair process and repair capacity.

Variable	Baseline Damage (r-values)	30 minutes after H_2O_2 induced Damage (r-values)	60 minutes after H_2O_2 induced Damage (r-values)	Repair Capacity (r-values)
Acetone	0.18	0.38	0.21	**-0.59***
Ethanol	-0.10	0.10	0.10	-0.41
Ethyl acetate	-0.08	0.13	-0.06	-0.37
Ethylmethacrylate	0.12	-0.28	-0.52	-0.36
Methylmethacrylate	0.18	-0.16	-0.18	-0.29
Toluene	-0.06	-0.12	-0.17	-0.50
n-buthyl acetate	0.09	**0.61***	0.32	0.28
Xylene	0.21	**0.62***	0.24	-0.38
Formaldehyde	0.26	0.20	0.42	-0.21
# Total chemical exposure	0.21	0.39	0.10	**-0.65***

The sum of all exposure to chemicals measured

* Values indicated in bold represents statistical significant values with p≤0.05

Table 5 renders the DNA damage and repair capacity of the control and exposed groups. Tail intensities were used to quantify the level of DNA damage (Keretetse *et al.*, 2008) in lymphocytes of both the control and exposed groups. Pyrimidine (Fpg) damage of the control group was significantly higher than the pyrimidine (Fpg) damage of the exposed group (p=0.02). The purine (Endo III) damage of the exposed group was elevated when compared to the control group, although not statically significant. Total damage caused by hydrogen peroxide was slightly higher in the control group than in the exposed group. From the results it may be concluded that the total damage caused in both groups increased when compared with the baseline damage.

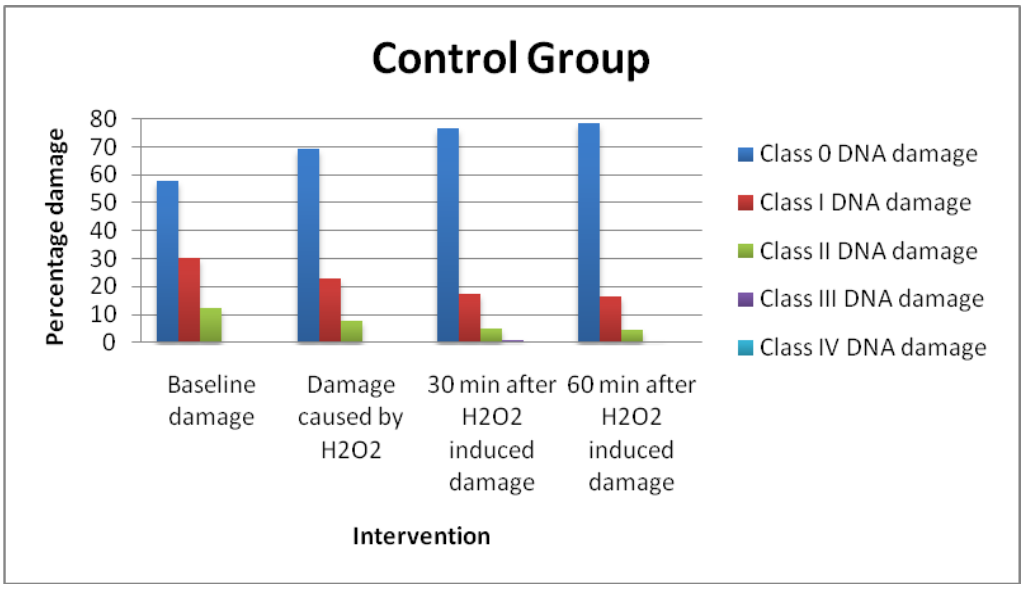
Table 5: Results of DNA damages and repair capacities of the exposed and control groups.

		Control Group		**Exposed Group**	
		Tail intensity		**Tail intensity**	
		Mean (± SD)	**Minimum / Maximum**	**Mean (± SD)**	**Minimum / Maximum**
Damage	Baseline damage	6.08 ± 2.41	2.62 / 9.91	4.78 ± 7.98	1.19 / 33.10
	Pyrimidine damage (Fpg)	**14.09 ± 10.56***	**0.51 / 33.23**	**8.92 ± 5.65***	**0.73 / 16.78**
	Purine damage (Endo III)	14.79 ± 6.05	7.83 / 26.67	17.70 ± 12.46	5.21 / 47.73
Repair measured	Baseline damage	4.99 ± 1.62	3.02 / 8.23	3.59 ± 2.44	1.37 / 11.06
	Total damage caused (H_2O_2)	12.07 ± 10.06	3.70 / 34.03	8.99 ± 6.88	1.89 / 28.62
	Damage caused after 30 minutes	6.47 ± 4.54	2.21 / 21.35	13.37 ± 8.88	0.98 / 30.51
	Damage caused after 60 minutes	8.46 ± 2.66	4.30 / 13.37	9.66 ± 6.24	1.39 / 21.30
Repair capacity		1.16 ± 0.78	1.16 ± 0.78	0.15 / 2.68	1.34 ± 0.94

* Values indicated in bold represents the significant difference (p=0.02).

Figure 1 a and b illustrates the baseline damage, total damage caused by H_2O_2, as well as the damage at 30 and 60 minute intervals after repair was initiated, of both the exposed and control groups respectively. Repair is depicted by a shift in damage class from Class IV to Class 0. The exposed group showed more Class 0 damage in the baseline damage, but also showed Class IV damage compared to the control group. The exposure of lymphocytes to hydrogen peroxide increased the damage in all classes of the exposed group. Thirty minutes after the exposure to H_2O_2 there was an increase in the types of damage and a shift to the right is seen. Sixty minutes after repair was initiated, Class III and IV damage was eliminated and only Class 0, Class I, and Class II damage could be seen. In the control group there was a shift to the left after 30 minutes as seen in the increase of Class 0 damage, as well as a shift to the right as seen by the appearance of Class III damage. This Class III damage was eliminated as seen at 60 minutes and no Class IV damage was seen in the control group.

(a)



(b)

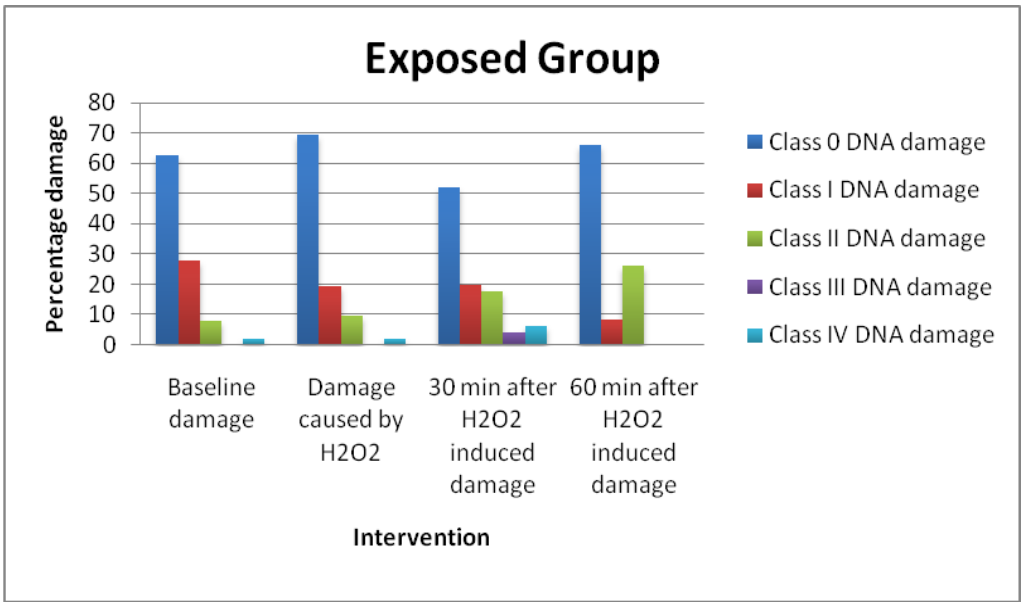


Figure 1: Mean baseline DNA damage, damage caused by H_2O_2, as well as damage after 30 and 60 minutes of the repair process for the exposed group (b) and the control group (a).

Figure 2 shows the effect of smoking on DNA damage in both the exposed and control groups. A T-Test and Mann Whitney U Test between smoking and non-smoking groups showed no statistical significance. However, pyrimidine (Fpg) DNA damage was higher in the control group when compared with the exposed group for smokers. Purine (Endo III) damage was higher in the exposed group compared to the control group (for smokers).

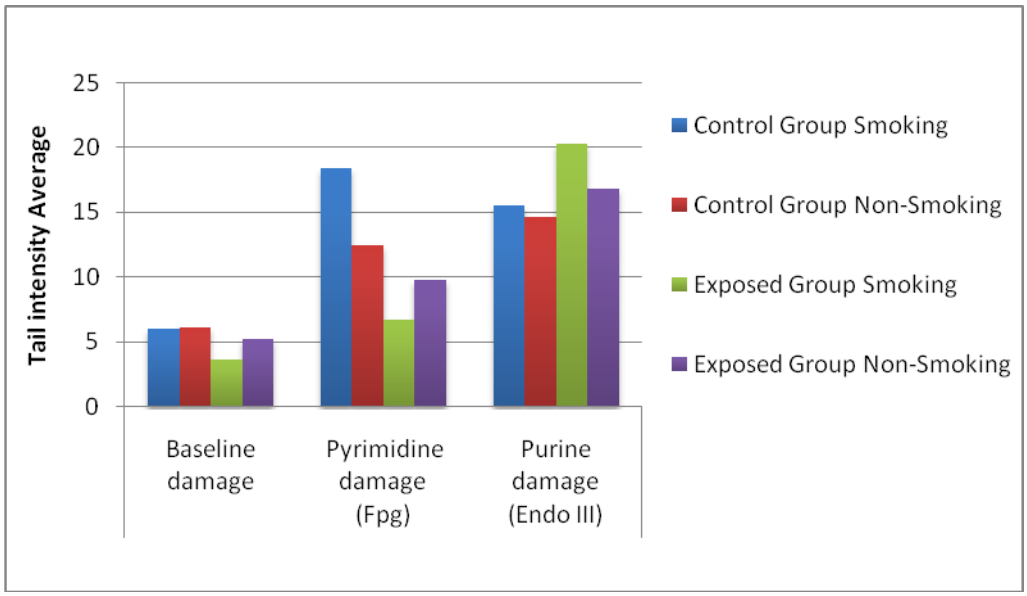
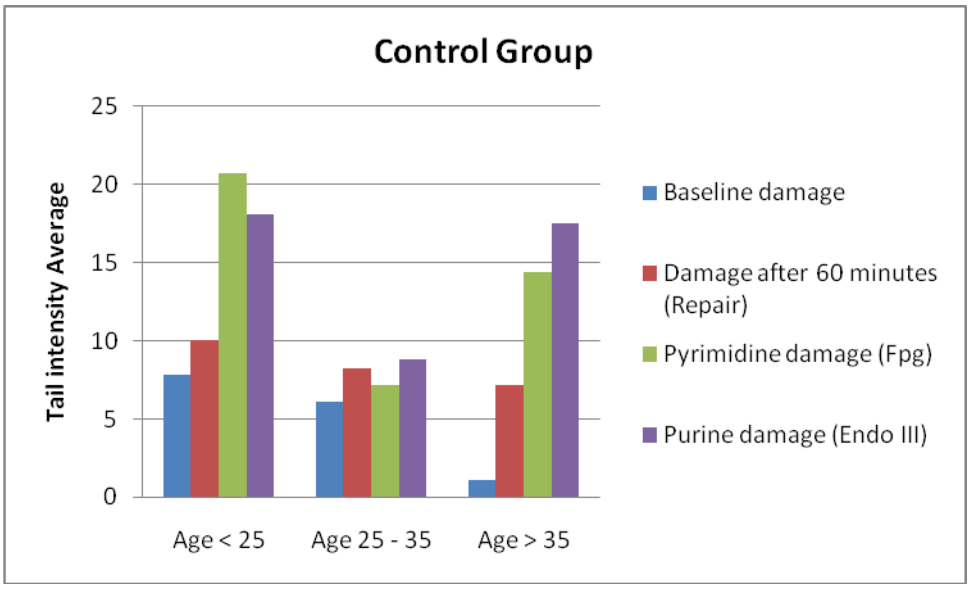


Figure 2: DNA damage compared between smokers and non-smokers.

Figure 3 a and b represents the effect of age on DNA damage and were also compared to the control group using the ANOVA analysis. The results indicate that the control group subjects with an age < 25 years had elevated pyrimidine (Fpg) damage when compared to the individuals of the exposed group of the same age group, although not statistically significant. The baseline damage of the exposed group for the age group > 35 was higher than the control group.

(a)



(b)

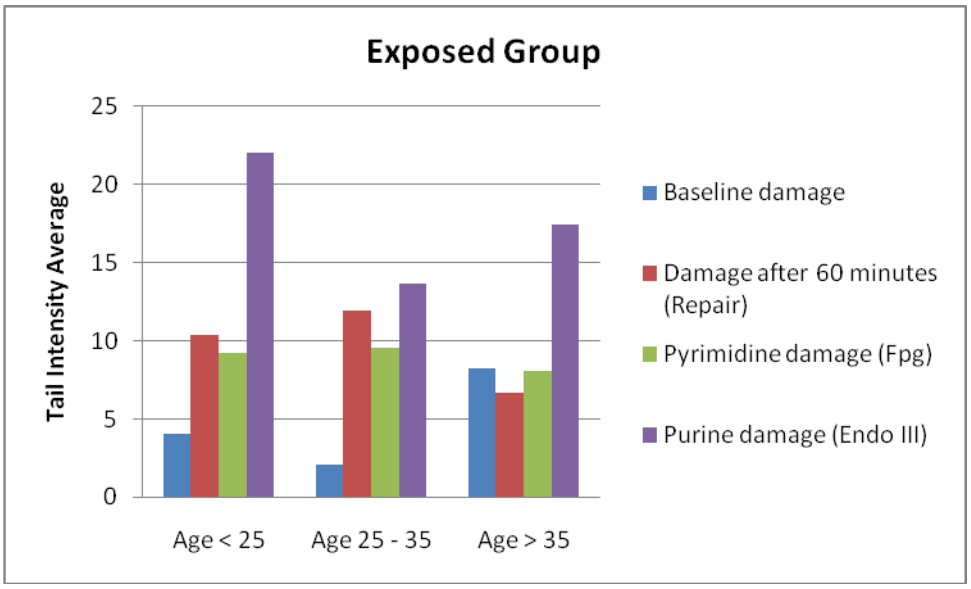


Figure 3: DNA damage of various age groups of the exposed group (b) and control group (a).

Figure 4 indicates the correlations between the repair capacities of the control and exposed groups, with regards to age and smoking habits. Previously mentioned smoking habits and age did not have a statistical significant on DNA damage and repair capacity. This figure shows a noteworthy difference between the control group and exposed group regarding repair capacity of the age group > 35. The remaining age groups and smoking habits showed no notable difference in repair capacities.

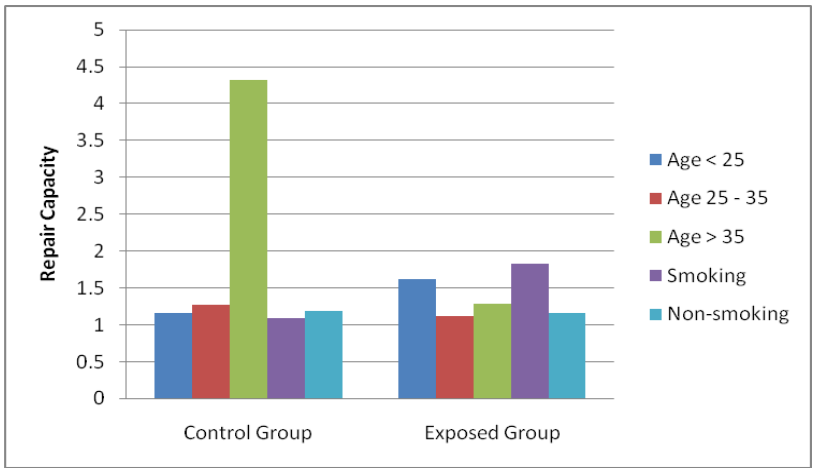


Figure 4: Repair capacities of the exposed group and control group with regards to age and smoking habits.

Discussion

The results of this survey revealed that none of the measured chemicals exceeded their individual Occupational Exposure Limits, or the action levels (50% of the OEL). Although nail technicians may not be presented with an immediate risk, long term exposure may lead to various health effects, as well as DNA damage (Keretetse *et al.,* 2008; Dreiem *et al*., 2002). Studies on the long term exposure to volatile organic compounds in nail salons have not been conducted in South Africa, or other countries. For this reason volatile organic compounds must be continuously monitored to determine the exposure risk of nail technicians, as well as the implementation of control measures.

A significant correlation was found between the level of exposure to methylmethacrylate and ethylmethacrylate and baseline damage. Baseline damage is the damage determined before any experimental influence. This finding therefore indicates that exposure to ethylmethacrylate and methylmethacrylate leads to DNA damage. A study done by Moore *et al*. (1988) on the effect of ethylmethacrylate and methylmethacrylate on L5178Y mouse lymphoma cells found that these chemicals caused DNA damage. Exposure to methylmethacrylate also indicated a significant correlation with the level of pyrimidine (Fpg) damage ($p=0.02$). Therefore it may be concluded that methylmethacrylate causes DNA damage by specifically damaging pyrimidine bases. These findings are in concurrence with previous studies done by Dreiem *et al*. (2002, 2003); Keretetse *et al*. (2008) and Mattia *et al*. (1993), who found that exposure to elevated levels of volatile organic compounds lead to increased DNA damage. However a negative correlation was identified between the exposure to xylene and toluene and the level of purine (Endo III) DNA damage. The negative correlation is in contradiction with the studies done by Dreiem *et al*. (2002, 2003); Keretetse *et al*. (2008) and Mattia *et al*. (1993). It may be concluded that xylene and toluene may not lead to purine damage, but may still lead to pyrimidine and baseline damage, although this finding could not be derived from this study. The remaining chemicals did not indicate statistically significant correlations between the level of exposure and the level of DNA damage. However, many of the volatile organic compounds found in nail salons has the same target organs and may therefore cause elevated damage with long term, continuous exposure. Chemicals such as acetone is one of the most used chemicals in nail salons as seen in this study (the average acetone exposure level was measured at 12.75 ppm), which may lead to increased exposure to the above mentioned chemical (Porter, 2009). Acetone containers are often left open on work tables, which may lead to the evaporation of acetone into the working environment of the nail technicians. Acetone showed a

negative correlation with repair capacity and will therefore impair the DNA repair capacity, this enhances the risk involved with exposure to acetone in nail salons.

Repair capacity was not directly measured in this study, but repair was initiated by adding HAMS solution to the cells, after maximum damage was caused by H_2O_2. By adding HAMS solution, the repair process was stimulated and slides were made 30 and 60 minutes after the cells were left to incubate with the HAMS solution. These slides were used to determine tail intensity, and therefore determining DNA damage still occurring at these time intervals. Results at these intervals consequently still represents damage, and repair capacity was calculated using these results. If the quantity of damage decreased at these intervals, it can be concluded that repair had taken place. Therefore a distinction must be made between repair capacity that was calculated, and repair that can be observed from the decrease in damage measured at the 30 and 60 minute intervals.

N-buthyl acetate and xylene showed a significant positive correlation with the DNA damage 30 minutes after hydrogen peroxide induced damage and repair was initiated ($p=0.02$). Consequently, as exposure to these chemicals increase, the DNA damage will also increase. At 60 minutes after repair was initiated there was a positive correlation between the level of exposure to n-buthyl acetate and xylene and DNA damage, however this correlation was not significant. This may indicate that repair had taken place. It may therefore be concluded that Xylene and n-buthyl acetate prolonged the repair process, and that repair took place after 60 minutes. Acetone, as mentioned previously, and the total chemical exposure indicated a significant negative correlation with repair capacity, indicating that increased exposure lead to decreased repair capacity, thus DNA repair capacity was impaired. These findings are in concurrence with the study done by Keretetse *et al.* (2008). The higher the DNA damage at 30 and 60 minute time intervals, the lower the level of repair was during that time. Therefore, n-buthyl acetate and xylene may impair the DNA repair mechanism, whereas acetone and total chemical exposure impaired overall DNA repair capacity. These results are in concurrence with the study done by Keretetse et al. (2008) who found that exposure to volatile organic compounds lead to impaired DNA repair capacity. Studies done by Andreoli *et al.* (1997) and Franceschetti *et al,* (2005) also support this finding. The other chemicals did not have a significant correlation with the level of repair capacity.

Baseline DNA damage, as well as damage caused after treatment with H_2O_2 of the control group, was higher than that of the exposed group, although not statistically

significant. Purine (Endo III) damage of the exposed group was elevated when compared to the control group. Pyrimidine (Fpg) DNA damage of the control group was significantly higher than the exposed group. A study done by Moller *et al*. (2008) on the effect of air pollution on DNA damage, revealed that pyrimidine (Fpg) damage increased with exposure to volatile organic compounds and other air pollutants, which is in contrast with the results found in this study. The level of exposure to different chemicals may be the cause of conflicting results, as seen previously, chemicals such as methylacrylate lead to increased pyrimidine (Fpg) damage.

The classes of DNA damage of the exposed group was compared with the control group. Damage increases from Class I to Class IV as indicated by the increasing tail migration of the DNA (class 0:0-6 %; class I:6,1-17 %; class II:17,1-35 %; class III:35,1-60 %; class IV:60,1-100 %) (Giovannelli *et al.,* 2002). More types of classes of baseline DNA damage was found in the exposed group when compared to the control group. After treatment with hydrogen peroxide, damage in Classes II and IV increased. 30 minutes after exposure to H_2O_2 an increase in Classes I to IV DNA damage could be seen, with the inclusion of Class III and IV. After 60 minutes, Class III and IV were repaired and only Class 0, I and II could be seen in the exposed group. It was also noticed that Class II damage was visible from 30 minutes, and increased at 60 minutes. DNA repair occurred from 30 minutes onwards, as Class III and Class IV was eliminated, and Class 0 and Class I increased. The control group showed little or no Class III DNA damage (only at 30 minutes) and no Class IV damage. Therefore repair took place after H_2O_2 induced damage and Class 0 increased. A study done by Van Dyk and Pretorius (2005) found that the distribution of the comets suggest that DNA repair takes place at a rapid rate in the period beyond the 40 min repair time since after 60 min almost all the comets were found to be in Class I. In the control group, repair took place immediately after H_2O_2 induced damage, whereas repair could only be seen in the exposed group after 60 minutes (damage was still visible after 30 minutes). The study supports our findings that DNA repair took place in the time interval between 30 and 60 minutes, due to the fact that no Class III and Class IV damage could be seen in either group. It may also be concluded that the exposed group showed more DNA damage, as Class III and Class IV DNA damage were notably visible.

The effect of smoking between the exposed group and the control group did not reveal a significant difference on the level of DNA damage or repair capacity. Pyrimidine (Fpg) damage was slightly higher in the smoking control group when compared with the smoking exposed group (as also seen in table 5, not taking smoking into account).

Thorne *et al*. (2009) found that smoking lead to increased pyrimidine (Fpg) sensitive oxidative DNA lesions founded by high levels of strand breaks at alkali labile sites, but did not have a visible effect on purine (Endo III) damage. The results of this study revealed that the smokers of the control group had an increased level of pyrimidine (Fpg) damage, which is in concurrence with previous studies. However, the smokers of the exposed group had higher purine (Endo III) damage than pyrimidine (Fpg) damage, which is in contradiction with earlier studies. The elevated purine (Endo III) damage may be a result of the occupational exposure to volatile organic compounds, in addition to smoking habits (Novotna *et al*., 2010).

Age indicated no significant influence on DNA damage and repair between the control group and the exposed group. The effect of age did however indicate that the control group subjects with an age of < 25 had elevated pyrimidine (Fpg) damage when compared to the individuals of the same age group for the exposed group. The pyrimidine (Fpg) damage of the exposed group was therefore less than the control group, although the purine (Endo III) damage was higher in the exposed group for the same age group. Baseline damage for the age group > 35 in the exposed group was higher than the baseline damage of the control group, which may be the result of long term occupational exposure to volatile organic compounds. The study done by Minikantan *et al.* (2010) found no significant correlation between DNA damage with age. Nevertheless, these findings are in contrast with data proposed by similar studies, who found that DNA damage was more frequent in elderly persons, when compared with younger subjects (Rossi et al., 2007). A study done by Mota *et al.* (2010) found that pyrimidine (Fpg) DNA damage increased with age. These findings are contradictory to the findings of this study, as indicated by the pyrimidine (Fpg) damage of the age group < 25 that was higher than the pyrimidine (Fpg) damage of the age group > 35 of both the control and exposed groups.

As previously mentioned, smoking habits and age had no significant influence on the DNA repair capacity or DNA damage. However, the mean repair capacity of the exposed group (1.34) was higher than the repair capacity of the control group (1.16), although not statically significant. Results have also indicated that DNA was still damaged after 60 minutes (after DNA damage caused by H_2O_2), thus repair has not significantly taken place in the exposed group, whereas the control groups mean DNA damage decreased after 60 minutes, indicating DNA repair has successfully taken place. These results are in concrrence with studies done by Keretetse *et al.* (2008), as well as

studies in other countries (Andreoli *et al.,* 1997 and Franceschetti *et al.,* 2005), who found that individuals exposed to volatile organic compounds showed an impaired DNA repair capacity. Repair capacities of the age group > 35 in the control group was noticeably higher than the repair capacities of the exposed group, although no literature could be found to support this finding.

Conclusion

From the results of this study it may be concluded that nail technicians are exposed to long term low levels of volatile organic compounds during their everyday work routine, as none of the chemicals evaluated exceeded their occupational exposure limit. Chemicals such as ethylmethacrylate and methylmethacrylate causes DNA damage, whereas n-buthyl acetate and xylene may prolong the DNA repair process. The first hypothesis is accepted, as chemicals such as ethylmethacrylate and methylmethacrylate lead to DNA damage. The second hypothesis is accepted as n-buthyl acetate and xylene may impair DNA repair mechanisms and acetone impaired DNA repair capacity overall.

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CHAPTER 4

SUMMARY AND CONCLUSION

1. Summary and Conclusion

Nail technicians are exposed to volatile organic compounds in their daily work routine which may lead to genotoxic consequences. These compounds include acetone, formaldehyde, ethylmethacrylates, methylmethacrylates, toluene and xylene, which are present in the products that nail technicians use daily, some of which have been identified as human carcinogens (formaldehyde) (IARC, 2006) and group three carcinogens (toluene and xylene) (IARC, 1999). A study done by Hipakka and Samimi (1987) found no evidence of these substances causing DNA damage, however further studies were recommended.

The results of this study indicated that none of the chemicals investigated exceeded their individual Occupational Exposure Limits, or the action levels (50% of the OEL), although some subjects in this study did show elevated levels of DNA damage. Methylmethacrylate and ethylmethacrylate had a significant positive correlation with the level of baseline damage. Mehylmethacryate also had a significant positive correlation with the level of pyrimidine (Fpg) damage. Therefore, acrylates such as ethylmethacryate and methylmethacrylate may cause DNA damage, whereas methylmethacrylate may also lead to base specific pyrimidine damage. In the USA, the Food and Drug Administration banned the use of methylmethacrylates in nail salons, due to the health hazards the chemical presents, such as severe skin irritation, sensitization, respiratory irritation and infections around the nail plate (FDA, 2005). The use of methylmethacrylate in New Zealand nail salons has also been banned (ERMA, 2006). This is problematic as methylmethacrylate exposure was found in the nail salons, and therefore is present in products used in South Africa despite its well-known health hazards.

N-buthyl acetate and xylene had significant correlations with the level of DNA damage 30 minutes after H_2O_2 induced damage, thus with increased exposure the DNA damage will increase. After sixty minutes, a insignificant positive correlation was found between n-buthyl acetate and xylene and DNA damage, indicating that repair had taken place. It may therefore be concluded that exposure to n-buthyl acetate and xylene may prolong the DNA repair process. The other chemicals did not have a significant correlation with the level of repair capacity.

The mean DNA repair capacity in the exposed group was slightly higher than the repair capacity of the unexposed group, which indicates that the repair mechanism of the

exposed group may have been affected by the exposure to chemicals. These results are in contradiction with studies done by Keretetse *et al.* (2008), as well as studies in other countries (Andreoli *et al.,* 1997 and Franceschetti *et al.,* 2005), who found that individuals exposed to volatile organic compounds showed an impaired DNA repair capacity. However, some nail technicians did show decreased repair capacity. Thus the effect of long term exposure on repair capacity is still unclear and should be investigated further. As previously noted, the level of DNA damage after 30 minutes of incubation for the exposed group was higher than that of the control group. Only after 60 minutes was DNA damage repaired, indicating that the repair mechanism of the exposed group may be impaired, whereas repair in the control group could already be seen after only 30 minutes of incubation with HAMS. However, the calculated average repair capacity of the control group was higher than the repair capacity of the exposed group.

Mean baseline DNA damage, as well as damage caused after treatment with H_2O_2 of the control group was slightly higher than that of the exposed group. This finding may be due to various other confounding factors that have not been taken into account in this study. Purine (Endo III) damage of the exposed group was elevated when compared to the control group, which may be attributed to the exposure to volatile organic compounds. Pyrimidine (Fpg) DNA damage was significantly higher in the control group than in the exposed group, therefore it may be concluded exposure to certain chemicals does not lead to pyrimidine DNA damage.

The comparison between the different classes of DNA damage of the exposed group indicated that Class III and Class IV damage was visible 30 minutes after repair was initiated, and completely repaired after 60 minutes, which indicated that repair had taken place, although the extent of damage in the exposed group was higher than in the control group, as Class III and Class IV damage was more visible. In the control group, no Class III or Class IV DNA damage was visible at any stage and repair took place at a faster rate. After 60 minutes, only Class 0, I and II DNA was visible in both the control and the exposed groups.

The effect of smoking and age between the exposed group and the control group did not reveal any significant differences on the levels of DNA damage or repair capacity.

The aim of this study was to investigate the level of exposure to volatile organic compounds nail technicians are exposed to daily, as well as to determine if these

chemicals might cause DNA damage, and impair DNA repair capacity. The first hypothesis of this study is accepted, as occupational exposure to ethylmethacrylate and methylmetacrylate lead to DNA damage. The second hypothesis is accepted as n-buthyl acetate and xylene may impair DNA repair mechanisms and acetone and total chemical exposure impaired DNA repair capacity overall.

2. Limitations

Shortcomings of this study include the small study group that was used. Due to the small group, sufficient statistical analysis could not be performed. A considerable amount of studies have not yet been conducted on the effect of VOC's on nail technicians and none of these studies were conducted in South Africa. Short Term Exposure Levels could not be obtained from test subjects the time period given to complete the study. All the chemicals used in nail salons could not be sampled in this study due to the different sampling strategies required for various substances, as well as the exposed groups unwillingness for further evaluation. Chemicals was chosen depending on the frequency, consequence and amount used on a daily basis.

3. Further Studies

Further studies are recommended which include different confounding factors such as period working as a nail technician, diet, exercise and ethnic group. Studies should also include a larger group of nail technicians and control subjects to ensure statistical significant results.

A broader range of chemicals should be investigated to determine the exposure of nail technicians to all of the hazardous chemical substances present in nail salons. Task specific short term exposure should be measured i.e. during soaking of nails, when acetone levels may be elevated to determine the contribution of each task in a nail salon to overall VOC exposure. It is also recommended that task specific exposure include all tasks during nail application and removal of artificial and gel nails.

4. Recommendations

Due to the nature of the occupation, sufficient local extraction ventilation systems are recommended to prevent inhalation of dangerous volatile organic compounds and particulates. General heating, ventilating, and air-conditioning systems (HVACs) may be used to exchange the air inside a salon. A nail salon should use a system intended to effectively capture and remove vapours and dust or expel them from the workplace (SAS, 2010). The unit should have a capture velocity of at least 0.5 m/s, according to the General Safety Regulations, Occupational Health and Safety Act, Act 85 of 1993.

Figure 1: An example of local extraction ventilation systems that may be used in nail salons, which can either be wall mounted (Left) or on the floor (Right).

The above mentioned extraction ventilation systems does not take up the much needed space and are equipped with a semi self-supportive flex hose so that the operator can position the source capture area where it is needed. Providing adequate extraction ventilation in nail salons will eliminate the need to use respirators to provide protection against the inhalation of volatile organic compounds. Latex or nitrile gloves may be worn to provide protection against skin contact with chemicals.

In the event where extraction ventilation systems are used, these systems should be inspected at least once every three months. All mechanical parts must be inspected for signs of deterioration such as wear and tear, corrosion and damage. This will ensure continuous sufficient extraction and limit excessive maintenance costs.

Due to the fact that many nail salons are located inside malls (doors and windows cannot be opened), sufficient extraction ventilation is required. Where extraction ventilation is not probable the following are recommended: openable windows and doors

should be kept open during warmer periods to allow for the introduction of fresh outside air and to create a natural draft through the work area. Fans may be provided in order to increase the amount of linear air movement, thereby ensuring a measure of thermal comfort and to assist with the removal of air contaminants.

5. References

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