EVALUATION OF SELECTED IMMUNOLOGIC INDICES AMONG OCCUPATIONALLY EXPOSED MALE PHOTOCOPIER OPERATORS

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OCTOBER, 2025.

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE, SCHOOL OF BASIC MEDICAL SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, EDOSTATE IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF MEDICAL LABORATORY SCIENCE DEGREE (B.MLS) IN MEDICAL LABORATORY SCIENCE.

SUPERVISED BY

DR. (MRS) . J. C. OTIKOR

OCTOBER, 2025.

CERTIFICATION

This is to certify that the project was carried out by **OTERI, OGHENEBRORHIE SARAH** with the matriculation number **BMS2101527** under the supervision of **Dr. (Mrs). J. C. Otikor** in the Department of Medical Laboratory Sciences, School of Basic Medical Sciences, University of Benin, Benin City in partial fulfillment of the requirement for the award of Bachelor of Medical Laboratory Science (BMLS) Degree.

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DEDICATION

I dedicate this work to God almighty, who is my creator, my steadfast supporter, my wellspring of insight, understanding and wisdom and also to my Mom for her great investment into my academic pursuit.

ACKNOWLEDGMENTS

I give thanks to God almighty for His grace upon my life and for seeing me through this research work.

My profound gratitude goes to my supervisor Dr. (MRS) J.C. OTIKOR for her concern, constructive and supportive idea which has aided this research work.

Special thanks to the Head of Department, Medical Laboratory Science, Dr. (MRS.) Z. OMORUYI and also to the entire staff and lecturers of the department for investing so much in my academic and character development.

My very special thanks go to Dr. N.S. Owie for his support, guidance and counsel throughout the preparation of this work.

My appreciation goes to my wonderful and blessed parents, Late Sunday A. Oteri and Mrs. Esther E. Oteri for their love, all round support, encouragement and prayers.

My profound gratitude goes to my lovely siblings; Christiana, Samson, Gloria and shedrack. May God bless you all

I sincerely appreciate the unwavering support and encouragement of my friends, Maxwell and Kate, whose kindness, understanding, and motivation greatly contributed to the successful completion of this project.

Special thanks to my wonderful friends; Valentina, Victor and Onome Who stood by me all through my journey in this school and supported me with prayers and love. God bless you all so much. You have a special place in my heart

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ABSTRACT

Photocopying which is the act of paper duplication has become an indispensable activity in academic and business settings which is increasingly been recognized as a source of environmental and occupational hazards. Photocopier machines release emissions containing ultrafine toner particles, volatile organic compounds (VOCs), ozone and heavy metals. Studies reveals that prolonged exposure to these chemical agents among occupationally exposed individuals is associated with respiratory problems, oxidative stress, inflammatory responses and haemotoxicity. Hence, this research was aimed to evaluate selected immunological indices in male photocopier operators who are occupationally exposed to photocopier emissions in university of Benin campus in Benin City. A total of ninety (n=90) participants were used for this study; comprising of sixty (n=60) exposed and thirty (n=30) non-exposed after administering well structural questionnaires. Eight (8) milliliters of venous blood was collected from ante cubital fossa after routine daily work (6-8 hours) using vacutainer and were dispensed into EDTA and Plain containers for total white blood cell count, Differential cell count, Cluster cell of differentiation count ($CD4^+$ T- Lymphocyte cell) and serum levels of Immunoglobulin E (IgE) respectively. Total white blood cell and Differential cell count were analyzed using SFRI haematology auto-analyzer, $CD4^+$ count using Partec Cyflow counter and serum IgE level was assay using ELISA technique. Data were analyzed using Statistical package for social science (SPSS) version 28. Analysis of variance (ANOVA), Chi-square and analysis of variance Statistical significance level was set at $p < 0.05$. Total white blood counts (Twbcs), Lymphocytes and monocytes, Absolute Lymphocyte and serum IgE level were significantly higher ($p< 0.001$) among occupationally exposed group (5.42 ± 0.18, 48.47±1.03, 16.45±0.42, 2.55±0.07 and 2.159±5.92) compared with the non-exposed group (4.01 ± 0.07, 34.31±0.54, 9.61±0.18, 2.11±0.15 and 1.580±3.95) respectively. While Granulocytes and $CD4^+$ cell count were Significantly lower($p<0.001$) among occupationally exposed group (35.16±1.02 and 658.31±19.10) when compared with non-exposed group (56.08±0.70 and 955.69±97.48). From this study we observed that continuous exposure to photocopying emissions resulted in increased levels of some immunologic parameters and decreased levels in others. We therefore advocate the use of proper personal protective equipment and strict adhere to occupational ethics during work process among photocopier operators in University of Benin campus.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Technological advancement has significantly transformed the modern work environment, with photocopying machines playing an essential role in academic, administrative, and commercial settings. These machines, although useful, emit various chemicals and pollutants during operation including ozone, volatile organic compounds (VOCs), nitrogen dioxide (NO_2), and toner dust that may pose potential health risks to operators (Chung *et al.,* 2011). Occupational exposure to environmental pollutants is a significant contributor to health challenges among workers across various industries. In academic institutions, a less-discussed but potentially serious occupational hazard is exposure to emissions from photocopiers and related document-printing equipment. Photocopier machines are widely used in universities for administrative, academic, and commercial purposes, often in enclosed or poorly ventilated spaces. Operators of these machines are frequently exposed to a range of chemical and particulate emissions during the copying process, including ozone (O_3), volatile organic compounds (VOCs) such as benzene and formaldehyde, toner particles, and ultrafine particles (UFPs) (Bello *et al.,* 2010). Over time, this chronic exposure may have subtle but significant effects on their health, particularly on the immune system. The immune system plays a critical role in defending the body against pathogens and maintaining internal balance. Immunological indices—such as total and differential white blood cell (WBC) counts, IgE, CD4 and Total lymphocyte count—serve as valuable biomarkers for assessing immune status and possible inflammatory or immune-modulating effects of environmental exposures (Janeway *et al.,* 2020). Occupational exposure to certain environmental agents has been shown to either suppress immune function or cause excessive immune activation, both of which can increase susceptibility to infections, autoimmune disorders, or chronic

inflammation. Occupational exposure to such emissions is a public health concern, especially in developing countries where workplace safety standards are often poorly implemented. Photocopier operators, particularly those working in enclosed or poorly ventilated spaces, are continuously exposed to low-level emissions for extended hours. Studies have shown that exposure to ozone and other chemicals released during photocopying may lead to oxidative stress, respiratory symptoms, and immunologic alterations (Meacher *et al.,* 2020). The immune system is a sensitive and complex network that responds to environmental insults. Chronic exposure to air pollutants and toxic substances has been associated with changes in leukocyte counts, immune cell dysfunction, and increased susceptibility to infections and autoimmune conditions (Rylance *et al.*, 2015). Assessing immunologic indices such as white blood cell (WBC) count, lymphocyte percentage, and neutrophil levels can provide insight into potential immune disturbances due to occupational exposure. Several studies conducted in industrial and office settings have demonstrated that prolonged exposure to emissions from printers and photocopiers is associated with respiratory problems, oxidative stress, and inflammatory responses (Vianello *et al.,* 2016). These health effects are thought to be mediated, at least in part, by the interaction of airborne pollutants with immune cells, leading to changes in leukocyte counts, immunoglobulin production, and cytokine release. However, there is a notable lack of data from African settings, particularly in Nigeria, where workplace safety regulations and environmental health monitoring may be less strictly enforced. In institutions like the University of Benin, photocopying services are in high demand, and operators often work long hours in confined spaces without adequate ventilation or protective equipment. Given the growing health concerns related to occupational exposure in office and academic environments, there is a critical need to evaluate whether photocopier operators are at risk of developing immunological alterations that could affect their overall health and productivity. The immune system acts as the body's defense against foreign pathogens and harmful substances. Occupational exposure to chemicals and air pollutants can suppress or dysregulate immune function by altering white blood cell (WBC) activity,

damaging immune cells, or increasing pro-inflammatory cytokine levels (Rylance *et al.,* 2015). In particular, studies have shown that workers exposed to indoor air pollutants have significant changes in their immunologic indices—such as increased total WBC count, altered lymphocyte-to-neutrophil ratios, and reduced natural killer (NK) cell activity—indicating an underlying immune system imbalance (Ndebele *et al.,* 2017).

In Nigeria, the level of awareness and monitoring of occupational hazards in informal and semi-formal work environments is low. Photocopier operators rarely undergo routine medical checkups, and few institutions have policies or protective measures such as fume extractors, masks, or work rotation systems to reduce exposure. This puts such workers at risk of developing long-term health complications, many of which may not be immediately evident but could present over time through reduced immunity or susceptibility to disease. While global literature has explored the health effects of photocopier emissions in general office workers, there is a scarcity of region-specific data—particularly in Sub-Saharan Africa—on the immunological impact of such occupational exposure. This study, therefore, seeks to fill that gap by examining the immunologic indices of photocopier operators in the University of Benin. It aims to compare their immune profiles with those of non-exposed individuals and assess whether continuous exposure to photocopier emissions has a measurable effect on immune function. By identifying potential immune alterations among photocopier operators, this research will contribute valuable data to the fields of occupational health, environmental toxicology, and preventive medicine, while also informing safety policies and workplace health interventions for similar environments in Nigeria and beyond.

1.2 Statement of the problem

Photocopier machines are indispensable tools in academic environments such as the University of Benin, where students, staff, and departments rely on them for the rapid duplication of academic and administrative documents. However, the frequent use of

these machines, especially in small, poorly ventilated rooms, poses a significant occupational hazard to operators. Research has shown that photocopiers release various airborne pollutants, including ozone, ultrafine particles, formaldehyde, and volatile organic compounds (VOCs), which can adversely affect the respiratory and immune systems of individuals with prolonged exposure (Madureira *et al.,* 2016). Occupational exposure to these emissions may not produce immediate symptoms, yet long-term exposure has been linked to systemic effects such as inflammation, allergic reactions, and immune dysregulation (Li *et al.,* 2019). Studies have identified significant changes in immune parameters such as total white blood cell (WBC) count, lymphocyte and neutrophil distribution, and pro-inflammatory cytokine levels in individuals exposed to environmental and occupational air pollutants (Suresh *et al.,* 2019). Despite these known risks, little attention has been given to the health status of photocopier operators in many developing countries, including Nigeria. Most of these workers are employed informally, work long hours, and have limited access to protective measures such as proper ventilation, personal protective equipment (PPE), or routine medical assessments. This leaves them vulnerable to chronic exposure without awareness of the long-term consequences. Additionally, very few local studies have been conducted to evaluate the immunologic profiles of such occupational groups, and there is a general lack of data on immune alterations related to photocopier emissions in Sub-Saharan Africa.

The absence of scientific data on the health risks facing photocopier operators hinders the implementation of effective workplace safety guidelines. Without such data, institutions may unknowingly expose workers to harmful agents, resulting in progressive immune impairment that may lead to frequent infections, autoimmune tendencies, or even long-term diseases. Thus, there is a critical need to investigate the immunologic indices among occupationally exposed photocopier operators in the University of Benin. This study aims to bridge the knowledge gap by comparing immune parameters such as WBC count, differential count, and possibly serum

cytokines between exposed individuals and unexposed controls. The findings will inform preventive health strategies, encourage policy formulation, and highlight the importance of occupational health surveillance in academic institutions.

1.3 Justification of the study

The rapid use of photocopying machines in academic environments such as the University of Benin has created occupational niches where workers are potentially exposed to chemical and particulate pollutants on a daily basis. Substances such as ozone, fine particulate matter, benzene derivatives, toluene, and formaldehyde are released during photocopying, especially in enclosed or poorly ventilated areas (Madureira *et al.*, 2016). Despite increasing global awareness of indoor air pollution and its impact on health, very little attention has been paid to the immunologic health of photocopier operators—a group particularly vulnerable to chronic exposure. Existing studies have highlighted the detrimental effects of such emissions on the respiratory, dermal, and immune systems (Li *et al*., 2019). Prolonged exposure to photocopier-generated pollutants may disrupt the body's immune surveillance mechanisms, potentially leading to immunosuppression, increased susceptibility to infections, or autoimmune reactions (Kim *et al.,* 2018). Yet, in developing countries like Nigeria, occupational health surveillance for workers in informal and low-risk job categories—such as photocopying operators—remains inadequate or entirely absent. There is currently a scarcity of data in Nigeria and Sub-Saharan Africa regarding the effect of occupational photocopier exposure on immune parameters such as white blood cell (WBC) count, lymphocyte percentages, and neutrophil activity. Without such data, these workers continue to face health risks without targeted interventions or adequate policy protection. Most operators work in environments with limited air conditioning or ventilation, spend prolonged hours near operating machines, and are unaware of the potential long-term risks they face.

This study is, therefore, justified by its potential to fill a critical gap in occupational health research in Nigeria. By evaluating the immunologic indices of photocopier

operators compared to a control population, the findings could help to:

Highlight the health implications of sustained exposure to photocopier emissions. Provide evidence for the development of institutional health and safety guidelines. Support advocacy for routine medical screening and provision of personal protective equipment (PPE) in university workplaces. Encourage further research on indoor air quality and occupational safety in academic settings.

1.4 Aim of the Study

This study was aimed to evaluate selected immunological indices in male photocopier operators who are occupationally exposed to photocopier emissions in university of Benin campus in Benin City.

1.5 Specific Objectives

The Specific Objectives are;

1. to evaluate total WBC and differential count among male photocopier operators that are occupationally exposed and control subject.
2. to evaluate the CD4 T cells and total lymphocyte count among male photocopier operators that are occupationally exposed and control subject.
3. to assess the serum concentration level of IgE among occupationally exposed male photocopier operators and control subject.
4. To compare results of control subject and occupationally exposed male photocopier operators

1.6 Research Questions

1. Are there changes some haematological parameters (Total WBC and Differential count) among occupationally exposed male photocopier operators

at the University of Benin?

2. Are there changes in the $CD4^+$ T lymphocyte cells and Total lymphocyte count among occupationally exposed male photocopier operators at the University of Benin?
3. Are there elevated levels of IgE among occupationally exposed male photocopier operators at the University of Benin elevated?
4. Is there a significant difference in the selected immunological indices between occupationally exposed male photocopier operators and non-exposed control subjects?

1.7 Research Hypotheses

Null Hypothesis (H_0):

There is no significant difference in the immunological indices (including white blood cell counts, IgE and CD4) between male photocopier operators and non-exposed individuals at the University of Benin.

Alternative Hypothesis (H_1):

There is a significant difference in the immunological indices between male photocopier operators and non-exposed individuals at the University of Benin.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Photocopying

Photocopying (also called xerography or electrophotography) is a dry document-reproduction technique that produces an exact paper copy of an original by creating an electrostatic latent image on a photoconductive surface, developing that image with powdered toner, transferring the toner to paper, and then fusing it with heat and pressure (Britanniaca, 2017).

Modern systems capture images digitally and commonly use drum or belt photoconductors and laser or LED exposure systems. The process primarily relies on xerography, a dry electrostatic method where light creates an electrostatic image on a photoreceptor drum, attracting toner particles to form a visible image. This toner is then transferred and fused onto a blank sheet of paper using heat and pressure, resulting in a quick, cost-effective copy of the original document. The Xerography process creates dry copies using static electricity by first charging a photoconductive drum, then exposing it to an image to create a charged latent image. Negatively charged toner particles are attracted to the charged areas of the drum, which then transfers the toner image to a sheet of paper. Finally, heat is applied to fuse the toner onto the paper, producing a permanent copy of the original (Britanniaca, 2017). Photocopiers, laser printers, and related office machines emit a wide variety of airborne contaminants during operation, especially when used for long hours in poorly

ventilated environments. These emissions include ozone, volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs), ultrafine particles (toner dust), nitrogen oxides, and aldehydes (Rathinasabapathy and Subramanian, 2013). Continuous inhalation of these pollutants contributes to oxidative stress, respiratory irritation, and systemic toxic effects. Importantly, such exposures can alter hematological and immunologic indices, which are sensitive biomarkers of early biological changes in exposed populations.

2.1.1. Economy Importance of photocopying

Photocopying is a core reprographics and document services offering in cybercafés and internet/telecentres. In many low- and middle-income settings cybercafés bundle internet access with value-added services such as printing, scanning, photocopying, document binding and lamination collectively called print-for-pay or reprographic services. These services generate direct retail revenue, attract students and small businesses, enable document-dependent transactions (applications, exams, government forms), and increase footfall (customers who then pay for internet time or other services). Studies of telecentres/cybercafés highlight printing/photocopying as a frequent and often primary non-internet revenue stream (Lopez-Bonilla *et al*., 2016). Photocopying is a direct source of income for cybercafes, especially in areas where residents may not have personal photocopiers, it provides customers or students with a convenient, one-stop-shop for both digital needs (like internet access) and physical document needs, It serves a broad customer base, from students needing copies of

notes to individuals applying for government licenses or submitting project reports (Sharma, 2018)

2.1.2. Importance of Adequate Safety Measures During Photocopying

Photocopiers and laser printers can release airborne particulates (toner dust), ozone, nitrogen oxides and a range of volatile/semi-volatile organic compounds during operation. Short- and long-term exposure to these emissions can cause respiratory irritation and other symptoms to occupationally exposed individuals; thus, safety guidance emphasizes adequate ventilation, routine maintenance, spill-clean procedures for toner, limiting direct operator exposure, and use of certified equipment with low ozone emissions. Employers and small-business operators (including cybercafé owners) are advised to place machines in ventilated areas, follow manufacturer maintenance schedules, use of personal protection equipment when servicing (gloves, avoid inhaling toner), and keep toner cartridges and waste collected for proper disposal or recycling. Despite the advantages and commercial benefits of photocopiers, they are also sources of air pollution. During operation, photocopiers emit toner particles, toxic gases namely ozone, nitrogen dioxide, volatile organic compounds, semi-volatile organic compounds, radiation, particulate matter, paper particles, nano particles and extremely low-frequency electromagnetic fields. These safety measures includes cleaning spills of toner carefully (avoid dry sweeping; use damp cloth or HEPA vacuum), educating staff about symptoms of exposure and safe service practices (gloves, power-off before repairs), running scheduled maintenance

and use manufacturer approved consumables (reduces particulate/ozone) (Lopez-Bonilla *et al*., 2016).

2.1.3. Health Effects of Photocopying

Photocopying machines release various emissions such as fine particulate matter, volatile organic compounds (VOCs), ozone, and chemical components from toners, all of which can negatively affect human health. Pirela *et al.,* (2017) analyzed the chemical components found in toner dust to contain substances like carbon black, resins, and trace metals which can irritate the respiratory tract and cause oxidative stress. Additionally, ozone and VOCs produced during the photocopying in an enclosed environment has been found to cause eye, nose, and throat irritation as well as headaches and nausea, e. Several studies have highlighted acute health effects among photocopier operators. These include respiratory symptoms such as cough, phlegm, wheezing, nasal blockage, and sore throat, which are significantly more frequent among exposed workers compared to unexposed groups (Elango *et al.,* 2013). Eye irritation, skin rashes, and dryness of mucous membranes have also been reported due to direct exposure to toner dust and chemical vapors (HealthXchange, 2022). Headaches and dizziness are common complaints in operators working long hours in confined spaces without proper air circulation. Chronic exposure to photocopying has been associated with systemic health effects. Biomarker studies have shown that long-term photocopier operators exhibit increased oxidative stress and elevated inflammatory cytokines such as IL-8 and ICAM-1, which suggest a higher risk for

cardiovascular diseases (Elango *et al.,* 2013). Furthermore, genotoxic effects have been reported among occupational operators and maintenance staff showing significantly increased DNA damage when compared to non-exposed groups with cumulative years of exposure strongly associated with genotoxicity (Kasi *et al.,* 2018). While some studies did not find significant impairment in lung function, they consistently reported a higher prevalence of respiratory symptoms, which may progress to chronic respiratory disease with longer exposure durations (Elango *et al.,* 2013). Environmental and occupational factors influence the severity of these health effects. Poorly ventilated rooms, the use of older photocopier models, and low-quality toners increase emissions, thereby raising health risks (Pirela *et al.,* 2017). Workers with pre-existing respiratory conditions such as asthma, as well as smokers, are more vulnerable to photocopying-related health hazards (Elango *et al.*, 2013). On the other hand, proper ventilation, the use of sealed toner cartridges, adherence to safety practices, and regular machine maintenance can significantly reduce harmful exposures.

2.2 Constituent of Photocopying Machines, Mode of Entry and Toxicological Effects

Photocopiers, laser printers, and related office machines emit a wide variety of airborne contaminants during operation, especially when used for long hours in poorly ventilated environments. These emissions include ozone, volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs), ultrafine particles (toner dust),

nitrogen oxides, and aldehydes (Rathinasabapathy and Subramanian, 2013). Photocopying poses both short-term and long-term health risks ranging from irritation and discomfort to oxidative stress, inflammation, and DNA damage. Although the severity of these effects depends on exposure levels and workplace conditions, cumulative exposure among operators highlights the importance of safety measures and environmental controls in photocopying environments. The constituents of the machines varies but the major ones are as follows.

2.2.1 Ozone Formation

Ozone (O_3) is a highly reactive oxidant gas that is generated as a by-product of electrical discharges in many office machines, including photocopiers and laser printers. While stratospheric ozone protects against ultraviolet radiation, tropospheric (ground-level) ozone is a respiratory irritant and a potent inducer of oxidative stress when inhaled. In indoor copy-center environments, even low but chronic ozone exposures produced by corona wires and other high-voltage components can produce biologically relevant effects on the respiratory tract and systemic immunity (Devlin *et al.,* 2012). When ozone is inhaled it reacts immediately with airway surface lining fluids and epithelial cell membranes to produce reactive oxygen species (ROS) and lipid oxidation products. These primary oxidative events activate intracellular signaling pathways (notably NF-κB and AP-1), which in turn upregulate pro-inflammatory genes and chemokines such as IL-6, IL-8 (CXCL8), and CXCL1 that recruit neutrophils to the airways (Mumby *et al.,* 2019). The resulting epithelial

injury increases permeability and facilitates translocation of particles and inflammatory mediators into the systemic circulation, producing measurable systemic inflammation (Mumby *et al.,* 2019). In addition to local oxidative injury, ozone exposure can trigger a neuroendocrine stress response (corticosteroid and catecholamine release) that transiently redistributes leukocyte populations — a mechanism proposed to explain ozone-induced lymphopenia observed in several human and animal studies (Henríquez *et al.,* 2021). This stress response can produce short-term reductions in circulating lymphocytes while increasing neutrophils and monocytes as part of an acute inflammatory/stress signature. Controlled human exposure experiments and occupational studies consistently report increased neutrophilic inflammation after ozone inhalation. Bronchoalveolar lavage (BAL) and bronchial biopsies taken after controlled exposures show rapid neutrophil influx and increases in IL-6 and other inflammatory mediators within hours (Devlin *et al.,* 2012). These acute neutrophilic responses are paralleled in peripheral blood by modest but reproducible rises in total WBC and neutrophil percentages in many studies (Devlin *et al*., 2012). Conversely, lymphocyte counts—particularly certain T-cell subsets—may transiently fall after ozone exposure. The lymphopenia is thought to arise from (a) stress-hormone mediated redistribution of lymphocytes into lymphoid tissues, and (b) direct oxidative injury and apoptosis of susceptible lymphocyte populations reported in animal models (Henríquez *et al.*, 2021). The time course is important: acute lymphopenia can appear within hours, whereas chronic repeated exposures may produce more complex alterations in adaptive immunity. There is mechanistic and

experimental evidence that ozone exposure affects antigen presentation and T-cell priming. Ozone activates and matures pulmonary dendritic cells, increasing their migration to draining lymph nodes and altering the quality of T-cell activation; this has been shown to promote Th2-biased responses in animal models, which enhances allergic inflammation on subsequent allergen exposure (Hollingsworth *et al.,* 2010). The resultant skewing and impaired T-cell proliferation can reduce effective $CD4^+$ T-cell function in some contexts, potentially undermining adaptive immune surveillance over time. Although human epidemiologic data directly linking chronic low-level ozone exposure to sustained reductions in circulating CD4 counts are limited, recent studies have reported associations between ambient ozone and altered CD4+ metrics in vulnerable populations, and experimental human exposures show compromised T-cell related functions after ozone challenge. These findings are biologically plausible given dendritic cell activation, oxidative DNA damage, and altered cytokine milieus produced by ozone (Chen *et al*., 2024). While ozone itself is not an allergen, it acts as an adjuvant that amplifies responses to inhaled antigens. Animal studies and controlled exposures have shown ozone can increase airway hyperresponsiveness and enhance eosinophilic airway inflammation when coupled with allergens (Depuydt *et al.*, 2021). Mechanistically, epithelial damage and dendritic cell activation promote Th2 polarization, which favors IgE class switching in B cells—thereby increasing total and allergen-specific IgE under the right sensitizing conditions. Human data are mixed: ozone exposure does not always raise IgE levels by itself, but it can increase allergic inflammation and exacerbate

pre-existing allergic disease. The concentrations of ozone produced by photocopiers are usually much lower than outdoor urban peaks, but photocopier operators may be exposed continuously in small, poorly ventilated rooms — resulting in meaningful cumulative dose. Controlled exposure studies establish the biological plausibility that repeated low-level ozone exposure produces chronic airway inflammation, periodic lymphocyte redistribution, and immune modulation that could appear as altered WBC differentials, transient lymphopenia, or enhanced allergic markers (Devlin *et al.*, 2012).

2.2.2 Volatile Organic Compounds (VOCs)

Volatile organic compounds (VOCs) are a heterogeneous group of carbon-based chemicals with high vapor pressures at room temperature, enabling their easy volatilization into indoor air. They are emitted from a wide range of materials, including paints, adhesives, solvents, and office equipment such as photocopiers and laser printers. Common VOCs identified in copy centers include formaldehyde, benzene, toluene, xylene, ethylbenzene, and styrene, many of which are known irritants, sensitizers, or immunotoxicants (Wensing *et al.*, 2020). Due to poor ventilation and prolonged working hours, photocopier operators are at risk of sustained exposure to low-to-moderate VOC concentrations, raising concern about their potential effects on the immune system. The immunological effects of VOCs are mediated through multiple, interrelated mechanisms. Oxidative Stress and Inflammatory Signaling: VOC metabolism often generates reactive oxygen species

(ROS) and electrophilic intermediates that damage airway epithelial cells. This cellular stress activates transcription factors such as NF-κB and AP-1, upregulating pro-inflammatory cytokines (e.g., IL-6, TNF-α, IL-8) and chemokines that recruit leukocytes into the airways and systemic circulation (Ogbodo *et al.*, 2022). Sustained activation of these pathways contributes to chronic low-grade inflammation. Formaldehyde and other aldehydes compromise tight junction integrity, enhancing allergen penetration. VOC-protein adducts can act as neoantigens, activating dendritic cells and skewing T-helper (Th) differentiation toward Th2 responses, which are associated with IgE production and hypersensitivity (Bönisch *et al.*, 2012). Some VOCs alter DNA methylation and histone modification in immune cells, particularly $CD4^+$ T cells, leading to long-term changes in immune programming. such alterations have been implicated in autoimmune tendencies and allergic susceptibility (Ogbodo *et al*., 2022). Aromatic hydrocarbons such as benzene are hematotoxic. Benzene metabolites (e.g., hydroquinone, benzoquinone) interfere with hematopoietic stem cells, causing bone marrow suppression, leukopenia, and selective depletion of lymphocyte subsets, including $CD4^+$ T cells (Guo *et al.,* 2020).

2.2.3 Ultrafine particles (Toner Dust)

Photocopiers and laser printers not only emit ozone and volatile organic compounds (VOCs) but also release ultrafine toner-derived particulates into the surrounding air. Toner powder is typically composed of carbon black, polymer resins, pigments, silica, and trace metals such as iron, titanium, or nickel. During high-volume copying and

printing, mechanical stress and heat can release fine and ultrafine particles (<1 μm) into indoor environments, where they are readily inhaled and deposited in the respiratory tract (Kagi *et al.,* 2019). Concerns regarding toner particulates are increasing because of their capacity to induce systemic haematological alterations in chronically exposed workers. The biological impact of toner particulates arises from their size, composition, and surface reactivity. toner particles reach the pulmonary alveoli, where they trigger macrophage activation, neutrophil recruitment, and cytokine release (IL-6, TNF-α). These mediators can leak into the systemic circulation, influencing bone marrow hematopoiesis and peripheral blood cell counts (Murphy *et al.*, 2011). Toner particles, especially those containing metals or carbon black, generate reactive oxygen species (ROS) which are capable of inducing oxidative DNA damage in leukocytes and hematopoietic progenitors, leading to apoptosis, impaired proliferation, or mutation (Endo *et al*., 2010). Animal studies suggest that inhaled ultrafine toner particles can translocate from the lungs into the bloodstream and accumulate in bone marrow, directly interfering with hematopoiesis (Lee *et al.*, 2012). Toner particles may act as adjuvants, altering lymphocyte subsets and immune balance that is a shift in $CD4^+$ and $CD8^+$ T-cell ratios, as well as elevations in inflammatory monocytes, have been observed in experimental exposure models. Direct human studies linking toner nanoparticle exposure specifically to reduced peripheral $CD4^+$ counts are limited. However, occupational surveillance and cross-sectional work on printer/copier operators report respiratory symptoms, oxidative stress biomarkers, and changes in circulating inflammatory markers that

plausibly precede or accompany $CD4^+$ functional changes. Importantly, many real-world exposures are mixed (nanoparticles + VOCs + ozone), complicating attribution to toner nanoparticles alone but reinforcing plausibility for combined immunotoxic effects in photocopier operators (Bello *et al.,* 2016). For photocopier operators working long hours in poorly ventilated rooms, repeated inhalation of toner nanoparticle particularly from modern nano-enabled toners — creates a sustained exposure scenario in which multiple mechanisms may operate simultaneously.

2.2.4 Lead (Pb)

Lead exposure occurs mainly through inhalation of lead dust/fumes (occupationally important) and ingestion (contaminated water, food, or hand-to-mouth behaviour). Dermal absorption of inorganic lead is low but possible for some organic lead compounds. Lead distributes widely in the body and accumulates in bone, serving as a long-term reservoir and a marker of cumulative exposure (Tchounwou *et al.*, 2012). Lead exerts immunotoxicity through multiple mechanisms: oxidative stress, disruption of intracellular signaling, impairment of antigen-presenting cell function, skewing of T-helper differentiation toward Th2 responses, and induction of apoptosis in immune cells. Lead may preferentially impair cell-mediated immunity (Th1 responses) while promoting humoral/Th2-type responses — a shift that can manifest as reduced CD4+ functional capacity even when absolute counts are variably affected (Fenga et al., 2017). Several occupational and environmental studies documents decrease in CD4+ percentages and absolute counts with increasing blood lead levels.

Mishra *et al*., (2010) reported significantly lower percentages of CD4+ cells in lead-exposed groups and a negative correlation between blood lead concentration and CD4 percentage. Li et al. (2020) described reduced CD4+ T-cell percentages in children with high environmental lead exposure. Reviews conclude that chronic lead exposure impairs Th1/Th2 balance and can reduce CD4 function (Mishra *et al.*, 2010b). Lead-exposed animal models demonstrate depressed T-cell proliferation, altered cytokine production (reduced IFN-γ, IL-2, increased IL-4/IL-10), and decreased T-helper function which all consistent with impaired CD4 activity. These functional deficits may precede or accompany numerical declines. Human epidemiological data provide some of the clearest evidence that lead exposure is associated with reduced CD4 percentages and impaired CD4 function; this is supported by consistent mechanistic and animal data showing disrupted T-cell proliferation, skewed helper differentiation, and apoptotic loss of lymphocytes. For occupational groups with chronic lead exposure, monitoring CD4 (both numeric and functional assays) is scientifically justified (Fenga *et al*., 2017b).

2.2.5. Manganese (Mn)

Manganese exposure occurs mainly by inhalation (welding fumes, mining dust, ferromanganese production) and ingestion. Occupational inhalation is the primary concern for immune outcomes because airborne Mn particulates (especially welding fumes) deliver Mn directly to the lung and systemic circulation. Dermal absorption is minimal (X. Chen *et al*., 2020). Manganese can generate oxidative stress and

modulate neurotransmitter systems at high exposures (neurotoxicity is well documented). Immunotoxic actions include changes in lymphocyte subsets, impaired lymphocyte proliferation, and altered cytokine expression. Mn inhalation can produce systemic suppression of T-lymphocyte populations and affect naïve T-cell compartments (e.g., CD4+CD45RA+), possibly via direct marrow effects or redistribution/trafficking changes after pulmonary inflammation (Nakata *et al.*, 2020). Welders and workers with elevated manganese exposures have been shown to have lower counts of several lymphocyte subsets including CD4+ T-cells (notably naïve CD4+CD45RA+ cells) and CD8+ cells, with inverse correlations between manganese tissue/blood measures and lymphocyte subsets (Nakata *et al.,* 2020; Araki *et al*., 2020). Recent occupational cohorts reinforce associations between Mn exposure and decreased CD3+, CD4+ and CD8+ T-lymphocyte numbers. Experimental inhalation studies indicate that manganese or manganese-containing fumes can depress T-cell populations and impair T-cell proliferative responses in experimental animals, supporting a causal effect of Mn on adaptive immunity (X. Chen *et al*., 2020). Occupational and experimental evidence shows that manganese exposure is associated with reductions in CD4+ T-cell counts (including naïve subsets) and impaired T-cell function. The weight of evidence in welding/fume-exposed cohorts makes Mn an important candidate metal for monitoring CD4 changes in exposed workers (Nakata *et al.,* 2020). Inhalation of toner-associated dust/nanoparticles and maintenance-related aerosols are the most plausible pathways for trace metal entry in copy-center settings. Hand-to-mouth ingestion (poor workplace hygiene) and dermal

contact during cartridge handling are secondary but relevant (Mishra *et al.*, 2010b). Human epidemiology gives the strongest, most consistent signal for lead (clear negative correlations with CD4 metrics), and substantial evidence for manganese in occupational inhalation cohorts (welders). Chromium (especially Cr(VI)) has strong mechanistic and animal evidence for T-cell effects and some human data indicating lymphocyte subset perturbation; regulatory toxicology reviews also list immune outcomes among Cr(VI) concerns. Therefore, lead and manganese are high-priority metals for CD4 surveillance in occupational settings, with chromium also warranting attention where exposures are plausible (Nakata *et al.,* 2020).

2.3.6 Chromium (Cr)

Chromium exists in several valence states; the two most relevant to health are trivalent chromium (Cr(III)) and hexavalent chromium (Cr(VI)). Cr(VI) is more mobile, readily passes cell membranes, and is a recognized occupational hazard (IARC group 1 carcinogen for some Cr(VI) compounds). Humans are exposed to chromium by inhalation (industrial fumes, dust), ingestion (contaminated water, food), and dermal contact (solutions or contaminated surfaces). Occupational inhalation of Cr(VI) is particularly important in welding, chrome plating, pigment manufacture and certain printing/industrial scenarios where chromates are used or present as impurities (Wilbur *et al.,* 2012). Cr(VI) is a strong oxidant that generates reactive oxygen species (ROS) and binds to cellular macromolecules after intracellular reduction to lower oxidation states. These processes produce DNA damage, oxidative stress, and cell

death pathways that affect rapidly dividing cells and immune cells. Chromium compounds alter antigen-presenting cell function and lymphocyte activation in vitro, and they can modulate cytokine production — mechanisms that can compromise T-cell proliferation and function. Animal studies show Cr(VI) exposure reduces thymic and splenic T-cell populations and impairs mitogen-induced lymphocyte proliferation (Dai *et al.,* 2017). Experimental inhalation and oral exposure to Cr (VI) in rodents has produced reductions in splenic T-cell counts and impaired T-cell function in several studies, including decreased mitogen-driven proliferation and altered cytokine profiles findings consistent with a toxic effect on CD4+ subsets (Dai *et al.,* 2017). Occupational biomonitoring studies and cross-sectional investigations of chromium-exposed workers have reported alterations in lymphocyte subsets (including changes in activated T and B cell markers); some studies show inverse associations between urinary chromium and specific lymphocyte subpopulations, though direct, consistent reports of CD4 numerical depletion are more limited than for lead or manganese. Still, recent toxicological reviews and regulatory assessments recognize immune alterations (including T-cell effects) among the endpoints affected by Cr (VI) (Boscolo *et al*., 2021). Mechanistic and animal data reported that Cr (VI) exposure can impair T-cell viability and function, and human occupational studies show altered lymphocyte phenotypes consistent with immunomodulation. Because CD4+ cells are sensitive to oxidative insults and signaling disruption, Cr (VI) exposures are biologically plausible contributors to reduced CD4 function and, in some contexts, reduced CD4 counts (Shin *et al.,* 2023).

Table 2.1 Heavy Metal Constituents in Toner and Their Immune Effects

Heavy Metal	Source in Photocopier	Immune Effect	Affected Indices
Nickel (Ni)	Toner pigment stabilizer	Allergen, promotes IgE response, neutrophilia	↑ IgE, ↑ neutrophils
Chromium (Cr)	Machine parts, toner additives	Decreases CD4/CD8 ratio, allergic asthma	↓ $CD4^+$ T cells, ↑ IgE
Lead (Pb)	Mechanical components, dust	Immunosuppression, anemia, lymphopenia	↓ Lymphocytes, ↓ hemoglobin
Cadmium (Cd)	Toner stabilizer, machine wear	Apoptosis of lymphocytes, Th2 skewing	↓ $CD4^+$ T cells, ↑ eosinophils
Manganese (Mn)	Toner trace element	Pro-inflammatory cytokine release	↑ WBC, ↑ inflammatory markers

(Lavicoli *et al*., 2017)

2.3 Total White Blood Cell Count

The WBC count represents the overall leukocyte population and serves as a general marker of immune reactivity. Occupational exposure to photocopier emissions can either cause leukocytosis or leukopenia, depending on the nature and duration of exposure. WBC count may rise (leukocytosis) as a systemic reaction to pulmonary inflammation (spillover of cytokines such as IL-6 and IL-8) after inhalation of ozone or toner particles, or fall (leukopenia) when exposures include bone-marrow toxicants (e.g., benzene or high cumulative chemical dose) that suppress hematopoiesis. The direction of change therefore reflects the dominant toxicological mechanism: inflammatory mobilization (↑WBC) vs. marrow suppression (↓WBC) (Aksoy, 1989). Increased WBC counts have been reported in photocopier operators, attributed to systemic inflammation secondary to chronic inhalation of ozone and ultrafine particles. Inflammatory mediators such as IL-6 and TNF-α are upregulated, stimulating leukopoiesis in the bone marrow (Awodele et al., 2015). On the other hand, exposure to hematotoxic chemicals like benzene, occasionally present in printing environments, can suppress bone marrow activity, leading to leukopenia (Aksoy, 1989). Cross-sectional studies of photocopier operators report elevated markers of systemic inflammation (e.g., IL-8, oxidative stress) and occasionally higher total WBC compared with non-exposed, supporting the inflammatory spillover model. However, not all field studies find statistically significant WBC differences which are likely reflecting differences in exposure intensity, machine age, ventilation,

and sample sizes (Awodele *et al*., 2015).

Animal inhalation studies of printer-emitted nanoparticles (Pirela et al., 2016) demonstrated systemic leukocyte alterations consistent with inflammatory and oxidative mechanisms, further validating human occupational findings. Experimental models confirm this dual pattern which are; short-term exposures yield neutrophilia, whereas long-term or high-dose exposures impair neutrophil survival and function (Stiegel *et al.,* 2016). This study observed a decreased neutrophils (neutropenia) which is epidemiologically important and may indicate either a later-stage effect where cumulative oxidative injury and marrow compromise reduce circulating neutrophils, or redistribution/functional suppression rather than true marrow failure. Both possibilities require follow-up (repeat CBC, check bone-marrow toxicant exposures such as benzene, review medications/infections). Controlled exposure literature and animal toner instillation studies document the time-course and functional impairment that can underlie such findings (Tomonaga *et al.,* 2020).

2.4 White Cell Differential Count

A white cell differential (also called differential white blood cell count) is a laboratory test that breaks down the different types of white blood cells (WBCs) present in the blood, usually expressed as percentages of the total WBC count. It provides important diagnostic information about infections, immune responses, allergies, blood cancers, and other hematologic conditions. Photocopier operators are often exposed to toner particles, ozone, volatile organic compounds (VOCs), and other emissions, which can

cause oxidative stress, airway irritation, and immune responses. These exposures may influence the white cell differential in the following ways:

2.4.1 Neutrophils

Neutrophils are typically the most abundant white blood cells, usually comprising about 40-70% of circulating leukocytes; they are the body's main defense against bacterial and fungal infections, removing pathogens by phagocytosis and using reactive oxygen species in the process. When neutrophils are elevated (neutrophilia), this often signals acute bacterial infection, inflammation, acute stress, or sometimes myeloproliferative disorders. When neutrophils are decreased (neutropenia), it may be due to viral infections, certain drugs (including chemotherapy), bone marrow suppression, or severe overwhelming infections. Photocopier emissions (particularly ozone and fine toner dust) can irritate the respiratory tract, leading to inflammatory responses. This may cause neutrophilia (increased neutrophils), indicating the body is mounting a defense against inhaled irritants and oxidative stress. Chronic exposure could also impair neutrophil function, reducing their effectiveness in clearing infections (Professional, 2025). Neutrophilia (relative or absolute ↑) is a consistent early sign of acute airway irritation and oxidative stress produced by ozone or particle inflammation. Controlled human ozone exposures and animal inhalation/instillation studies consistently show neutrophil recruitment to the lung and often a parallel rise in peripheral neutrophils hours after exposure (Stiegel *et al.*, 2016). Acute exposures (hours to days) typically cause neutrophilia and transient lymphocyte redistribution;

chronic, repeated exposures can lead to persistent immune dysregulation, genotoxicity, and in some cases marrow suppression. Ozone and particle-derived ROS activate airway epithelium and macrophages to release chemokines (e.g., IL-8/CXCL8, LTB4) that recruit neutrophils. Systemic cytokine release can stimulate bone-marrow granulopoiesis and transiently redistribute neutrophils from marginated pools into circulating blood. Chronic, repeated exposures can also exhaust neutrophil function (oxidative-burst impairment) or, in some toxicant combinations, depress bone marrow production leading to neutropenia in severe/longer-term scenarios (Arjomandi *et al*., 2015). Experimental models confirm this dual pattern: short-term exposures yield neutrophilia, whereas long-term or high-dose exposures impair neutrophil survival and function (Stiegel *et al.,* 2016). This study observed a decreased neutrophils (neutropenia) which is epidemiologically important and may indicate either a later-stage effect where cumulative oxidative injury and marrow compromise reduce circulating neutrophils, or redistribution/functional suppression rather than true marrow failure. Both possibilities require follow-up (repeat CBC, check bone-marrow toxicant exposures such as benzene, review medications/infections). Controlled exposure literature and animal toner instillation studies document the time-course and functional impairment that can underlie such findings (Tomonaga *et al.,* 2020).

2.4.2 Lymphocytes

Lymphocytes make up about 20–40% of the white blood cells; these include B cells, T cells, and natural killer (NK) cells, and are primarily responsible for mounting

responses against viral infections, producing antibodies, and coordinating immune memory. An increased lymphocyte count (lymphocytosis) is often observed in viral infections, certain chronic infections, and lymphoid leukemias (Crna, 2020). A decreased count (lymphopenia) may be seen in immunodeficiency (e.g. HIV), with corticosteroid use, certain cancers, or after treatments like chemotherapy. Exposure to chemicals and oxidative stress may trigger immune dysregulation. Some studies report altered lymphocyte counts in occupationally exposed workers, with either lymphocytosis (from chronic antigenic stimulation) or lymphopenia (due to immune suppression). This can make photocopier operators more vulnerable to viral infections and may indicate long-term immune imbalance. VOCs and certain metal exposures (and chronic oxidative stress) can impair lymphocyte proliferation, induce apoptosis, and reduce CD4 function. Experimental and epidemiologic work shows functional suppression (reduced proliferation and IL-2 production) may occur even when absolute counts are not dramatically changed (He *et al*., 2024). Animal inhalation and instillation work (printer-emitted particles, toner) demonstrate reduced lymphocyte proliferation and altered splenic T-cell populations after repeated exposures.

2.4.3 Monocytes

Monocytes typically account for 2–8% of white cells; they circulate in the blood and then move into tissues where they become macrophages or dendritic cells, helping to phagocytose debris and pathogens, and to present antigen to lymphocytes to stimulate adaptive immune responses (Tigner *et al.,* 2022). Monocytosis (high monocyte count)

can occur in chronic infections (e.g. tuberculosis), inflammatory disorders (e.g. autoimmune diseases), or in recovery phases of acute infections. Monocytopenia (low monocyte count) is less common but may accompany bone marrow suppression, aplastic anemia, or severe infections overwhelming monocyte production. Toner/particle inhalation stimulates monocyte recruitment and differentiation in lung tissue; systemic monocytosis is seen in several occupational/inhalation models. Chronic activation of monocytes/macrophages is a mechanism for ongoing tissue oxidative damage (Pirela *et al.,* 2017). Elevated monocytes with raised inflammatory cytokines point to persistent low-grade systemic inflammation that can have downstream effects (e.g., atherosclerosis risk) and complicate respiratory disease. Monocytic activation may also skew adaptive responses and contribute to antigen presentation that drives allergy or autoimmunity in susceptible people. Elevated monocyte counts (monocytosis) in photocopier operators indicate sustained activation of the phagocytic system. This is consistent with chronic low-grade inflammation induced by particulates. Animal studies show that ultrafine toner particles are capable of directly stimulating monocyte recruitment and differentiation into pro-inflammatory macrophages within lung tissue (Pirela *et al*., 2015).

2.4.4 Eosinophils

Eosinophils generally compose about 1–4% of the white blood cells; they are involved in defense against parasitic infections, in allergic responses, and in modulating inflammatory processes by releasing mediators. Eosinophilia (elevated

eosinophils) is often seen with parasitic infections, asthma, allergies, and certain skin diseases. A low eosinophil count is generally less clinically significant, but may occur during periods of stress, with corticosteroid administration, or certain infections (Tigner *et al.,* 2022). Photocopier emissions can worsen allergic responses or airway hypersensitivity. Some operators may show eosinophilia, reflecting allergic-type reactions, asthma, or airway irritation triggered by ozone and dust. This is especially important because eosinophil elevation correlates with respiratory symptoms like wheezing, coughing, and shortness of breath. Chronic co-exposure to ozone plus VOCs and adjuvant action of particles (which increase epithelial permeability) can favor Th2 polarization and eosinophilic inflammation; occupational reports document eosinophilia and elevated IgE in exposed workers consistent with allergic sensitization. Studies of photocopier operators demonstrate eosinophilia, which correlates with increased serum IgE levels and allergic airway inflammation (Elango et al., 2013). Mechanistically, ozone and VOCs enhance Th2 polarization, favoring IL-5 release, which recruits eosinophils into circulation and respiratory tissues. Animal models of ozone exposure reveal eosinophilic infiltration of lung tissues, reinforcing the hypothesis of allergen-sensitization pathways in exposed workers (Williams et al., 2011).

2.4.5 Basophils

Basophils are the rarest of the white cell types, commonly forming around 0.5–1% of leukocytes; they play roles in allergic responses and inflammation, particularly by

releasing histamine and other mediators and bind IgE on their surfaces. Basophilia (elevated basophils) may be seen in hypersensitivity reactions, certain myeloproliferative disorders, after splenectomy, or in chronic inflammation. Basopenia (low basophils) is less often clinically worrisome but can occur with acute stress or during treatment with certain medications. Though rare, basophils may also increase (basophilia) in response to allergic or hypersensitivity reactions induced by exposure. They release histamine and other mediators, which could worsen rhinitis, itchy eyes, and airway irritation reported in photocopier operators.

2.4.5 $CD4^+$ T-lymphocyte count (T-Helper Cell)

Another important immunologic index is the CD4 T-cell count, a marker of adaptive immunity and immune competence. While CD4 count is widely recognized in the context of infectious disease monitoring, its role in occupational exposure studies is equally important, as reduced counts may reflect immune dysregulation or suppression. Research has shown that chronic exposure to indoor air pollutants, including those generated by photocopying machines, can lead to systemic oxidative stress and immune dysfunction, potentially contributing to lower CD4 counts (Brook *et al.,* 2010). Human occupational studies of chemical exposures (benzene, mixed VOCs) document lymphopenia and depressed CD4 percentages in some cohorts. Monitoring CD4 in photocopier operator studies is valuable because a fall in CD4 cells suggests impaired cell-mediated immunity and increased infection risk (Pirela *et al.,* 2016). Animal studies corroborate these findings; toner nanoparticle instillation in

rats led to impaired splenic lymphocyte proliferation and altered T-cell subsets (Tomonaga *et al.,* 2020). Photocopier operators are routinely exposed to a variety of emissions, including ozone, volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs), and fine toner particles, especially in poorly ventilated environments. These emissions have been linked with oxidative stress, DNA damage, and hematological alterations (Awodele *et al ,* 2013). Prolonged oxidative stress results in excessive generation of reactive oxygen species (ROS), which can induce apoptosis or functional impairment of lymphocytes, including CD4+ T-cells. Studies in toner-exposed industrial workers and office staff have demonstrated alterations in immune function, including abnormal lymphocyte proliferation and inflammatory responses (Kasai *et al.,* 2017; Hasegawa *et al.*, 2018). Longitudinal follow-up data suggest that persistent exposure to photocopier emissions may not only alter white cell differential counts but also reduce CD4 cell levels, leading to immune suppression or chronic immune activation (Yanagi *et al.*,2021).

2.5. Immunoglobulin E (IgE)

Immunoglobulin E (IgE) is a central biomarker of allergic responses and hypersensitivity reactions. Elevated serum IgE levels are typically associated with atopic conditions such as asthma, allergic rhinitis, and dermatitis, all of which may be triggered or exacerbated by occupational exposure to airborne irritants. In photocopying environments, emissions from toner particles, ozone, and volatile organic compounds can act as allergens or irritants, stimulating the production of IgE

and promoting hypersensitivity reactions (Kelly and Fussell, 2012). Workers with elevated serum IgE often present with symptoms such as sneezing, watery eyes, nasal congestion, and skin rashes, which have been documented in photocopier operators with prolonged exposure (Elango *et al*., 2013). Brook *et al.*, (2010) highlighted that air pollutants contribute to immune dysregulation by enhancing IgE-mediated allergic pathways. Hence, measuring serum IgE levels in photocopier operators is an important immunologic index, as it provides evidence of allergic sensitization and the burden of occupational exposure on immune health.

In occupational settings with allergen or chemical exposures, serum IgE levels are consistently elevated among exposed vs non-exposed groups. The tannery workers in Bangladesh exhibited mean IgE levels of ~339.6 IU/mL versus ~207.5 IU/mL in non-exposed controls even those without overt allergic symptoms showed elevated IgE (~313.5 IU/mL) compared to non-exposed individuals (~90.4 IU/mL) (Hossain & Islam, 2016). Elevated IgE is frequently accompanied by eosinophilia and respiratory symptoms in exposed workers, suggesting that hypersensitivity pathways are activated in response to low-level chronic exposures to chemical mixtures, particulate matter, or irritant gases (Occupational Asthma Studies; Urban Pollution Studies) (UK Occupational Asthma Study, 2021; MDPI Urban Exposure, 2015).

2.6 Some Haematological Changes Associated with Occupational Exposures

Haematologic indices are measurable parameters derived from the complete blood count (CBC) that provide insight into the quantity, morphology, and functional

capacity of circulating blood cells, namely red blood cells (RBCs), white blood cells (WBCs), and platelets. These indices are indispensable diagnostic tools in clinical practice, often used to detect anemia, infections, bone marrow suppression, and alterations in immune status (Bain, 2015; Hoffbrand and Moss, 2019;). Within occupational health research, particularly among photocopier operators exposed to ozone, volatile organic compounds (VOCs), nanoparticles, and heavy metals such as chromium, manganese, and lead, haematologic indices serve as key biomarkers for assessing systemic toxicity and immune dysregulation (Chakrabarti *et al.*, 2019).

The full blood count (FBC) is one of the most widely applied investigations in occupational health studies as it simultaneously reflects both the quantitative and qualitative status of blood cells. Among photocopier operators, chronic exposure to emissions such as ozone, VOCs, and fine particulate matter has been associated with measurable alterations in FBC parameters. Awodele *et al*., (2015) reported that photocopier operators in Lagos, Nigeria, exhibited significantly elevated WBC counts compared to non-exposed controls, indicating persistent immune activation and systemic inflammatory stress. Other reports have demonstrated fluctuations in hemoglobin and hematocrit levels, suggesting oxidative damage to erythrocytes and potential hematopoietic toxicity associated with long-term exposure to photocopier emissions (Bestari *et al.*, 2020). Photocopier emissions (ozone, volatile organic compounds, and ultrafine toner particles) can alter haematologic indices via pulmonary inflammation, oxidative stress, and direct effects on hematopoiesis.

Controlled human ozone exposure studies and animal models of printer-emitted particles demonstrate an early neutrophilic inflammatory response with systemic cytokine spillover (IL-6, IL-8) and later functional impairment of immune cells; animal inhalation/instillation studies further show particle translocation to lymphoid organs and marrow changes. Field studies of photocopier operators report elevated oxidative stress and some leukocyte changes, while genotoxicity assays (Comet) indicate DNA damage in exposed workers; however, results are heterogeneous across studies because of differences in exposure intensity, toner chemistry, and workplace ventilation. When marrow-toxic VOCs (e.g., benzene) are present, classic marrow suppression (leukopenia, anemia, thrombocytopenia) is observed and warrants biomonitoring and medical surveillance. These findings emphasize the importance of white cell differential analysis in detecting subclinical immune disturbances among occupationally exposed workers. In addition to elevated leukocyte counts and differential white cell changes seen in operators of printing and photocopying machines, recent evidence from Ekpoma, Edo State, indicates that prolonged environmental exposure (e.g. roadside dust) may not always produce statistically significant changes in total WBC, CD4 count or differential counts; this underscores variability by exposure level, control group, and other risk modifiers (Babatope *et al*., 2024). Despite several studies of haematological indices among photocopier operators for example in Lagos, Nigeria and various centers in India — changes in white blood cell counts, neutrophils, lymphocytes and eosinophils have often not reached statistical significance (Awodele et al., 2015). However, some red cell indices such as

hematocrit, mean corpuscular volume and red cell distribution width have been reported to be elevated among photocopier operators compared to controls.

Table 2.2: Common Substances Emitted by Photocopiers and Their Potential Immunologic Effects

Substance	Source	Immune Impact	Affected Indices
Ozone (O_3)	Corona wires in photocopiers	Inhibits T-cell activation, reduces lymphocyte counts, induces oxidative stress	↓ $CD4^+$ T-cell, ↑ oxidative stress markers
Volatile Organic Compounds (VOCs)	Toner, ink, heated components	Stimulates IgE production, chronic inflammation, lymphocyte epigenetic changes	↑ IgE, ↓ lymphocytes, ↑ eosinophils
Toner Particulate Matter	Toner dust, drum unit	Phagocytosed by macrophages, causes lung irritation, increases neutrophils	↑ Neutrophils, ↑ cytokines
Carbon black	Toner pigment base	Respiratory irritation, systemic inflammation	↑ Total WBC, ↑ inflammatory cytokines
Nitrogen oxides (NOx)	Electrical discharges	Triggers pro-inflammatory cytokine release	↑ TNF-α, ↑ IL-6, ↑ total WBC

(Elango *et al.*, 2013)

2.7 Mechanism of Ozone immune modulation

Inhaled ozone readily penetrates the respiratory tract and initiates oxidative stress, which is the overproduction of reactive oxygen species (ROS) beyond the capacity of the body's antioxidant defenses. This triggers a cascade of cellular events involving lipid peroxidation, protein oxidation, and DNA damage in airway epithelial cells (Chuang *et al.,* 2020). This oxidative stress activates inflammatory pathways such as nuclear factor-kappa B (NF-κB) and activator protein-1 (AP-1), leading to the production of pro-inflammatory cytokines like interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and interleukin-8 (IL-8) (Zhou *et al.,* 2019). These cytokines recruit immune cells which include neutrophils, macrophages, and dendritic cells—to the site of inflammation, thereby altering both innate and adaptive immune responses. The immunotoxic potential of ozone arises primarily from its high oxidative reactivity when it comes into contact with airway surfaces. Unlike many other pollutants that require metabolic activation, ozone reacts immediately with the epithelial lining fluid and cell membranes upon inhalation. This interaction generates reactive oxygen species (ROS), lipid ozonation products (LOPs), and aldehydes that act as secondary messengers in initiating immune and inflammatory responses (Mudway and Kelly, 2021). Another important mechanism involves epithelial barrier disruption. Ozone exposure increases epithelial

permeability and reduces tight junction integrity, allowing allergens and particulates to penetrate more deeply into submucosal tissues. This enhances antigen uptake by dendritic cells and amplifies immune activation (Hollingsworth *et al.,* 2010). Additionally, dendritic cells exposed to ozone undergo phenotypic changes that bias T-helper cell differentiation toward a Th2 response, which is associated with allergic sensitization and elevated immunoglobulin E (IgE) production (Naclerio *et al*., 2010). On a systemic level, ozone has been shown to cause lymphocyte apoptosis and redistribution. Acute exposures often result in transient lymphopenia, attributed partly to corticosteroid-mediated sequestration of lymphocytes in lymphoid tissues and partly to direct oxidative damage to T lymphocytes (Henríquez *et al.*, 2021). This reduction in circulating $CD4^+$ T cells impair immune coordination, potentially compromising adaptive immunity in chronically exposed individuals. Collectively, these mechanisms illustrate how ozone exerts a dual effect on immunity which include immune activation, characterized by neutrophilic inflammation, cytokine release, and oxidative stress and immune suppression, involving lymphocyte apoptosis, reduced $CD4^+$ proliferation, and impaired antigen presentation. This paradoxical response explains why ozone exposure can simultaneously promote chronic inflammation while weakening host defense mechanisms (Jaspers *et al.,* 2010).

VOC exposure and higher total WBC counts, with an increase in neutrophil percentages. Kwon et al. (2018) found that elevated indoor VOC concentrations

correlated with airway inflammation biomarkers and neutrophilia in exposed populations.

- Leukopenia in High-Level Exposures: Conversely, chronic occupational exposure to benzene often results in leukopenia and lymphopenia. Epidemiological studies of factory and refinery workers have consistently documented depressed WBC counts in association with benzene exposure (Guo *et al*., 2020).

Thus, the leukocyte response depends on the VOC type and exposure intensity: irritant VOCs elicit inflammatory leukocytosis, while hematotoxic VOCs (benzene) suppress bone marrow function.

2.8 VOC-Induced $CD4^+$ T Cell Modulation

Several VOCs have been found to impact $CD4^+$ T lymphocyte function, especially through oxidative DNA damage and dysregulation of cytokine networks. Chronic exposure to benzene and formaldehyde may impair T-cell proliferation and differentiation, particularly in $CD4^+$ T cells, which coordinate adaptive immune responses (Zhang *et al.,* 2018). VOCs also impact adaptive immunity through effects on $CD4^+$ T lymphocytes:

- Reduced Counts: Workers exposed to benzene show decreased circulating $CD4^+$ counts, attributed to marrow suppression and direct oxidative DNA damage in lymphocytes (Brandão *et al.,* 2020).

- Functional Suppression: In vitro studies indicate reduced $CD4^+$ proliferation

and impaired IL-2 production following VOC exposure, highlighting functional compromise even when cell counts remain stable (Ogbodo *et al.,* 2022).

- Helper Subset Skewing: Exposure to formaldehyde and related compounds enhances Th2 differentiation while impairing Th1-mediated immunity. This skewing not only weakens host defenses against infections but also promotes allergic responses (Bönisch *et al.,* 2012).

- Regulatory T-Cell Dysregulation: Some VOCs interfere with Treg activity, tipping the balance toward unchecked inflammation or hypersensitivity.

These immunosuppressive effects compromise immune surveillance, increasing susceptibility to infections and autoimmune disorders. Studies among factory and laboratory workers have consistently shown lower $CD4^+$ counts in individuals with chronic VOC exposure (Wang *et al.,* 2020).

2.9 Serum Immunoglobulin E (IgE) Elevation and Allergic Immune Response

VOCs can act as adjuvants, enhancing the body's sensitivity to environmental allergens. Through disruption of epithelial barriers and promotion of Th2 polarization, they stimulate B-cell class switching to IgE production, contributing to allergic diseases such as asthma and rhinitis (Rumchev *et al.*, 2017).VOCs have been implicated in IgE-mediated allergic sensitization:

- Animal Studies: Formaldehyde-exposed mice demonstrated enhanced allergen-specific IgE responses and increased eosinophilic infiltration compared with

unexposed controls (Gu *et al.,* 2018).

- Human Evidence: Nurmatov et al. (2015) systematically reviewed epidemiological studies and concluded that indoor VOC exposure is consistently associated with higher risks of asthma, allergic rhinitis, and atopy, often linked with elevated IgE levels.

- Clinical Implications: Elevated total and allergen-specific IgE in VOC-exposed populations reflects a Th2-skewed immune state. Occupational settings with VOC exposure have reported higher prevalence of rhinitis, conjunctivitis, bronchial hyperreactivity, and asthma among workers.

Increased IgE levels are often observed in tandem with higher eosinophil percentages, reinforcing the link between VOC exposure and allergic immune responses. Additionally, indoor VOCs may amplify the sensitizing effects of other allergens like dust, mold, or ozone (Arif and Shah, 2020).

2.10 Occupational Photocopier Exposure

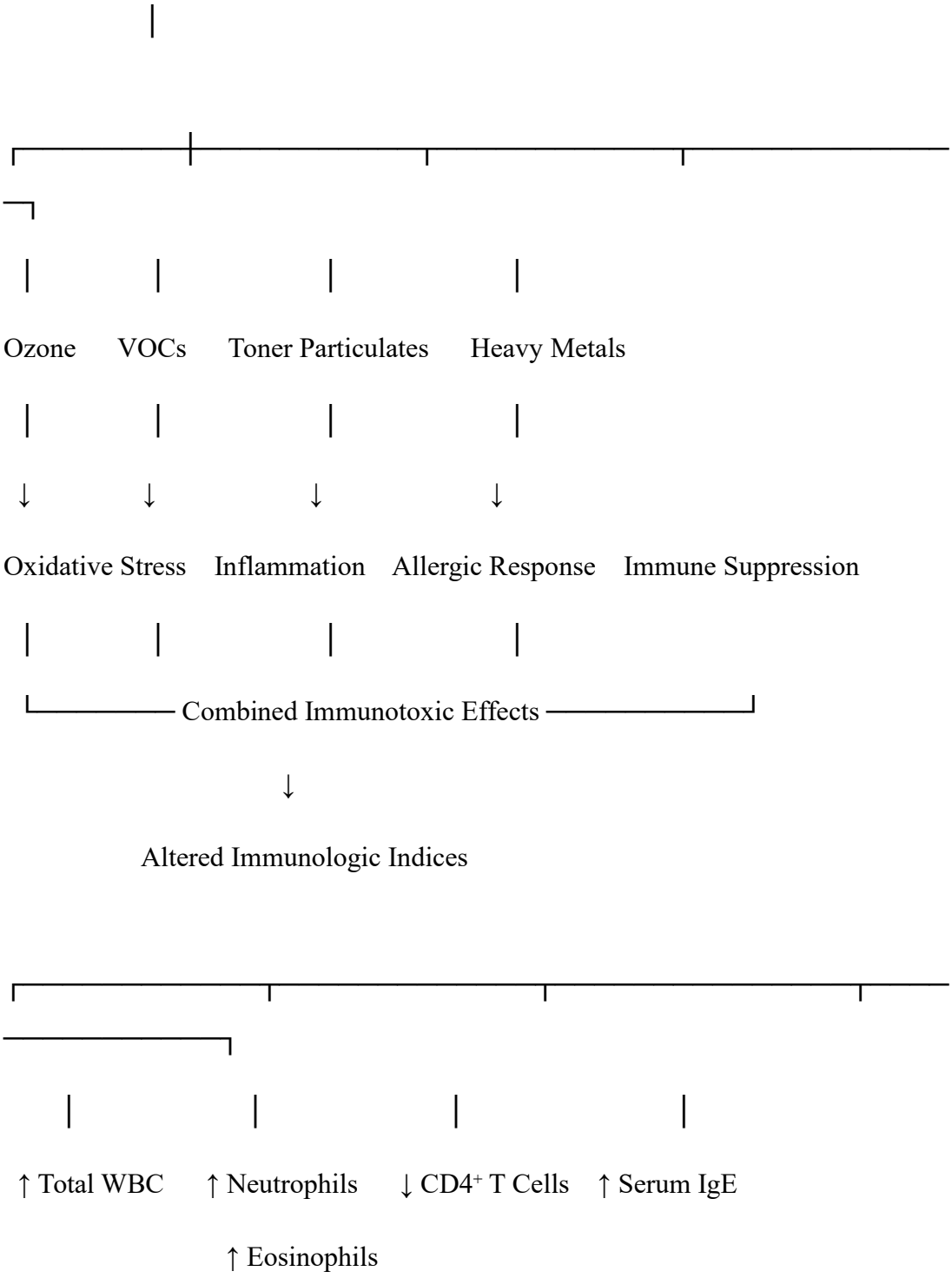

Figure 2.1 Conceptual Model Diagram (Manisalidis *et al.*, 2020; Tchounwou *et al.*, 2012).

CHAPTER THREE

MATERIAL AND METHOD

3.1 Study Design

This study is a comparative cross-sectional study, designed to evaluate and compare immunological indices between male photocopier operators (exposed group) and non-exposed individuals (control group) within the University of Benin campus. The study participants consist of male individuals who have been operating photocopying machines and are occupationally exposed to photocopier emissions (such as ozone, toner particles, and volatile organic compounds) for a period of one(1) year and above within the age range of 18–60 years.

3.2 Study Area

The research was conducted at the University of Benin Campuses located in Ovia North East and Oredo Local Government Areas of Edo State, Nigeria. Participants were drawn from both the Ugbowo and Ekenwan campuses, where male photocopier operators are actively engaged in daily copying and printing activities in academic and administrative departments.

3.3 Study Population

Male photocopier operators who have been actively working within the University of Benin for a minimum of one(1) year and above which make up the exposed group while the control group participants, comprises of university administrative staffs and other non-exposed students who work in similar environments but are not

involved in photocopying activities.

3.4 Inclusion Criteria

- Individuals aged 18–60 years
- Male photocopier operators who have worked for at least one(1) year and above i
- Controls who have not been exposed to photocopier emissions occupationally
- Willingness to participate and sign informed consent

3.5 Exclusion Criteria

- Individuals with pre-existing chronic illnesses (e.g., HIV, cancer, autoimmune diseases)
- Those on immunosuppressive medications (e.g., corticosteroids)
- Recent history of infection or hospitalization (within the past month)
- Females

3.6 Ethical Considerations

Ethical approval for this study was sought and obtained from the research and ethics committee from school of Basic Medical Science with CMS/REC/01/VOL.2/826. See Appendix 1

3.7 Sample Size Determination

The sample size for this study will be determined using the sample size determination for health studies:

$N=Z^2pq/d^2$(Daniel *et al*., 2005)

Where:

N = the desired sample size

Z = the standard deviation set at 1.96

P = the estimated prevalence of altered immunological markers among occupationally exposed workers is 50% (Adeyeni *et al.*, 2023)

50/100 =0.50

q = (1-P) = (1-0.50) =0.5

d = the margin of error or precision sets at 13%

13/100 =0.13

Therefore, $Z^2 = (1.96^2) = 3.842$

$d^2 = (0.13^2) = 0.0169$

Then n= 3.842 x 0.50 x 0.5/ 0.0169

=56.8 which is approximately 60

Therefore, a total of 60 participants was recruited for this study as exposed while 30 non-exposed healthy controls.

3.8 Blood Sample Collection and Separation

About Eight milliliters(8mL) of venous blood was collected from ante-cubital fossa of

each participants using vacutainer blood collection set under aseptic conditions after each daily work (6-8 hours). Four milliliters (4) mL was dispensed into an EDTA anticoagulated container for Haematological parameters, $CD4^+$ T Lymphocytes cells count analysis. While the remaining 4mL was dispense into plain containers and allow to clot, centrifuged at 3,000 rpm for 10 minutes to obtain serum. Serum was then separated and frozen in a well labelled container at -70^{o}c until ready for analysis of IgE.

3.9 Laboratory Analysis

3.9.1 Total WBC and differential Count Analysis

The SFRI auto-haematological analyzer was used to do a full blood count immediately upon collection. This auto-analyzer is used to perform a comprehensive blood count to determine the quantity of blood cells. Calibration and standardization of the equipment, processing, and analysis were performed exactly in accordance with the manufacturer's instructions.

Principle

It works on the premise of counting and quantifying cell types utilizing radio frequency and direct current (Gopal *et al.* 2020). It analyses blood using three detector blocks, a cell pack, and stromatolyser reagents. The WBC detector block measures the number of white blood cells (WBC), whereas haemoglobin detectors use the direct current (DC) detection method to measure the number of red blood cells and platelets.

Procedure

The procedure is such that the sample for analysis is mixed using a vortex mixer and the lid of the sample container is opened and the sample fed into the Auto-analyzer via the probe, the analysis was done by the machine and the results of the analysis displayed at the read-out screen which can also be printed.

3.9.2 CD4 T cells

3.9.2.1 Determination of CD4

Blood specimen was analyzed for CD4 T-lymphocyte count using Partec Cyflow counter (Partec, Germany) following the manufacturer's instruction as previously described by (Akinbo *et al*., 2015).

Principle

The principle is based on the passage of cells in single file in front of a laser so they can be detected, counted and sorted. Cell components are fluorescently labelled and then excited by the laser to emit light at varying wavelengths detected by photodetector which is converted in signal pulses.

Procedure

Twenty microliters (20µL) of CD4 phycoerythrine antibody was placed into a partec test tube and 20µL of well mixed whole EDTA specimen was added. The content of the test tube was mixed gently and allowed to stand in the dark for

15minutes at room temperature. This mixture was agitated every 5 minutes. Eight hundred microliter (800µL) of CD4 buffer solution was added to the mixture of antibody and blood specimen and mixed gently which was insert into the counter for counting.

3.9.3 Manual determination of Total Lymphocyte count (TLC)

Principle

TLC was calculated from CBC by multiplying the percentage of lymphocytes with total white-blood cell count (Angelo *et al*., 2019)

3.9.4 Determination of Serum Immunoglobulin E(IgE) levels using enzyme-linked immunosorbent assay (ELISA) kits (Hamilton, R. G et al., 2020)

Principle:

The microelisa strip platee kit which has been pre-coated with an antibody specific to Immunoglobulin E (IgE) Standards or samples are added to the appropriate microelisa strip plate wells and combined to the specific antibody. Then a horseradish peroxidase (HRP)-conjugated antibody specific for IgE is added to each microelisa stripplate well incubated. Free components are washed away. The tetramethylbenzidine (TMB) substrate solution A and B are added to each well. Only those wells that contain IgE antibody will appear blue in colour and then turn yellow after addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450nm. The OD value is proportional to the concentration of IgE in the samples are calculated by comparing the OD of the samples to the standard curve.

Procedure:

0.025ml (25µl) of the diluted serum (test and control sample) was pipette into their assigned wells. Then, 0.025ml (25µl) of the IgE enzyme reagent was added to the wells. The mixture was swirled for 30 secs after which it was covered and left to incubate at room temperature for 45mins.Contents of the microplate was discarded via decantation and the micro titre wells was tap and blotted dry using adsorbent paper.0.350ml (350µl) of wash buffer was added, decanted and blotted dry. This procedure was repeated 2 more times after which 0.100ml (100µl) of the working substrate solution was added to the wells and the plate was incubated for extra 15mins.After incubation, 0.050ml (50µl) of the stop solution was added to each well, mixed gently and the absorbance of the content of each well was read at 450nm using a microplate reader.

3.10. Statistical Analysis

Data from the study were analyzed using statistical package for social sciences (SPSS) version 28.0 software (IBM Inc. USA). The comparison of mean values of measured parameters between occupationally exposed Photocopiers and apparently healthy non-exposed control was performed using unpaired student's T-test, Chi-square and analysis of variance (ANOVA). Statistical significance level was set at $p < 0.05$.

CHAPTER FOUR

RESULTS

Table 4.1: Demographic Characteristics of the study Groups.

The demographic data characteristics of the study groups (exposed) and control (non-exposed) as illustrated in Table 4.1 A total of 98 questionnaires were administered to all male participants but only 90 (Photocopiers =60 and control=30) met the inclusion and exclusion criteria. From this study, the frequency of distributions for the various age groups suitable matched suitable matched while it was also observed at the educational level that secondary has the highest participants across the studied groups (16.7%. 66.7%, 8% and 2% respectively) and was statistically significant ($\mathbf{x^2}$=61.015, **P**<0.000). Concerning the use of protective devices, due to widely varying sets of the use of different protective devices, it was not possible determine use of personal protective equipment (PPE) for 'dose' levels over the periods of photocopy machines exposure for the purposes of this study and as such, the results herein should be seen as reflective of either 'YES' or 'NO' use of protective device only. In all, majority of the occupationally exposed photocopiers were found not to be using any type of personal(self)protective equipment (PPE) and was statistically significant ($\mathbf{x^2}$=90, **P**<0.000). They had a poor socio-economic backgrounds and low

level of awareness concerning the hazardous effects of exposure to photocopy machines and as such are posed to adverse effects resulting from exposure to Photocopy machines. Also, on the years on job, it was observed that occupationally exposed photocopiers across the various groups were 55.0% of exposed participants which made up < 5 years of exposure, 18.3% had between 5-10 years of exposure, another 18.3% of exposed participants had between 11-15 years of exposure while 8.3% had >15 years of exposure highly significant (χ^2<0.000) as shown in table 4.1.

Table 4.1. Distribution of Demographic Characteristics of the study group (Exposed and Non-exposed)

Characteristics	Exposed (n=60)	Non-exposed (n=30)	χ^2	p
Age group (Years)				
				0.00
18-30	22 (36.7%)	18 (60.0%)	9.508	9
31-44	16 (26.7%)	10 (33.3%)		
45-60	22 (36.7%)	2 (6.7%)		
Level of Education				
				0.00
Primary	10 (16.7%)	0 (0.0%)	61.015	0
Secondary	40 (66.7%)	0 (0.0%)		
Tertiary	8 (13.3%)	11 (36.7%)		
Undergraduate	2 (3.3%)	19 (63.3%)		
Use of protective device				
				0.00
Nil	0 (0.0%)	30 (100.0%)	90.000	0
No	41 (68.3%)	0 (0.0%)		
Occasionally	1 (1.7%)	0 (0.0%)		
Yes	18 (30.0%)	0 (0.0%)		
Duration of Exposure (Years)				
<5	33 (55.0%)	0 (0.0%)		
5 - 10	11 (18.3%)	0 (0.0%)		
10 - 15	11 (18.3%)	0 (0.0%)		
15 Above	5 (8.3%)	0 (0.0%)		

Table 4.2. Shows the comparison of Mean±SD of Total white Blood cell and Differential count (LYM, MID, and GRA) parameters among occupationally exposed photocopiers and non-exposed individual. The WBC count, Lymphocytes count, Monocyte count and Granulocytes count among occupationally exposed group (5.42, 48.47, 16.45 and (35.16) were significantly higher ($p<0.000$) when compared with the non-exposed group (4.01, 34.31, 9.61) respectively. Conversely, Granulocyte value was significantly lower with a ($p<0.000$) among occupationally exposed when compared with non-exposed individual (35.16 vs 56.08) as shown in table 4.2 below.

Table 4.2. Mean Comparison of Total white Blood cell and differential Count Parameters of the studied Groups

Parameters	Exposed (n=60)	Non-exposed (n=30)	t-value	p-value
WBC (10^9/L)	5.42±0.18	4.01±0.07	5.550	0.000
LYM(%)	48.47±1.03	34.31±0.54	9.361	0.000
MID(%)	16.45±0.42	9.61±0.18	11.230	0.000
GRA(%)	35.16±1.02	56.08±0.70	-13.663	0.000

Key:

P<0.05= significant

P<0.0001=Highly Significant

Table 4.3: shows the comparison of Mean±SD of CD4-T count, Absolute Lymphocyte count and serum level of immunoglobulin E(IgE) parameters among occupationally exposed photocopiers and non-exposed individual. The $CD4^+$ cell count, Absolute lymphocytes and serum IgE were significantly higher ($p<0.0001$) among exposed (2.55±0.07 and 2.1597±5.92) when compared with non-exposed individual (2.11±0.15 and 1.5807±3.95) While $CD4^+$ cell count was significantly lower ($p<0.001$) among exposed (658.31±19.10) when compared to the non-exposed group (955.69±97.48) non-exposed group as ahown in table 4.3 below

Table 4.3. Mean Comparison of $CD4^+$ cell count, Absolute Lymphocyte count and serum Immunoglobulin E(IgE) level of studied groups

	Exposed(n=30)	Non-exposed (n=60	t	P
CD4 T COUNT(cells/μL)	658.31±19.10	955.69±97.48	-4.725	0.000
ABSOLUTE LYMPHOCYTE ($x10^9$ Cell/L)	2.55±0.07	2.11±0.15	3.068	0.003
IgE(Pg/mL)	2.159±5.92	1.580±3.95	6.542	0.000

Key:

P<0.05= significant

P<0.0001=Highly Significant

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1. Discussion

Photocopying, while an indispensable activity in academic and business settings, has increasingly been recognized as a source of environmental and occupational hazards. It release emissions containing ultrafine toner particles, volatile organic compounds (VOCs), ozone, and trace metals, which are inhaled during prolonged operation. These pollutants are capable of inducing oxidative stress, inflammatory responses, and immune dysregulation (Murphy *et al*., 2011). The Immunological parameters provide sensitive biomarkers in biomonitoring of occupationally exposed workers. Hence, this study was aimed to address changes in some immunologic indices using total and differential, absolute lymphocyte count $CD4^{+}$T cells and serum IgE levels among photocopier operators (exposed group) and non-exposed individuals. The results, presented in Tables 4.1, 4.2, and 4.3, reveal clear alterations in hematological and immunologic parameters attributable to occupational exposure.

Demographic characteristics of all male participants and we observed at the educational level that secondary has the highest participants across the studied groups (16.7%, 66.7%, 8% and 2% respectively) and was statistically highly significant (X^2 = 61.015, P = 0.000). The reason for this could be due to the fact that photocopying business has become a lucrative source of livelihood in sub-Saharan Africa where there are high rates of unemployment. Young adults after secondary education are left

with no choice rather than to engage themselves in photocopying and related machine operation work as a source of income, thus accounting for the predominance of this educational category and age group in the present study.

The total white blood cell count was significantly higher ($p<0.000$) in the exposed group (5.42 ± 0.18) compared with the non-exposed group (4.01 ± 0.07). This is in agreement with work done by (Awodele *et al*., 2015) and (Elango *et al.,* 2013) in Indian among occupationally exposed photocopying machine operators. This finding indicates systemic immune activation and low-grade inflammation likely driven by inhaled particulates matters and formed ozone in a poorly ventilated environment in the respiratory pathway which stimulate inflammatory cytokines. Interleukin-6 (IL-6) is majorly produced by tissues macrophages in the lung, hence the elevated monocytes seen in this study. Grimbaldeston *et al*., (2020) reported that late phase response to toxicant as the migration of leukocytes such as neutrophils, lymphocytes, eosinophils and macrophages to the initial site and usually occurs 2–24 hours after the original reaction. Cytokines from mast cells may play a role in the persistence of long-term effects and these cytokines regulate IgE synthesis and promote eosinophil development, thus contributing to allergic inflammatory responses (Donald, 2021). Since painters are occupationally exposed to paint fumes this cause continuous degranulation of mast cells or basophils cells thus continuous release of vasoamines mediators (histamine, prostaglandin, serotonin, cytokine and heparin) from their granules into the surrounding affected tissues. Hence, the reason for the statistical

highly significant (P<0.0001) decrease in basophils value seen in this study. Activated regulated eosinophils also migrate to this site and release its granules contents such as myeloperoxidases and cytokines which further complicate the inflammatory process hence the reasons for high significant(P<0.05) decrease in eosinophil observed in this study. Similarly, neutrophils with a short life span of 2-3days migrate along endothelial walls and against blood gradient to the injured sites. its main function is to phagocyte foreign substances in the compromised/affected sites, this could account for the significantly lower (P < 0.0001) neutrophil among exposed group (35.16 ± 1.02) compared with non-exposed (56.08 ± 0.70) observed in this study. Controlled ozone exposure experiments in humans similarly demonstrated neutrophil elevation (Devlin *et al.,* 2012), while animal models of toner inhalation confirmed neutrophil recruitment in lung and systemic compartments (Pirela *et al*., 2017). A reduction in neutrophils (neutropenia) is clinically important because it compromises innate immunity and increases susceptibility to bacterial infections (Hoffbrand and Moss, 2019). Conversely, this finding contrasts with the report of Elango *et al*., (2013), who observed neutrophilia among Indian photocopier operators, attributing it to acute airway irritation and systemic inflammation caused by ozone and particulate matter. The discrepancy may be explained by exposure duration, machine type, room ventilation, and immune adaptation. Chronic exposure can exhaust bone marrow reserves or induce apoptosis of circulating neutrophils, leading to decreased counts as observed in this study (Stiegel *et al*., 2016). Animal experiments also support this duality: short-term toner dust instillation in rats induces neutrophilic infiltration of the

lungs, whereas prolonged exposure reduces circulating neutrophils due to redistribution and oxidative damage (Pirela *et al.,* 2016; Tomonaga *et al.,* 2020). The decreased neutrophil count in the present study may therefore reflect a shift from acute inflammatory activation to chronic immune suppression, a finding with serious occupational health implications. Interestingly, lymphocyte percentages were markedly significantly higher($p<0.001$) in the exposed group (48.47 ± 1.03) than in the non-exposed group (34.31 ± 0.54). Several studies have reported lymphocyte fluctuations in exposed workers. Awodele *et al.*, (2015) found elevated lymphocyte counts, while Babatope et al. (2024) reported no significant differences in Ekpoma, Edo State. They observed increased lymphocytes count and decreased neutrophils count could possibly be attributed to the presence of hexavalent chromium (Crvi) used in the manufacture of ultrafine toners used in photocopying machines.The variability suggests that environmental, genetic, and exposure-related factors influence lymphocyte dynamics. A plausible explanation is that, in this cohort, chronic antigenic stimulation may have induced compensatory proliferation of lymphocytes, although further subset analysis shows that $CD4^+$ T cells were significantly reduced (as discussed below). This indicates that not all lymphocyte subsets are equally affected, and some populations such as B cells or CD8 T cells may expand even as $CD4^+$ cells decline. The present study also revealed a reduction in CD4 count among operators compared with controls. This is consistent with findings from Lee et al. (2012) and Mishra et al. (2010), who documented decreased CD4 percentages in workers exposed to heavy metals such as lead and manganese. Mechanistically,

photocopier-related pollutants may impair CD4 cells via oxidative stress, DNA damage, and apoptosis triggered by heavy metals (Cr, Pb, Mn) and nanoparticles (Dai et al., 2017; Nakata et al., 2020). Reduced CD4 count is clinically significant as it reflects impaired cell-mediated immunity and increased risk of opportunistic infections. Animal models confirm these findings. Tomonaga et al. (2020) reported that rats chronically exposed to toner nanoparticles exhibited reduced splenic T-cell proliferation, while lead-exposed mice showed diminished CD4 activity and Th1/Th2 imbalance (Fenga et al., 2017). Collectively, these results support that lymphocyte and CD4 alterations observed in this study are biologically plausible outcomes of chronic occupational exposure. On the other hand, the antigen presenting cells (APC) also presence is T-cell lymphocytes of the innate immune system through the MHC II on the antigenic surface and this cause the cells to be activated and differentiate into T helper cells (T_{H2}). Hence, this may be the reason for the resultant increase in lymphocytic cells in this study. The T-helper cells produce chemokines (IL-4) which signal plasma cells to produce immunoglobulin E. IgE produced have affinity to binds through the Fc receptors on the surfaces mast cells and basophils. The cause of degranulation of these cells to release their contents to the surrounding tissues. Histamine causes vasodilation and leukotriene attracts neutrophils to the injured sites. All these processes could be the reason for the haematological alteration seen in occupational exposed photocopiers and if this variation is not resolve by the immune system and could contribute so the pathobiology of the respiratory system. Monocytes were significantly elevated among exposed operators (16.45 ± 0.42%) compared with

non-exposed individuals (9.61 ± 0.18). This aligns with the role of monocytes in persistent inflammatory signaling and antigen presentation. Endo et al. (2010) observed similar elevations in experimental rats exposed to toner, highlighting the link between particulate inhalation and monocytosis. Monocytosis has also been associated with increased cardiovascular risk, as activated monocytes contribute to oxidative vascular injury (Brook et al., 2010). By contrast, some occupational studies report no significant monocyte alterations (Babatope et al., 2024), reflecting inter-study variability. The monocyte increase observed here underscores the role of photocopier emissions in sustaining low-grade chronic inflammation. It also showed elevated eosinophil counts in photocopier operators compared with controls. Eosinophilia is often linked to allergic sensitization and elevated IgE levels. This finding is consistent with Elango et al. (2013), who reported higher eosinophils in Indian photocopier operators, suggesting Th2 polarization and allergic airway inflammation due to ozone and VOCs. Animal models support this mechanism: Williams et al. (2011) demonstrated ozone-induced airway eosinophilia in mice, mediated by IL-5 and Th2 cytokines. Elevated eosinophils in the present study therefore highlight the potential of photocopier emissions to induce allergic-type immune responses, contributing to respiratory symptoms such as rhinitis and asthma-like manifestations reported among operators.

The mean (SEM $CD4^+$ T-cell count was significantly lower in exposed operators (658.31 ± 19.10 cells/μL) compared with controls (955.69 ± 97.48 cells/μL). This marked reduction is a strong indicator of immune suppression, as $CD4^+$ helper T-cells

play a central role in coordinating adaptive immune responses, including activation of B-cells, cytotoxic T-cells, and macrophages. Reduced CD4 counts are therefore associated with impaired immune regulation and increased vulnerability to infections, malignancies, and autoimmune dysregulation (Hoffbrand and Moss, 2019). The findings of this study are consistent with previous occupational investigations. Mishra *et al*., (2010) demonstrated that battery workers exposed to lead. Similarly, Nakata *et al*., (2020) observed that welders exposed to manganese fumes had significantly reduced naïve $CD4^+$ ($CD4^+CD45RA^+$) subsets, suggesting selective depletion of helper T-cells by inhaled metal particulates. Experimental studies also corroborate these observations: Dai et al. (2017) reported that exposure to hexavalent chromium (Cr(VI)) reduced splenic T-cell numbers and proliferation in mice, an effect mediated by oxidative stress and mitochondrial damage. These reports support the biological plausibility that pollutants from photocopier emissions—ozone, volatile organic compounds (VOCs), and heavy metals such as lead, chromium, and manganese—can contribute to $CD4^+$ depletion via oxidative stress, apoptosis, and cytokine imbalance (Kasai *et al*., 2017; Fenga *et al*., 2017). Serum IgE levels were also elevated in exposed individuals (2.16 ± 5.92) compared with controls (1.58 ± 3.95 pg/mL). Elevated IgE is a recognized marker of allergic sensitization and Th2-skewed immune responses. Pollutants such as ozone and VOCs act as immunological adjuvants by enhancing antigen presentation, upregulating IL-4 and IL-13, and stimulating IgE class switching in B-cells (Kelly and Fussell, 2012). These effects contribute to hypersensitivity disorders, particularly allergic rhinitis and asthma. Elango et al.

(2013) similarly reported elevated eosinophils and IgE among photocopier operators in India, consistent with pollutant-induced allergic airway inflammation. Animal studies further reinforce this mechanism: Williams et al. (2011) demonstrated that ozone exposure increased eosinophilic airway infiltration and IgE production in murine models through Th2 cytokine activation. The combined findings—reduced CD4 counts, increased lymphocytes overall, and elevated IgE—reflect a dual phenomenon of immune suppression and allergic sensitization. On one hand, helper T-cell depletion signals weakened cell-mediated immunity and reduced host defense. On the other, elevated IgE and eosinophilia indicate heightened risk for hypersensitivity reactions. This duality suggests that photocopier emissions not only predispose operators to infections but also increase their risk for allergic and atopic diseases. Such immune dysregulation has also been observed in other occupational cohorts exposed to metal fumes and indoor air pollutants, underscoring the occupational health relevance of these findings (Fenga *et al*., 2017).

5.2. Conclusion

The immunity of occupationally exposed male photocopier operators was compromised.

5.3 Recommendations

We therefore advocate the need for policy makers and employers of labour to embark on enlightenment campaign for regular checkup and the use of proper personal protective equipment should strictly be adhere to by occupationally exposed

photocopying machines operators in University of Benin campus.

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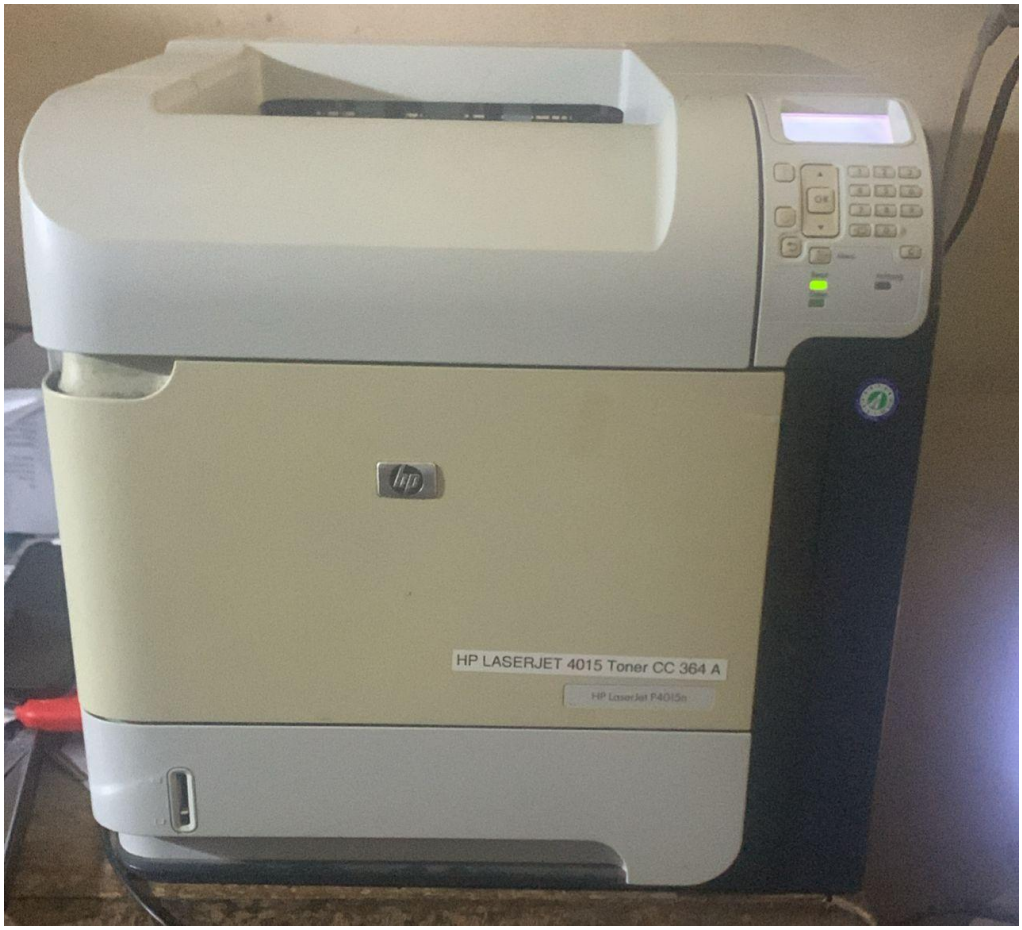
APPENDIX I

Photocoping Toner

Photocoping Machine



Printer

APPENDIX II

PHOTOCOPY MACHINE EMISSION FUMES EXPOSURES ASSESSMENT QUESTIONNAIRE

DEPARTMENT OF MEDICAL LABORATORY SCIENCE
SCHOOL OF BASIC MEDICAL SCIENCES
COLLEGE OF MEDICAL SCIENCES
UNIVERSITY OF BENIN
BENIN CITY

Dear respondent,

My name is…….. a AN UNDERGRADUATE Student of the Department of Medical Laboratory Science, University of Benin. I am carrying out research on the evaluation of some IMMUNOLOGIC IDICES AMONG MALE OCCUPATIONALLY EXPOSED PHOTOCOPIER OPERATORS AT UNIVERSITY OF BENIN CAMPUS. This questionnaire is aimed at getting primary data on participants who are occupationally exposed to machine emmission resulting from photocopiers at University of in Benin Campus. You are therefore kindly requested to fill in the answers in the space provided. With your information given, we will be able to recommend to appropriate authority with a view of increasing the wellbeing of the citizenry. Whatever information you provide will be treated with confidentiality. Thank you for anticipated co-operation.

Kindly respond by filling or ticking the spaces provided.

SOCIAL DEMOGRAPHIC CHARACTERISTICS

1. Age: _____
2. **Sex:** Male [ ] Female [ ]
3. Marital Status: Single [ ], Married [ ], Divorced [ ], Widow or Widower [ ]
4. Number of Children: None [ ], One [ ], Two [ ], Three [ ], Four [ ], Above four [ ]
5. Level of education: No formal education [ ], Primary [ ], Secondary [ ], Tertiary [ ], Undergraduate [ ]

LIFESTYLE

6. Do you smoke: Yes [ ], No [ ]
7. Do you drink alcohol: Yes [ ], No [ ]
8. Any programmed excesses: Yes [ ], No [ ]
9. If yes, which excesses _____

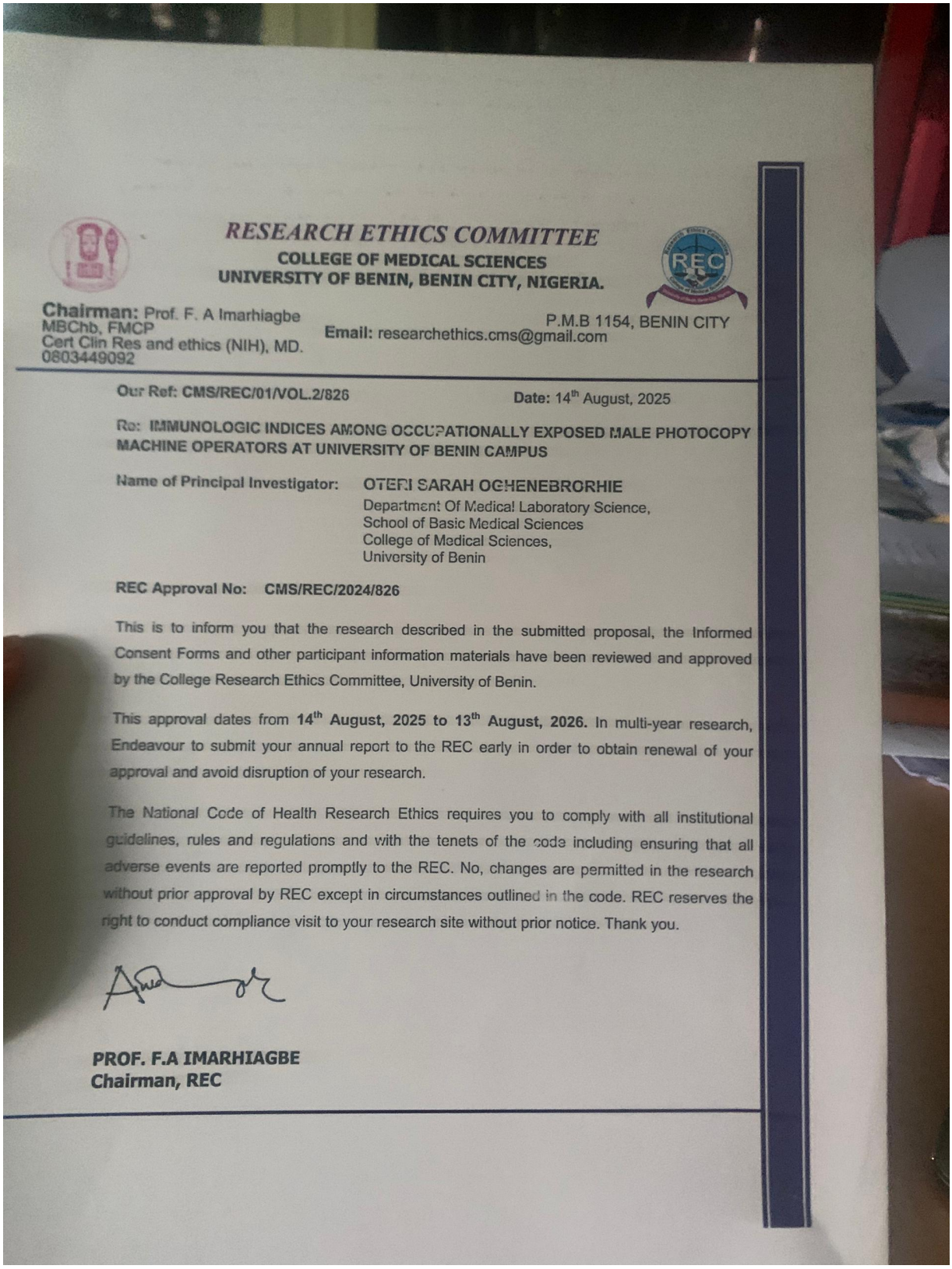
RISK ASSESSMENT INDICES

10. Do you have any knowledge of health hazard that you are associated with in the course of your job that can predispose you to any illness? Yes [ ], No [ ]
11. If yes, what hazard and which illness _____

EXPOSURE ASESSMENT, INDICATORS

12. How long have you been in this job_____
13. How many hours do you spend on the job on daily basis _____
14. Do you use protective device? Yes [ ], No [ ]
15. If yes, which type _____

APPENDIX III



RESEARCH ETHICS COMMITTEE
COLLEGE OF MEDICAL SCIENCES
UNIVERSITY OF BENIN, BENIN CITY, NIGERIA.

Chairman: Prof. F. A Imarhiagbe
MBChb, FMCP
Cert Clin Res and ethics (NIH), MD.
0803449092

P.M.B 1154, BENIN CITY
Email: researchethics.cms@gmail.com

Our Ref: CMS/REC/01/VOL.2/826 **Date:** 14th August, 2025

Re: IMMUNOLOGIC INDICES AMONG OCCUPATIONALLY EXPOSED MALE PHOTOCOPY MACHINE OPERATORS AT UNIVERSITY OF BENIN CAMPUS

Name of Principal Investigator: **OTERI SARAH OGHENEBRORHIE**
Department Of Medical Laboratory Science,
School of Basic Medical Sciences
College of Medical Sciences,
University of Benin

REC Approval No: CMS/REC/2024/826

This is to inform you that the research described in the submitted proposal, the Informed Consent Forms and other participant information materials have been reviewed and approved by the College Research Ethics Committee, University of Benin.

This approval dates from **14th August, 2025 to 13th August, 2026.** In multi-year research, Endeavour to submit your annual report to the REC early in order to obtain renewal of your approval and avoid disruption of your research.

The National Code of Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenets of the code including ensuring that all adverse events are reported promptly to the REC. No, changes are permitted in the research without prior approval by REC except in circumstances outlined in the code. REC reserves the right to conduct compliance visit to your research site without prior notice. Thank you.

PROF. F.A IMARHIAGBE
Chairman, REC