Photocopiers are an indispensable tool in today's world. Photocopiers can produce accurate and durable copies of an original text or graphic material by the use of electrostatic charges, light, heat or chemicals. However, during operation, photocopiers emit toner particles, toxic gases like ozone, nitrogen dioxide, volatile organic compounds (VOCs), semi-volatile organic compounds, particles emitted by the paper processed during printing and copying and extremely low-frequency electromagnetic fields (Kleinsorge et al., 2011). Photocopy workers are potentially exposed to high concentrations of a variety of these pollutants emitted from photocopiers (Yang and Haung, 2008).

The present work is aimed to assess the effects of long term exposure to these pollutants on the health of the workers in these photocopier units. The experimental procedure followed for the study on “Pulmonary Function and Markers of Oxidative Stress and Inflammation in Photocopier Operators” is discussed under the following heads:

3.1 Study Area
3.2 Study Design
3.3 Acquiring Informed Consent
3.4 Selection of Participants
3.5 Collection of Demographic Data
3.6 Anthropometric Measurements
3.7 Pulmonary Function Test
3.8 Blood Collection
3.9 Collection of Exhaled Breath Condensate
3.10 Assessment of Complete Blood Count
3.11 Assessment of Biochemical Markers in Blood
3.11.1 Estimation of Plasma Glucose
3.11.2 Estimation of Serum Total Protein
3.11.3 Estimation of Serum Albumin
3.11.4 Estimation of Serum Globulin

3.12 Assessment of Oxidative Status in Blood
3.12.1 Estimation of Total Lipid Peroxidation
3.12.2 Estimation of Total Antioxidant Activity
3.12.3 Estimation of Free 8-Isoprostane
3.12.4 Estimation of Ferric Reducing Antioxidant Capacity

3.13 Assessment of Inflammatory Biomarkers in Blood
3.13.1 Estimation of Clara cell protein
3.13.2 Estimation of Leukotriene B₄
3.13.3 Estimation of Interleukin 6
3.13.4 Estimation of Interleukin 8
3.13.5 Estimation of Eosinophil Cationic Protein
3.13.6 Estimation of C Reactive Protein
3.13.7 Estimation of Total Nitrates
3.13.8 Estimation of the Activity of Myeloperoxidase
3.13.9 Estimation of Intercellular Adhesion Molecule 1

3.14 Analysis of Cadmium, Selenium and selenoproteins in Blood
3.14.1 Estimation of Cadmium and Selenium
3.14.2 Estimation of the Activity of Glutathione peroxidase
3.14.3 Estimation of the Activity of Thioredoxin reductase

3.15 Assessment of Genotoxicity in Blood

3.16 Metabolomic Analysis of Exhaled Breath Condensate

3.17 Air Quality Monitoring in Photocopier Units

3.18 Statistical Analysis

3.1 Study Area
The study was carried out in Coimbatore district. Coimbatore is the second largest city in Tamilnadu, India. It is an industrial city with numerous academic institutions. Hence, a large number of photocopier units are functioning. Coimbatore was selected as the study area because of an existing good rapport with a few participants and proximity of the units.

3.2 Study Design

A cross sectional study involves simultaneous measurement of exposure and its outcome at a specific point of time for a defined group of individuals. Cross sectional studies provide data that reflect the prevalence of an exposure or outcome in a selected group (Merrill, 2008). Hence a cross sectional observational study was carried out to assess whether occupational exposure to photocopiers compromises the health of the operators of these units.

The study was carried out between January 2011 and March 2012. The investigator approached the proprietors and workers of photocopier units and explained the protocol of the study. Photocopier workers who voluntarily accepted and satisfied the selection criteria were included in the exposed group. Participants who were without any professional exposure to photocopiers and satisfied the selection criteria were included in the control group. All interviews and sample collection procedures from exposed participants were carried out at the photocopier units where the participants worked between 10.00 am and 2.00 pm on a working day. All data and sample collection from control participants were carried out at places convenient for them. The study was approved by the Human Ethical Committee of Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore (HEC.2011.24).

3.3 Acquiring Informed Consent

An informed consent form was evolved based on Indian Council of Medical Research (ICMR) guidelines (Appendix 1). Written informed consent was given by all the participants before the collection of data and blood, urine and exhaled breath condensate samples.

3.4 Selection of Participants
1. Inclusion criteria: Male and female workers in the age group of 18 – 50 years

2. Exclusion criteria: Participants with any ailments like cardiovascular diseases, diabetes mellitus, hypertension, etc.,

3. Exposed group: Operators who were working in photocopier units for ≥ 1 year

4. Control group: No professional exposure to photocopiers

Participants who were below 18 years were excluded from the study due to ethical reasons. Participants who were above 50 years in this occupation were poorly represented, since photocopiers have been in use in India only in the recent past. Hence, they were excluded. Participants who had self reported systemic diseases were also excluded from the study. Occupational exposure was defined as minimum one year employment in any photocopier unit. Participants who consented to take part in the study and fulfilled the selection criteria were included in the study.

A total of 329 participants were approached to take part in the study. The number of participants who accepted to take part was 227. They completed the interview schedule. But 66 were disqualified from the study. 8 participants withdrew from the study. Assessment of pulmonary function tests was carried out on 153 participants (110 photocopier operators and 43 controls). Blood samples could not be obtained from 13 participants. Biomarkers were assessed in 100 photocopier operators and 40 control participants.

3.5 Collection of Demographic Data

An interview schedule is a written list of questions, open ended or closed, prepared for use in an interview in a person to person interaction (Kumar, 2011). An interview schedule was considered appropriate for this study, since interview schedules allowed the interviewer to obtain complete data in greater depth and permitted the interviewer to establish and maintain a rapport with the subject. Hence, an interview schedule (Appendix 2) was evolved by the investigator with the help of a trained pulmonologist and approved by the ethical committee. The interviewer collected
personal, socio economic, demographic, professional and general health details including medical history and current medication from all participants.

According to Raaschou-Nielsen et al. (2010) confounding from smoking is a major concern in any study on air pollution because smoking is the major risk factor and it is more prevalent in urban areas. Hence, smoking histories and exposure to second hand tobacco smoke were recorded in the present study. Smoking histories included ages started and quit, years smoked and average number of cigarettes smoked per day. Second hand smoke was defined as someone smoking regularly in the same room at home or at work. Participants were also asked about the types of fuels used at home. Based on St. George’s respiratory questionnaire (Jones et al., 1992), a questionnaire on respiratory symptoms was also included in the interview schedule.

**Cumulative exposure to photocopiers:**

Number of working hours/day and number of working days/week varied widely among the selected participants. Hence, to compute the cumulative exposure to photocopiers, the following formula was used.

\[ \text{No. of hours exposed} = abc \times 50^* \text{ where } a= \text{No. of working hours/day}; b= \text{No. of working days/week}; c = \text{No. of years of exposure}. \times 50 \text{ weeks/year} \]

**3.6 Anthropometric Measurements**

Anthropometry is the study of measurement of human body in terms of the dimensions of bone, muscle and adipose tissue (NHANES III, 2007). Combined with the dietary and related questionnaire data and the biochemical determinations, anthropometry is essential and critical information is needed to assist in describing the data collected from persons (NHANES, 2005).

Weight was measured using a portable weighing scale to the nearest kilogram. Height was measured barefoot using a measuring tape fixed from the base of the floor at the photocopier units. Body Mass Index (BMI) is an important confounding factor in air pollution studies. Hence, BMI was calculated as body weight in kilogram divided by the square of height in meters.
3.7 Pulmonary Function Test

Spirometry plays an important role in an occupational respiratory health surveillance program. It can assist the health professional by determining if a worker demonstrates a specific pattern of respiratory impairment and can help to assess the effectiveness of measures implemented to protect the individual worker. In addition, results from defined groups of workers can be evaluated in relation to potential workplace hazards (CDC, 2011). In the occupational health setting, spirometry plays a critical role in the primary, secondary and tertiary prevention of workplace-related lung disease (Townsend et al., 2011).

Hence, pulmonary function of the selected participants was assessed by using the spirometer Vitalograph Alpha 6000, UK by the investigator who was trained for this purpose before initiating this study. Spirometry was performed according to the American Thoracic Society / European Respiratory Society (ERS) guidelines. Participants were instructed to take a deep breath and then blow as hard and long as possible into the spirometer. Following a demonstration and practice with the mouthpiece, they performed tests in a sitting position (Plate 1). The manoeuvre was repeated until the Vitalograph spirometer indicated that satisfactory results were achieved (e.g., FEV\(_1\) and FVC within 200 ml of previous values) or the participant chose to stop. The lung function values assessed were: Vital Capacity (VC), Forced Vital Capacity (FVC), Forced Expiratory Volume in 1 Second (FEV\(_1\)), FEV\(_1\)/FVC, Peak Expiratory Flow (PEF), Forced Expiratory Flow 25% - 75% (FEF\(_{25-75}\)), Peak Inspiratory Flow (PIF) And Maximal Ventilatory Volume (MVV). Each subject’s best trial (largest sum of FEV\(_1\) and FVC) was included in analyses. The percentages of predicted values for all values were calculated using published values for Asians (Quanjer et al., 1993). Airflow limitation was determined according to two alternative definitions, using FEV\(_1\)/FVC < 0.7 as a common definition of COPD and FEV\(_1\)/FVC less than lower limit of normal calculated for age and sex.

Plate 1
3.8 Blood Collection

Five ml of blood was collected from the participants by venipuncture. All vacutainers were purchased from Guangzhou Improve Medical Instruments, China. The collected blood was transferred in to 3 tubes viz., 2.5 ml into K$_2$ EDTA vacutainers for plasma separation, 2.0 ml into pro-coagulation tubes for serum separation and 0.5 ml into lithium heparin vacutainers for plasma separation. All samples were immediately stored on ice and transported to the laboratory. 100 µl aliquots of whole blood samples were taken for hematological analysis and single cell gel electrophoresis. All samples destined for serum and plasma collection were centrifuged at 5000 rpm at 4°C for 10 minutes. Plasma/serum was removed, aliquoted into labeled cryovials and stored at -80°C until analysis.

3.9 Collection of Exhaled Breath Condensate

Breathing air through a cooling system results in condensation, thereby rendering collection of exhaled breath in a liquid form. Exhaled breath condensate (EBC) is a suitable matrix to assess respiratory health status in workers exposed to pneumotoxic
substances, due to its ability to quantify lung tissue dose and consequent pulmonary effects. The collection of EBC may contribute to studying the pathological state of the airways of workers with acute and chronic exposure to pollutants. The analysis of EBC is one of the most promising methods currently available for the study of pulmonary biomarkers of exposure, effect and susceptibility in occupational settings. Being collected in a totally noninvasive way, it is particularly suitable to be applied in field studies (Corradi et al., 2010).

Exhaled breath condensate was collected from willing participants (n=28). An improvised device was assembled in the laboratory and tested to assure EBC collection. The improved EBC collection device (depicted in Figure 7) consisted of a mouth piece which also acted as a saliva trap, 1.5 m long, an improvised device xic polyvinyl chloride (PVC) tube, a thermocol box filled with ice and a labelled cryovial. One end of the PVC tube was connected to the mouth piece and the other end held close to the cryovial to collect the condensate. The middle part of the PVC tube was immersed in ice during EBC collection.

Figure 7

Improvised device for collection of exhaled breath condensate

Participants sat comfortably and supported the improvised EBC collection device at bust height. They were asked to inhale through their nose and blow their tidal volume through the mouth piece, adapted to the mouth closing the lips around it without a nose
A calm respiratory pattern was requested during sampling, which lasted for 15 - 20 minutes. Participants were asked to swallow their saliva before beginning and during collection to avoid contamination of EBC. After 10 minutes, the PVC tube was removed with both ends held up. The residual volume was also transferred into the cryovial. At least 1 ml of condensate was collected from each subject. Cryovials containing EBC were put on ice immediately and transported to the laboratory where they were stored at -80°C until analysis.

The mouth pieces and PVC tubes used for EBC collection were washed and reused for 3 sample collections. After soaking with 1 % labolene, they were washed thoroughly with running water and rinsed with distilled water and ethanol before reuse. 

1H Nuclear Magnetic Resonance (1H NMR) spectroscopy analysis of the EBC collected from washed EBC apparatus was carried out to ensure that residual detergents were not present. EBC samples were collected from 28 participants.

3.10 Assessment of Complete Blood Count

The complete blood cell (CBC) count is an inexpensive, frequently obtained blood test whose information content is potentially underused (Andersen et al., 2007). In occupational settings, complete blood count (CBC) must be performed routinely as exposure to solvents and metals may affect the kidneys, liver and bone marrow (http://www.epi.state.nc.us/epi/oii/pdf/methguidelines.pdf).

Complete blood count (CBC) was assessed in the whole blood of all participants by a three part differential analyzer (Sysmex KX 21, Sysmex Corporation, Japan). CBC metrics included Hematocrit, Hemoglobin (Hb), Red Cell Distribution Width (RDW), Mean Corpuscular Volume (MCV), Red Blood Cell (RBC) Count, Platelet Count (PLT), Mean Platelet Volume (MPV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Total White Blood Cell (WBC) Count, Lymphocytes, Mixed Cells, Neutrophils, Platelet Distribution Width (PDW) and Platelet Large Cell Ratio (P-LCR).

3.11 Assessment of Biochemical Markers in Blood
The following biochemical markers were assayed immediately after serum and plasma separation.

### 3.11.1 Estimation of Plasma Glucose

The prevalence of diabetes mellitus has been increasing. Lack of diagnosis of diabetes mellitus and poor control of diabetes mellitus or pre-diabetes among patients have been reported widely. These facts indicate that blood glucose screening is needed (Barasch et al., 2012). In epidemiological studies, blood glucose is an often measured parameter as a risk factor, mediator or confounder. In the present study blood glucose was estimated to rule out diabetes mellitus among the participants. According to Moebus et al. (2011) random blood glucose sample or a fasting duration of 3 hour seems sufficient for reliable blood glucose measurements. Hence, random blood glucose was assessed for the participants in the study to rule out diabetes mellitus. Random blood glucose levels were estimated by kit method (Agappe diagnostics, India) based on glucose oxidase method in the plasma of the participants (Appendix 3).

### 3.11.2 Estimation of Serum Total Protein

Total protein measurements are used in the diagnosis and treatment of a variety of diseases involving the liver, kidney, or bone marrow, as well as other metabolic or nutritional disorders (NHANES III, 2011). Hence, to rule out systemic diseases, serum protein levels were estimated by a kit (CPC Diagnostics, India) based on Biuret method (Appendix 4).

### 3.11.3 Estimation of Serum Albumin

Serum albumin levels were estimated using a kit (CPC Diagnostics, India) based on Bromocresol green method (Appendix 5).

### 3.11.4 Estimation of Serum Globulin

Serum globulin levels were calculated by subtracting serum albumin from total protein and expressed as g/l.
3.12 Assessment of Oxidative Status in Blood

Recent research has identified oxidative stress as one potential feature underlying the toxic effect of air pollutants. Toxicity may arise from an imbalance of biological pro-oxidant and antioxidant processes linked to increased exposure to oxidants or the presence of impaired antioxidant defenses (Romieu et al., 2008). Air pollutants have and continue to be, major contributing factors to chronic diseases and mortality, subsequently impacting public health. Chronic diseases include: COPD, cardiovascular diseases (CVD), asthma and cancer. By-products of oxidative stress found in air pollutants are common initiators or promoters of the damage produced in such chronic diseases. Such air pollutants include ozone, sulfur oxides, carbon monoxide, nitrogen oxides and particulate matter (Yang and Omaye, 2009).

Photocopiers produce ozone, carbon monoxide, nitrogen oxides and particulate matter during their operations. Hence, it is imperative to estimate the oxidative status of the participants.

3.12.1 Estimation of Total Lipid Peroxidation

Lipid peroxidation is the most commonly studied manifestation of oxidative stress, mainly evaluated in terms of malondialdehyde (MDA) and malondialdehyde like compounds. Thiobarbituric acid substances (TBARS) are frequently assessed as a measure of MDA, a commonly used marker of lipid peroxidation (Jayakumar, 2009). Hence, in the present study, TBARS was estimated in the serum of participants by colorimetry by the method of Jentzsch et al. (1996) (Appendix 6).

3.12.2 Estimation of Trolox Equivalent Antioxidant Activity

The total antioxidant power as an ‘integrated parameter of antioxidants present in a complex sample’ is often more meaningful to evaluate health beneficial effects because of the co-operative action of antioxidants (Apak et al., 2007). Total antioxidant capacity considers the cumulative effect of all antioxidants present in blood and body fluids (Suresh et al., 2009). Therefore, total antioxidant capacity of the participants was assayed in plasma by a Trolox Equivalent antioxidant kit
3.12.3 Estimation of Free 8-Isoprostane

Isoprostanoids are prostaglandin-like compounds that are formed as a result of free radical induced oxidation of essential fatty acids (primarily arachidonic acid) and are an established marker for lipid peroxidation (Bhatia, 2011). In particular, free 8-isoprostane is produced by prostaglandin in the presence of oxidative stress and is considered to be useful marker (Ishikawa, 2007). A competitive enzyme immune assay kit (Cayman Chemical, USA) was used to assess the levels of free 8-isoprostane in the plasma samples of the participants (Appendix 8).

3.12.4 Estimation of Ferric Reducing Antioxidant Capacity

Ferric reducing antioxidant capacity levels are indicative of the total amount of non-enzymatic antioxidants. The reducing power of the antioxidants in serum was measured by the ferric reducing antioxidant power method (Benzie and Strain, 1996) in serum (Appendix 9).

3.13 Assessment of Inflammatory Biomarkers in Blood

The inflammatory processes in the lung are characterized by the influx of neutrophils into the airways (Carpagnano et al., 2011). Neutrophil migration from circulation into an area of inflammation involves regulated expression of a number of adhesion molecules on the cell surface. According to Banerjee et al. (2012) up regulation of these surface molecules has been reported in patients with chronic obstructive pulmonary disease and cardiovascular diseases. Therefore, the following pulmonary and inflammatory biomarkers were assessed in the plasma of the selected participants.

3.13.1 Estimation of Clara Cell Protein

The lungs have developed their own defense mechanisms in response to the activity of the environmental pollutants, where a major role is played by Clara cells (Halatek et al., 2009). These cells secrete Clara cell protein (CC16, CC10 or
secretoglobin A1), an anti-inflammatory protein secreted along the tracheobronchial tree and the surfactant-associated protein D, which is secreted by the Clara cells and alveolar epithelium in the deep lung. CC16 is considered the most valuable lung biomarker (Haddam et al., 2009). Determination of CC16 in blood serum, to which it passively leaks through the bronchoalveolar barrier, enables its monitoring in the lungs and, indirectly, assessing the condition of the pulmonary epithelial cells (Halatek et al., 2009). The levels of Clara cell protein in the plasma samples of the participants were assessed by a sandwich enzyme linked immunosorbent assay (ELISA) kit (USCN Life Sciences Inc, USA) in order to determine the status of lung epithelial cells (Appendix 10).

3.13.2 Estimation of Leukotriene B₄

Leukotrienes are biologically active metabolites that are derived from arachidonic acid through the action of 5-lipoxygenase. Leukotriene B₄ (LTB₄), a potent chemo attractant for neutrophils, has been involved in the pathophysiology of inflammatory airway diseases such as COPD, cystic fibrosis, severe asthma and asthma exacerbation. The quantitative assessment of LTB₄ concentrations in biological fluids is essential for establishing the pathophysiological role of LTB₄ in patients with pulmonary diseases (Montuschi, 2009). LTB₄ is usually used as an indicator of the state of activation of neutrophils (Carpagnano et al., 2011). Identifying abnormal concentrations of LTB₄ can shed light on a specific pathway of inflammation or oxidative stress (Horvath and de Jongste, 2010). A competitive enzyme immune assay kit (Cayman Chemical, USA) was used to assess the levels of Leukotriene B₄ in the plasma of the participants (Appendix 11).

3.13.3 Estimation of Interleukin 6

IL-6 is a multifunctional pro-inflammatory cytokine that is linked to a number of disorders including systemic and pulmonary vascular diseases. IL-6 is now considered a major biomarker for cardiovascular risk and the main stimulant for hepatic production of C Reactive Protein, a compound widely used as a biomarker for atherosclerosis (Savale et al. 2009). Elevated plasma levels of inflammatory cytokine IL-6 is observed in various disorders like COPD, CVD, sepsis, etc., Hence, plasma
levels of interleukin 6 were assessed by sandwich ELISA kit (Koma Biotech, Korea) - (Appendix 12).

3.13.4 Estimation of Interleukin 8

IL-8 is a potent neutrophil chemo-attractant playing an important role in inflammation (Marik and Raghavan, 2009). IL-8 is one of the best biomarkers for lung epithelial barrier injury, inflammation and neutrophil chemotaxis (Ware et al., 2009). Levels of plasma interleukin 8 were estimated by sandwich ELISA kit purchased from Koma Biotech, Korea (Appendix 13).

3.13.5 Estimation of Eosinophil Cationic Protein

Eosinophils contain several basic granule proteins, the best known being the eosinophil cationic protein (ECP). ECP is a cytotoxic, pro-fibrotic ribonuclease, which is found deposited in these eosinophil-related diseases and is often used in parallel with blood eosinophilia to monitor those diseases (Bystrom et al., 2012). According to Quirce et al., (2010), ECP is one of the most studied markers of inflammation and may serve as a general marker of inflammation, both in processes of eosinophil and neutrophil activation. Levels of plasma ECP were estimated by sandwich ELISA kit purchased from USCN, China (Appendix 14).

3.13.6 Estimation of C Reactive Protein

C Reactive Protein (CRP) is a nonspecific marker of systemic inflammation. It is an acute phase reactant synthesized in the liver in response to cytokines, especially IL-6 (Qi et al., 2008). Plasma CRP levels undergo a rapid and robust rise in response to inflammatory stimuli. Because of this phenomenon, plasma levels of CRP have long been considered to be an important biomarker for detecting the presence of systemic inflammation. Measurement of CRP level has been shown to have prognostic and/or diagnostic value in a large number of disease states, including sepsis, pneumonia, appendicitis, coronary artery disease, stroke, diabetes and rheumatic disease, among others. In most cases, higher CRP levels have been associated with adverse outcomes (Bajwa et al., 2009).
CRP has also been shown to be a marker of inflammation in atherosclerosis and levels of CRP correlate with the degree of pulmonary inflammation in stable COPD (Dahl et al., 2011). Hence, CRP levels were assessed in the participants of the present study by a sandwich ELISA kit (Cayman Chemical, USA) as detailed in appendix 15.

### 3.13.7 Estimation of Total Nitrates

Nitric oxide (NO) is a multifunctional reactive mediator produced in mammalian cells, which may act as a vasodilator, neurotransmitter and an effector molecule of immune cells. In addition, NO mediates the expression of pro-inflammatory cytokines, including IL-8 (Alusik et al., 2008). NO is rapidly removed by its rapid diffusion through tissues into red blood cells, where it is rapidly converted to nitrate by reaction with oxyhaemoglobin (Pacher et al., 2007). Hence, plasma nitrate level is a surrogate marker of nitric oxide production and altered NO metabolism (Bloomer et al., 2011; Abraham and Singer, 2007). Plasma nitrate levels were estimated by a kit based on the Greiss reaction (Cayman Chemical, USA) - (Appendix 16).

### 3.13.8 Estimation of the Activity of Myeloperoxidase

Myeloperoxidase (MPO) is an enzyme found in myeloid cells, particularly in neutrophils and to a lesser extent in monocytes and tissue macrophages. It plays an important role in the host defense against bacteria and viruses. In neutrophils, MPO is located within the primary granules and its concentration is used as an indicator of neutrophil activation and corresponding inflammatory response (Banerjee et al., 2010). MPO is released from activated neutrophils and monocytes during inflammation and has been identified as a novel inflammatory marker (Shah et al., 2008b). Myeloperoxidase was estimated in lithium heparin plasma participants by a sandwich EIA kit (Enzo Life Sciences, Switzerland) - (Appendix 17).

### 3.13.9 Estimation of Intercellular Adhesion Molecule 1

Intercellular adhesion molecule (ICAM)-1 is an immunoglobulin like cell adhesion molecule expressed by several cell types including leukocytes and endothelial cells. ICAM-1 is a transmembrane protein that mediates endothelial transmigration of WBCs in inflammatory response (Bind et al., 2012). ICAM-1 has been shown to transmit
intracellular signals that lead to activation of pro-inflammatory responses that can perpetuate an inflammatory response. A soluble form of ICAM-1 (sICAM-1) has been found in plasma (Lawson and Wolf, 2009). sICAM-1 is an endothelium-derived inflammatory marker that has been associated with diverse conditions such as COPD, myocardial infarction, diabetes, stroke and malaria (Pare et al., 2011). Levels of ICAM-1 were assayed in the plasma of the participants by a sandwich ELISA (USCN Life Sciences) kit (Appendix 18).

3.14 Analysis of Cadmium, Selenium and Selenoproteins in Blood

Photoreceptor drums of photocopiers and laser printers contain selenium and cadmium (Woollins and Laitinen, 2011). Chronic exposure to dust from photocopiers may lead to altered cadmium and selenium levels among the operators.

3.14.1 Estimation of Cadmium and Selenium

Cadmium is a toxic metal. After inhalation or ingestion of Cadmium, it is transferred into the bloodstream (whole blood and serum Cadmium concentrations range between 0.2 and 20 nmol/L), where Cadmium is transported either as a free ion or protein-bound, eg, attached to albumin or metallothioneins (Messner et al., 2009). Cadmium in blood is considered the most valid marker of recent exposure (Rignell-Hydbom et al., 2009). Inductively coupled plasma is widely recognized as a suitable technique for the determination of trace elements, the particular advantages being the multi-element capability, large dynamic range and effective background correction (Massadeh et al., 2010).

Selenium is an essential micronutrient for humans, acting as a component of the unusual amino acids, selenocysteine and selenomethionine. The cell cannot synthesise selenoproteins when Se levels are low, although some selenoproteins and some tissues are prioritised over others. Characterised functions of known selenoproteins, include selenium transport (selenoprotein P), antioxidant/ redox properties (glutathione peroxidases (GPx), thioredoxin reductases and selenoprotein P) and anti-inflammatory properties (selenoprotein S and GPx4) (Ferguson et al., 2012). There is a narrow range between selenium intake levels required for selenoprotein synthesis and toxic levels (Laclaustra et al., 2009). Exposure to high levels via inhalation or ingestion may cause
adverse health effects (Risher, 2011). Hence, possible occupational exposure to selenium was studied by assessing the levels of serum selenium, plasma glutathione peroxidase and thioredoxin reductase. Serum selenium levels are directly related to recent selenium intake (Reilly, 2006). Serum cadmium and selenium were assessed by Inductively Coupled Plasma – Optical Emission Spectrometry (Appendix 19).

3.14.2 Estimation of the Activity of Glutathione peroxidase

Glutathione peroxidases (GPx) comprise a family of enzymes that scavenge peroxides. Eight known isoforms exist; however, only five isoforms contain selenocysteine and are capable of catalyzing the reduction of hydrogen peroxide and lipid hydroperoxides using glutathione (GSH) as a reducing cofactor. Of these, plasma glutathione peroxidase (GPx-3) is the only known selenocysteine-containing extracellular antioxidant isoform (Ottaviano et al., 2009). Plasma glutathione peroxidase is also one of the best known selenoprotein biomarkers that have been widely used in discriminating selenium status of the population (Reszka, 2012). GPx activity reaches a saturation state as selenium intake increases, thus serving as an index of selenium status in populations with low intake (Gropper et al., 2008). In the present context, plasma GPx is of great interest due to the twin lacunae, the one of prospective selenium exposure and the other of possible elevation in oxidative stress. Levels of plasma glutathione peroxidase activities of the participants were assayed using a colorimetric kit procured from Cayman Chemical USA (Appendix 20).

3.14.3 Estimation of the Activity of Thioredoxin reductase

 Thioredoxin reductase is a selenoprotein with three isoforms. All three isoenzymes of mammalian thioredoxin reductases contain an essential selenocysteine residue. Thioredoxin reductase has a large number of functions in DNA synthesis, defense against oxidative stress and apoptosis or redox signalling with reference to many diseases. Plasma levels of thioredoxin reductase have been used as a marker of inflammation (Holmgren and Lu, 2010). Thioredoxin reductase was estimated in the plasma of the participants using a colorimetric kit procured from Cayman Chemical, USA (Appendix 21).
3.15 Assessment of Genotoxicity in Blood

Occupational exposure to genotoxic agents is often associated with an elevated risk for DNA damage. Most exposures in an occupational setting are chronic or repeated and the induced DNA damage might be expected to be elevated above baseline levels (Garaj-Vrhovac and Kopjar, 2009). Comet assay has been found to be a sensitive method for human biomonitoring for detection of DNA damage and useful tool for detection of genetic damage at the individual cell level. Comet assay combines the simplicity of biochemical techniques for detecting DNA single-strand breaks (strand breaks and incomplete excision repair sites), alkali-labile sites and cross-linking, with the single-cell approach typical of cytogenetic assays (Costa et al., 2011).

Use of whole blood samples for the determination of DNA damage is judicious, since valuable time and resources are saved from the separation of peripheral blood lymphocytes (Al-Salmani et al., 2011). In the present study, 10 % Dimethyl sulfoxide was added as preservative to 100 µl of whole blood and stored at -80°C for single cell gel electrophoresis (SCGE). SCGE was carried out by the protocol of Dhawan et al. (2009) – (Appendix 22).

Two kinds of comet scoring are in common use, visual scoring and computer based image analysis. Comets are assigned to one of five classes according to the perceived intensity of the comet tail in visual scoring. Values of 0–4 are given according to the damage class, the overall score for 100 comets will be between 0 and 400 arbitrary units. The alternative method is computer based image analysis, which gives a value of percentage DNA in the tail (among other parameters) for each comet. The image analysis can be either semi-automated, where a gel is scanned and comets selected for analysis by the operator, or automated, with no involvement of the operator in selecting comets. In terms of objectivity, all three types of comet scoring are equally acceptable (Azqueta and Collins, 2011). In the present study, comet scoring was carried out by the semi-automated method using a free software viz., Auto comet score (Tritek Corporation, VA) following the manufacturers guidelines. Hundred comets were scored for each sample.
3.16 Metabolomic Analysis of Exhaled Breath Condensate

Exhaled breath condensate (EBC) is a simple, non-invasive and useful matrix to study the biochemical and inflammatory molecules in the airway lining fluid. Metabol(n)omics appears to be an important tool to gain qualitative and quantitative information on low-molecular weight metabolites present in cells, tissues and fluids (Sofia et al., 2011). Epidemiological metabonomics is likely to be one of the key new omics areas to assist translational research in the near future (Ala-Korpela et al., 2011).

Nuclear magnetic resonance (NMR) spectroscopy can provide biochemical profiles of metabolites in biological samples (de Laurentiis et al., 2008). NMR-based metabonomics can be used to analyze EBC samples from adults, allowing a clear-cut separation between healthy participants and patients with airway disease (Sofia et al., 2011).

In the present study, exhaled breath condensate samples stored at -80°C were lyophilized to 500 µl. The lyophilized samples were transported on dry ice to the H-NMR facility and 1D $^1$H NMR spectra was recorded on Bruker AV III 500 MHz FT NMR (Appendix 23). Spectra were water suppressed.

Samples were clustered into 2 groups (normal or obstructed / restricted) according to lung function. Data was fed into Microsoft excel in the form of peak signals and intensities. Data was analysed by Metaboanalyst 2.0 pattern recognised software. Peak signals of each sample was normalised by sum. Data were log transformed. Auto scaling was carried out by mean-centering and dividing by the standard deviation of each variable. Partial Least Squares – Discriminant Analysis was used to discriminate between normal and diseased participants (Pears et al., 2005).

3.17 Air Quality Monitoring in Photocopier Units

The quality of indoor air inside offices, schools and workplaces is important not only for comfort of the workers but also for their health. Poor indoor air quality has been tied to symptoms like headaches, fatigue, trouble concentrating and irritation of the
eyes, nose, throat and lungs. Also, some specific diseases have been linked to specific air contaminants or indoor environments. In addition, some exposures do not cause immediate symptoms but can lead to cancer after many years (http://www.osha.gov/SLTC/indoorairquality/). Hence, in the present study, ambient air quality was monitored in five selected photocopier units during a work day to assess the levels of exposure of photocopier workers to indoor air pollutants (Plate 2).

**Experimental Procedure**

**Plate 2**

Ambient air quality monitoring in a photocopier unit

The following parameters were assessed in the photocopier units by the methods indicated:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method</th>
<th>Appendix no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon monoxide (CO)</td>
<td>Electrochemical sensor</td>
<td>-</td>
</tr>
<tr>
<td>Nitrogen dioxide (NO₂)</td>
<td>IS 5182:P6:2006</td>
<td>Appendix 24</td>
</tr>
<tr>
<td>Ozone (O₃)</td>
<td>IS 5182:P9:1974</td>
<td>Appendix 25</td>
</tr>
<tr>
<td>Fine particulate matter (PM₂.₅)</td>
<td>IEPA 40 CFR : P50 : App. L</td>
<td>Appendix 26</td>
</tr>
</tbody>
</table>
3.18 Statistical Analysis

The results were analysed by SPSS 16.0 statistical software package. Non parametric comparisons were done by Chi square test or Fisher’s exact test. Data was tested for normal distribution using Shapiro Wilk test. Normal data were compared using student’s ‘t’ test. Non normal data were compared using Mann Whitney test. Correlations between variables were carried out using Spearman’s rank correlation. Significance is reported at p<0.05.

Pack years of smoking is calculated as average number of cigarettes per day / 20 x number of years smoked. To study the interaction between cigarette smoking and photocopier exposure, a new variable (PE x PY) was created by multiplying photocopier exposure/1000 and pack years smoked.

Wherever p values are mentioned in the tables for the statistical analysis namely Fisher’s exact test, Student’s ‘t’ test, Chi square test, Mann Whitney test, Spearman’s rank correlation p<0.05 indicates significant difference/correlation.