3

Experimental Procedure

Indoor air quality is a crucial factor that determines the health effects of workers in office environment with modern office equipments. Deducing human health in such an office environment is no simple matter, since the workplace environment is dynamic and complex with many office equipments. Adverse health effects caused by occupational exposure to particulate matter and toxic emissions from office equipments (Nakadate *et al*., 2006; McGarry *et al*., 2011) have been the root cause of surveillance and epidemiologic research in a multitude of exposure studies.

Photocopier industry being forerunners of servitization firm (Visintin, 2014) not only has a huge potential of employment to service engineers in maintenance and repair of the machines but also provides scope for many small scale service entrepreneurs. Maintenance, repair and renovation of electronic equipment is a lucrative business with small investment among technicians. In India, photocopy operation and maintenance is the source of livelihood of many families. As the source of investment, space requirement being less with higher returns, many educates have started to become service technician and are employed in revamp of photocopier machines.

Service personnel as part of their routine tasks clean and recondition the photocopier machine and fill toners without any personal protection. Hence, they are more prone to toner exposure and photocopier emissions via both dermal and inhalation routes. According to Pingle, (2005) in India with 22% of the workforce employed in the service sector, there is a need to improve the delivery of occupational health services even in the non-manufacturing sectors which is equally important in comparison to manufacturing sector that constitutes 14% workforce distribution.This could be achieved through air quality monitoring of the work place and health surveillance of the workers.

The experimental design of the present study is to monitor the ambient air quality of the photocopier units and to assess the effects of long term exposure to pollutants on the health of the service personnel in these photocopier units. The experimental procedurepertaining to the present study entitled "Air quality monitoring and health surveillance of photocopier service personnel in xerographic units" was performed in two phases:

Phase I

3.1 Ambient Air Quality Monitoring

3.1.1 Particulate Matter

3.1.2 Carbon monoxide

3.1.3 Nitrogen dioxides

3.1.4 Sulphur dioxide

3.1.5 Ammonia

3.1.6 Ozone

3.1.7 Volatile Organic Compounds

3.1.8 Benzene

3.1.9 Benzo (a) pyrene

3.1.10 Heavy Metals

3.2 Characterisation of Toners

Phase II

3.3 Health Surveillance of the Photocopier Service Personnel in Photocopier Units

3.3.1 Study Area

3.3.2 Study Design

3.3.3 Selection Criteria of the Participants

3.3.4 Collection of Demographic Data

3.3.5 Anthropometric Measurements

3.3.6 Measurement of Blood Pressure

3.3.7 Lung Function Test

3.3.8 Sample Collection

3.3.9 Complete Blood Count Profile
3.3.10 Random Blood Sugar Test
3.3.11 Systemic Biomarkers – An invasive approach
3.3.12 Excretory Biomarkers-A non-invasive metabolomic approach

3.4 Statistical Analysis

Phase I

3.1 Ambient Air Quality Monitoring

The quality of the indoor air in offices is influenced by changes in ventilation technology, building and carpeting materials, prevalence of construction related moisture problems and new office machinery. Among the office machinery, especially photocopiers and laser printers have deserved special attention, due to their build-up of heat load and they also bring in new organic and inorganic effluents (Tuomi *et al.,* 2000). Modern office conveniences emit harmful pollutants namely ozone, volatile organic compounds and sub micron sized respirable particles while in operation or through maintenance and service with the usage of cleaning agents (Burroughs and Hansen, 2004). Hence, the service professionals working with photocopiers are exposed to indoor air pollutants 8 -10 hours a day at different work sites as part of their every day work. Ambient air quality monitoring in these photocopier units is therefore essential to assess their exposure in this environment and their impact on health.

Ambient air quality was monitored in a subsample of twelve photocopy centers in Coimbatore as per Indian Standard Guidelines (IS 5182) in order to assess the impact of the photocopiers on the indoor air quality. This was done during March 2012 in order to assess the real field impact of the photocopiers on the indoor air quality.

Comment [K1]: Started as new paragraph

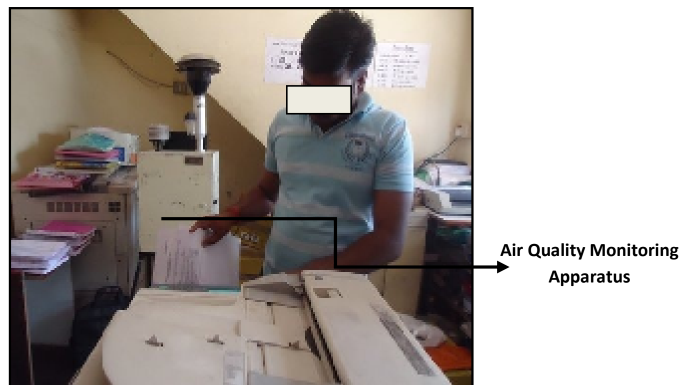
A questionnaire related to air quality assessment was prepared and information obtained from the proprietors that included basic details of the photocopier centre namely business working hours, environmental conditions that detail the room dimensions, building material (roof, wall and doors), room

style: number of entrances, types and modes of ventilation, number of photocopier machines, number of copies made per day, toner type, brand and category (dry / wet) used, furniture available, other VOC emitting sources as detailed in Appendix 1.

Air quality measurements were made on working days for a period of 8 hours by an International Organizational for Standardization (ISO 2001) certified commercial laboratory after obtaining consent from the proprietor of the photocopier units. Air sampling equipment was positioned directly opposite to the standing photocopier machines in all the centres (Plate 1). Table 2 lists the air quality parameters assessed and their assessment methods.

Plate 1

Ambient Air Quality Monitoring



3.1.1 Particulate Matter

Particulate Matter (PM) is defined as a suspended mixture of solid and liquid particles of various sizes, composition and origin as per the report of WHO, 2003). The measurement of particulate mass concentrations reflects the amount of the air pollutants inside the photocopier shops. It is widely accepted that PM includes crude components and indicators of toxicologically relevant pollutants. Thus, the local mix may influence the toxicological potency of PM, (Englert, 2004).

Table 2

List of air quality parameters assessed in the study

Air Quality Parameters	Sampling Air Flow	Methodology	Appendix No.
Particulate Matter (2.5 μ)	1000 L / minute for 8 hour	Gravimetric method (BIS 5182: P4, 2005)	2
Carbon monoxide (CO)	40 ml / minute for 1 hour	Silico molybdate method (BIS 5182: P10,1999)	3
Nitrogen dioxide (NO_2)	200 ml / Minute for 4 hour	Modified Jacobs and Hochheiser method (BIS 5182: P6, 2006)	4
Sulphur dioxide (SO_2)	1 L / minute for 30 minute	West and Gaeke method (BIS 5182: P2, 2001)	5
Ammonia (NH_3)	1 L / miute for 1 hour	Indophenol method (Lodge, 1988)	6
Ozone	1 L / minute for 1 hour	Chemical method (BIS 5182: P9, 1974)	7
Benzene	1.2 ml / minute	Active sampling by adsorption onto activated charcoal tube, desorption by carbondisulphide followed by Gas Chromatographic method (BIS 5182: P11, 2006)	8
Benzo (a) pyrene	1.2 ml / minute	Active sampling by adsorption onto activated charcoal tube, desorption by carbondisulphide followed by Gas Chromatographic method (BIS 5182: P12, 2004)	9
Heavy metals (Arsenic, lead, Nickel)	1L / minute for 8 hour	Atomic Absorption Spectroscopy (BIS 5182: P22, 2004); (USEPA – IO 3.2); (APHA, 1998)	10

3.1.2 Carbon monoxide

Carbon monoxide is one of the important organic gaseous pollutants that should be monitored for its indoor air level (http://www3.epa.gov/airquality /carbonmonoxide/).

3.1.3 Nitrogen dioxide

Gaseous pollutant nitrogen oxides are emitted as nitric oxides that rapidly react with ozone or radicals in the air forming nitrogen dioxide (Kampa and Castanas, 2008). According to Brown (1999), small pollutant emissions of nitrogen dioxide were observed from dry process photocopiers but were difficult to interpret and a procedure for assessing the same was recommended.

3.1.4 Sulfur dioxide

Sulfur dioxide (SO_2) is one of the group of highly reactive gases known as “oxides of sulfur.” It is one of the common pollutants in air. SO_2 is linked with a number of adverse effects on the respiratory system. (http://www3.epa.gov/ airquality /sulfurdioxide/#).

3.1.5 Ammonia

Anhydrous ammonia is a colourless nitrogen containing gas with a strong odour. It is generally used as a fixative in photocopiers. Anhydrous ammonia and ammonium hydroxide have an affinity for mucous membranes that lead to liquefactive necrosis and full tissue destruction. It is directly corrosive to airways at concentrations of 1000 ppm and above (Vijayan *et al*., 2011)

3.1.6. Ozone

Ozone, a highly reactive, unstable gas is produced from office equipments including photocopier machines and laser printers (Weschler, 2000) either when a paper and photocopier receptor are inserted or discharged or due to UV lamp operation during photocopying process (Brown, 1999).

3.1.7 Volatile Organic Compounds

Indoor organic contaminants are conventionally classified by volatility. Volatile organic compounds (VOCs) have boiling points from <0°C to 240–260°C and are present in the gas phase at typical indoor concentrations. Semi volatile organic compounds (SVOCs) have boiling points from 240–260°C to 380–400°C, partitioning between the gas phase and the particulate phase under typical indoor conditions. (Zhang and Smith, 2003). In general, chronic health effects of VOCs can be classified as either non-carcinogenic or carcinogenic. Their presence in the gaseous phase of the indoor air and their adverse health effects makes them an essential parameter to be identified and quantified both in the indoor and outdoor air (Ramírez *et al*., 2012)

3.1.8 Benzene

Benzene is classified as group I carcinogen, by the IARC (2012). Due to their possible health effects, benzene has been one of the chief volatile organic pollutants estimated in many photocopier-related studies (Wolkoff *et al*., 1993; Leovic *et al*., 1996, 1998; Brown, 1999; Stefaniak *et al*., 2000; Lee *et al*., 2001, 2006).

3.1.9 Benzo (a) pyrene

Benzo(a)pyrene (BaP) is a polycyclic aromatic hydrocarbon (PAH) that is a by-product of incomplete combustion or burning of organic (carbon-containing) items (ATSDR, 1995).They are semi volatile organic compounds highly hazardous to health (Samanta *et al*., 2002). All toner particles contain considerable amounts of the pigments carbon black and magnetite as well as small amounts of polycyclic aromatic hydrocarbons (Gminski *et al*., 2011).

3.1.10 Heavy Metals

The presence of metal content in the particulate matter contributes to toxicity (Kampa and Castanas, 2008).

3.2. Characterisation of Toner

Toner, a major consumable in photocopier industry is used as an indicator that would possibly identify the health hazard in the work place with photocopier machines (Lee and Hsu, 2007). Their consistent toxic emissions include VOC such as benzene, toluene, styrene, ethylbenzenes, xylenes, acetophenone, alkanes and aldehydes based on the characteristics of the toner and the fuser material (Leovic *et al*., 1996; Brown 1999; Lee *et al*., 2001).

Toner is the main component of the xerographic process to which service personnel are exposed in large proportion while refilling and in maintenance process. In refill process, they propel out used-up residual toner from cartridge for subsequent refilling (Plate 2). As part of maintenance activity they also empty the residual toner in the toner tank and cleanse the cartridge with a soft cloth and also apply organic gels and creams to wipe the roller.

Plate 2

Toner Refilling Process

The toner particle size and the morphology were studied using scanning electron microscopy (SEM) and composition of toner powders by energy dispersive X ray spectra (EDX) (Appendix 11). In particular, VOCs that possibly would emanate from toner powder were identified using head space GC- MS using the (USEPA method 8260 C) (Appendix 12), at the simulated usion temperature (180 – 200°C) of photocopier machines and matched with mass spectral library, National Institute of Standards and Technology Database (**NIST 11**).

Phase II

3.3 Health Surveillance of Photocopier Service Personnel in Photocopier Units

Health surveillance of workers is an essential task in preventive occupational health care (WHO, 2002). Information on bio-monitoring and exposure studies in photocopier units is limited among the service personnel in India. The study was conducted in photocopy shops situated in and around Coimbatore district, Tamil Nadu between the period January 2011 and December 2013 among the photocopier service personnel. The present health surveillance study was focussed to assess the impact of the exposure levels on the lungs and in the blood and urine among these workers in order to prevent and control occupational hazards and maintain their well-being.The participants were invited to participate through personal door-to-door approach. They were informed in their local language in prior about the study objectives, sample collection methods and other testing procedures before obtaining the voluntary signed informed consent forms. A copy of the same is given to them for their future reference. English version of the informed consent is attached (Appendix 13). With a good rapport created through regular visits and awareness service personnel gave their consent to participate in the study. With the appreciation and recommendation of the consented subjects further contacts were developed, to achieve the target participant population size of 100. The health surveillance programme was conducted during their course of work between 10.00 a.m and 3.00 p.m in the photocopier units.

3.3.1 Study Area

The study was carried out in Coimbatore district which is the second largest city in Tamilnadu, India. It is one among the industrially developed and commercially vibrant districts with high potential for service activities as per the brief Industrial profile of Coimbatore District compiled by MSME (2012 – 2013), Government of India. Being an educational hub with many academic institutions, Coimbatore is a home to large number of full fledged automated photocopier units operating near the academic institutions. Coimbatore was selected as the study area because of the proximity of the units.

3.3.2 Study Design

Epidemiological approach remains a key science in the study of associations between indoor air pollutants and diseases like asthma with few recommendations (Brown *et al*., 2010). A cross sectional study is an observational study that provides simultaneous snapshot of the exposure and outcome for each participant at a specific point in time (Carlson and Morrison 2009). Hence, an observational cross sectional health surveillance study was carried out to test the hypotheses whether occupational exposure to toners and emissions affect the health of the photocopier service personnel in xerographic units as they require shorter time commitment and fewer resources to conduct. A clear distinction between the exposure to toners and the emission could not be often made since exposure to either the toner or its by-product emissions are both complex health hazard in photocopier units. The crude indicator of exposure in this study is therefore occupation. The study was approved by the **Human Ethical Committee of Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore (HEC.2009.14).**

3.3.3 Selection Criteria of the Participants

- Inclusion criteria: Male workers in the age group of 18 – 50 years
- Exclusion criteria: Participants with any systemic diseases

- Exposed Group: Photocopier service professionals who were working in xerographic units with two or more years of exposure in machine maintenance
- Control group: Participants with no professional exposure to photocopier machines. Subjects in this group included shopkeepers, clerks, administrative, accountant and management executives.

Participants below 18 years were excluded from the study due to ethical constraints. Participants above 50 years are a poor representative study sample for any clinical study. Hence they were excluded. Participants who had self reported systemic diseases were also excluded from the study. Occupational exposure was defined as minimum of 2 years employment as photocopier service personnel. Participants who voluntarily consented to take part in the study and fulfilled the selection criteria were included in the study (Figure 11)

A total of 481 participants were approached to take part in the study. The number of participants who accepted to take part was 313. They completed the interview schedule. But 100 were disqualified. Thirty six participants withdrew from the study (Figure 12). Assessment of lung function test was carried out on participants (100 – service personnel and 77 – controls). Blood samples could not be obtained from 14 participants in the study. Systemic biomarkers were assessed in 90 service personnel and 73 controls and excretory biomarkers were assessed in 17 service personnel and 23 controls. After enrolment, the study protocol consisted of two parts: (1) interviewer administered questionnaire followed by (2) health surveillance.

Figure 11

Study Design

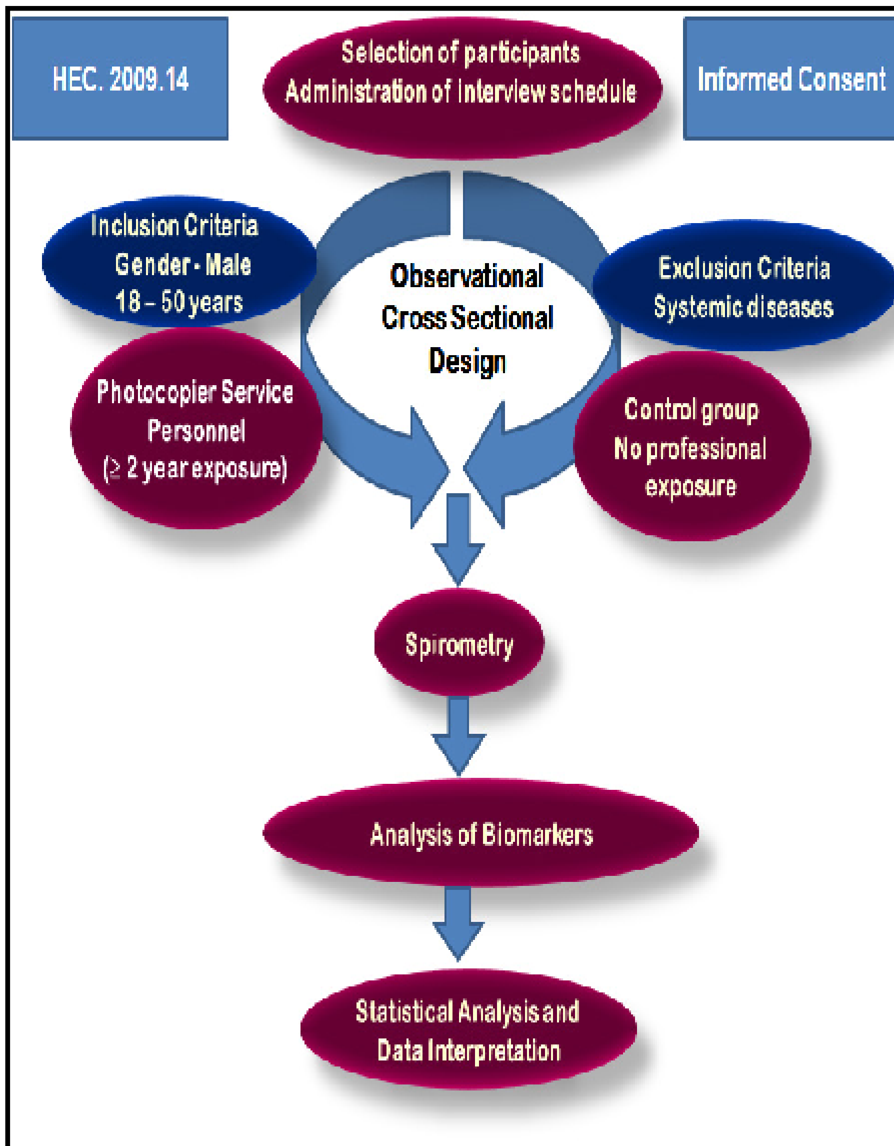
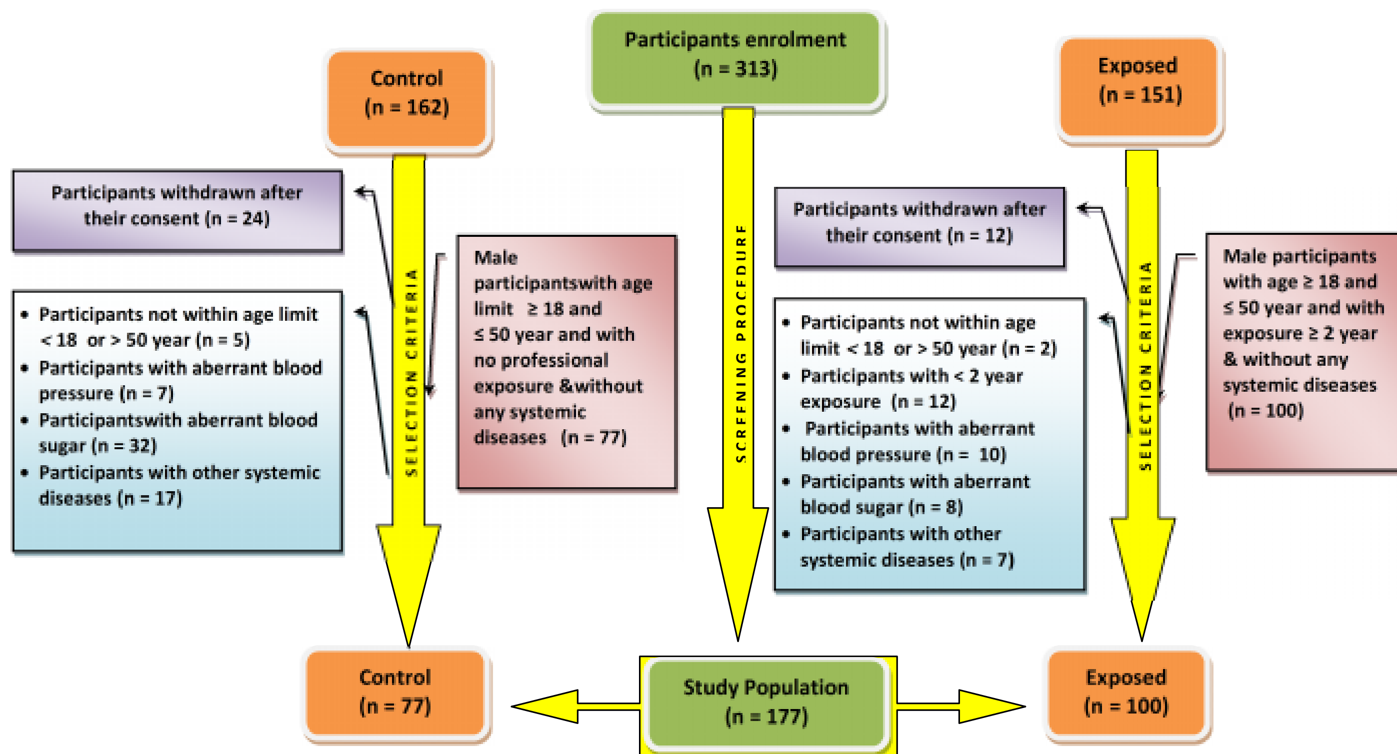


Figure 12

Study Population



3.3.4 Collection of Demographic Data

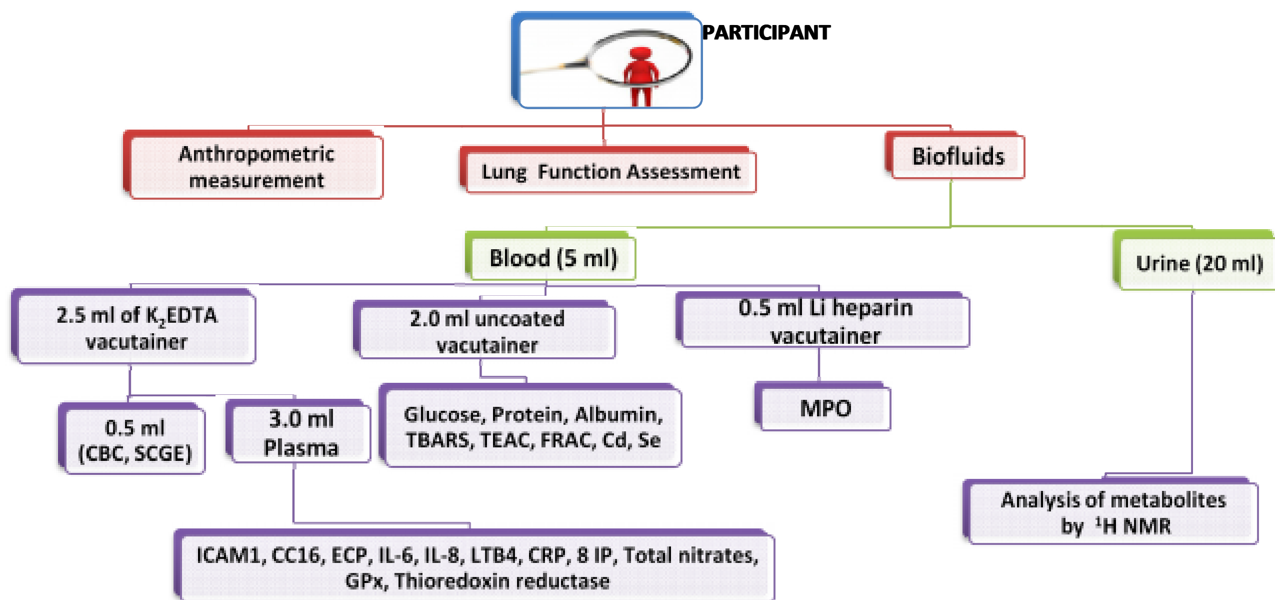
Questionnaires are frequently used in the exposure assessment of occupational and environmental epidemiological studies (Nieuwenhuijsen, 2005). This when administered by an interviewer is called interview schedule.

Interview schedule is a list of questions, open or closed prepared for use by an interviewer in person to person interactions. This is preferred for its data consistency and comparability and is a structured research data collection tool (Kumar, 2011). It was considered suitable for this study, since through this mode of face-to-face interview, the data obtained is complete with higher response rate and establishes a good rapport with the participant. The interviewer collected personal, socio economic, occupational, smoking history and general health details inclusive of medical history and current medication from all the participants.

The participants were also interviewed for their respiratory symptoms using a modified questionnaire based on St. George's respiratory questionnaire (Jones *et al*., 1992). Occupational history includes the number of working hours, work days and years in photocopie rmachine service and maintenance based on which the cumulative hours of exposure was calculated. The smoking history of the participant includes the number of years of smoking, number of cigarettes they smoked, their current smoking status on which they were categorized as ever smokers and non smokers. A copy of the interview schedule is presented in Appendix 14.

The participants underwent anthropometric measurements, blood pressure measurement and lung function tests. Consequently 5 ml of venous blood was collected to analyse complete blood count and assess the levels of biomarkers followed by collection of 20 ml of urine for metabolomics study as indicated in the flow chart (Figure13).

Figure 13. Flow chart showing the health surveillance programme among the study participants



K_2EDTA: Potassium Ethylenediaminetetraacetate; Li: Lithium; CBC: Complete Blood Count; SCGE: Single Cell gel Electrophoresis; TBARS: Thiobarbituric Acid Reactive Substances; TAOC: Total Antioxidant Capacity; FRAC: Ferric Reducing Antioxidant Capacity; Cd: Cadmium; Se: Selenium MPO: Myeloperoxidase; ICAM1 1: Inter Cellular Adhesion Molecule 1; CC16: Clara cell Protein 16 K Da;ECP: Eosinophilic Cationic Protein; IL: Interleukins; LTB4: Leukotriene B4; CRP: C Reactive Protein; 8 IP: 8 Isoprostane; Gpx: Glutathione peroxidise;; NMR: Nuclear Magnetic Resonance Spectroscopy

3.3.5 Anthropometric Measurements

Anthropometry is the study of the measurement of the human body in terms of the dimensions of bone, muscles and adipose fat tissues (NHANES III, 2007). Anthropometric measurements namely height in centimetres (measured using stadiometer without shoes in standing position, with the head in upright position, heels put together and with calve buttocks, heels and back touching the stadiometer), weight in kilograms (recorded without shoes and minimal clothing with the head in upright position using electronic weighing balance) and Body Mass Index in kg/m^2 (calculated by dividing weight by height in meter square) as given according to World Health Organisation (WHO, 1995).

3.3.6 Measurement of Blood Pressure

Blood Pressure was measured using Sphygmomanometer and represented as millimeter of mercury (mm Hg). Both systolic and diastolic blood pressure was noted to exclude those participants with aberrant blood pressure according to the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (Chobanian *et al*., 2003).

3.3.7 Lung Function Test

Lung function was assessed using a portable battery operated calibrated spirometer **(Vitalograph Alpha 6000, UK).** It was performed for every participant by a trained researcher as per the recommendations of American Thoracic Society/ European Respiratory Guidelines. The participants were called in batches of 2 - 3 for this test. Hence, when the demonstration and training was given for one participant the others could get familiarised with the procedure. They were asked to blast the air from the lungs through the mouthpiece in sitting position and digitally the manoeuvre of the lung function parameters was recorded. The test was repeated at least thrice and up to a maximum of eight forced expiratory manoeuvre were recorded in an effort to obtain the best flow volume curve which ever, is earlier based on the comfort of the participant. The lung function parameters were obtained from the printer, attached to the

Spirometer. It included the Vital Capacity (VC), Forced Vital Capacity, Forced Expiratory Volume in 1 second (FEV_1), the ratio of Forced Expiratory Volume in 1 second to Forced Vital Capacity (FEV_1/FVC), Peak Expiratory Flow (PEF) and Peak Inspiratory Flow (PIF). All these parameters were expressed as percentage of predicted corresponding values based on age, sex, height, weight and smoking status for Asians (Quanjer *et al*., 1993). Airflow limitation was based on two alternative standards, using FEV1/FVC < 0.7 as a common definition of Chronic Obstructive Pulmonary Disease COPD) and FEV1/FVC < lower limit of normal (LLN) calculated for age and sex.

3.3.8 Sample Collection

Blood and urine samples were collected from the willing participants for the current study to assess both systemic and excretory biomarkers in addition to the routine complete blood count profile and random blood sugar test.

5 ml of venous blood was collected from the participants by venipuncture and was dispersed into three different vacutainers for further assays with the following composition: 2.5 ml of blood into K_2 EDTA, 0.5 ml into lithium heparin containing vacutainers for plasma separation and 2.0 ml into uncoated vacutainers for serum separation. The tubes were subsequently centrifuged at 3000 rpm for 10 minutes at 4°C for plasma and serum separation. All these were aliquoted into labelled cryovials and stored at - 80°C until analysis.

Morning spot urine (20 ml) were collected from willing participants during work hours and stored in urine containers with sodium azide as preservative. They were sealed and frozen immediately at - 80ºC until analysis.

3.3.9 Complete Blood Count Profile

A complete blood count is a basic health-screening test in diagnostic laboratories (Kościelniak *et al*., 2015). It is a starting point for most medical investigations. It not only tests abnormalities of the blood but, as blood travels throughout the whole body it also gives an indication of disease present in other

vital organs (http://www.myvmc.com/investigations/full-blood-count-fbc-full-blood-test-or-complete-blood-count-cbc/).

Complete blood count was analyzed by Sysmex KX-21 three part analyser (TRANSASIA, Japan) in whole blood. Complete blood count metrics include: white blood cell count (WBC), lymphocytes count, neutrophils count, red blood cell distribution width (RDW), platelets, red blood cell count (RBC), haemoglobin (Hb), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet distribution width (PDW), mean platelet volume (MPV) and platelet large cell ratio (P-LCR).

3.3.10 Random Blood Sugar Test

Diabetes is a common and costly chronic disease that is increasing in prevalence and imposes a significant public health burden in recent days of modern life style intervention (Engelgau *et al*., 2000). India also being a diabetic capital of the world makes screening for random blood sugar an essential criteria for the study. It was assessed in the participants to exclude those with diabetes mellitus from the study.

Random blood glucose levels were estimated based on glucose oxidase peroxidase method (Trinder, 1969) in the serum of the participants using kit method (Agappe diagnostics, India) (Appendix 15).

3.3.11 Systemic Biomarkers – An invasive approach

Biological markers (biomarkers) are defined as “cellular, biochemical or molecular alterations that are measurable in biological media such as human tissues, cells, or fluids (Hulka, 1990). They are potentially useful tools for occupational health and safety research, practice and policy (Schulte and Hauser, 2012).

They are classified as 1) biomarkers of exposure that involve measurement of parent compound, metabolites and reflect the dose of exposure,

2) biomarkers of effect that are a measurable biochemical, physiological and behavioural alteration within an organism that can be recognized as associated with an established or possible health impairment or disease and 3) biomarkers of susceptibility that indicate an inherent or acquired ability of an organism to respond to specific exposure (Manno *et al.,* 2010).

The goal of occupational biomarker is practical to apply relevant and valid biomarkers of exposure, effect and susceptibility to worker populations at risk of exposure to hazardous substances (Christiani, 1996).

A crucial step in the health risk assessment process is to demonstrate that individuals have indeed been exposed to hazardous substances by showing evidence of a body burden from exposure. These biomarkers may range from the demonstration of chemicals and chemical metabolites in body fluid to the formation of chemical specific adducts on to cellular macromolecules (Au *et al*., 2005). Hence, identification of pragmatic biomarker in blood, an invasive approach is vital as it would explore further, the speculated exposures prevalent among the service personnel in the photocopier units and thereby achieve the goal of human risk assessment (i.e) protection of human health.

The genotoxicity due to occupational exposure of photocopier units was assessed by alkaline comet assay in the whole blood cells. Comet assay is a useful exposure biomarker of effect in human biomonitoring studies to identify environmental genotoxins (Singh *et al.,* 1988).

Workplace atmosphere contains metals and metalloids characterized by different degree of their harmful activity. Some are very toxic (arsenic, lead and cadmium), while others are less harmful to human health (Halatek *et al.,* 2009). It is therefore essential to check these toxic metal concentrations in serum in order to prevent their bioaccumulation. They were estimated as noted in table 2 in both the participant groups in the serum to find the effect of occupational exposure to photocopiers.

3.3.12 Excretory Biomarkers – A non-invasive metabolomic approach

Urine is a ‘‘favoured’’ biofluid among metabolomics researchers. As a biological waste material, urine typically contains metabolic breakdown products from a wide range of foods, drinks, drugs, environmental contaminants, endogenous waste metabolites and bacterial by-products (Bouatra *et al*., 2013).

Metabolomics 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). Nuclear magnetic resonance (NMR) spectroscopy is a commonly used analytical method to analyze the small molecule composition, that is, the metabolome, of body fluids such as urine and blood serum. Variations in metabolite concentrations have been associated with the biochemical status of organisms and reflect changes in metabolism arising from biologic conditions, including disease and response tochemical treatment (Tiziani *et al*., 2009). A pilot studyof ^{1}H NMR metabolomics approach was used to analyse the metabolic fingerprint of the urine to identify the occupational exposure biomarker among service personnel as explained in Table 2.

In metabolic biomarker discovery, an important issue is how to extract relevant information from the metabolic profile of the biofluids. All data are highly multivariate. Therefore, the use of chemometrics is required to find trends or significant information in the data i.e. relevant metabolites (Smolinska *et al*., 2012). Metaboanalyst (2.0) online web server version was used to to facilitate urine metabolomic data processing and statistical analysis (Xia *et al*., 2012).

The biomarkers that were assessed in the study are enlisted in Table 3 (Appendix 16 – Appendix 35).

Table 3

Biomarkers assessed in the health surveillance programme among the study participants

Name of the biomarker	Biofluid*	Importance	Method of analysis	Reference	Appendix
Total Protein	Serum	Inflammatory marker	Biuret Method (CPC Diagnostics, India)	Gornall *et al.*,1949	16
Albumin	-do-	Negative acute phase protein marker (Jain *et al.,* 2011)	Bromocresol Green Method (CPC Diagnostics, India)	Doumas *et al.*, 1971	17
Globulin	-do-	Positive acute phase protein marker (Jain *et al.,* 2011)	Bromocresol Green Method (CPC Diagnostics, India)	Doumas *et al.*, 1971 Globulin = Total Protein – Albumin	17
Lipid peroxides	-do-	Oxidative stress marker (Niki, 2008)	Thiobarbituric Acid Reactive Substances (**TBARS**), Colorimetric Method	Jentzsch *et al.*, 1996	18
Free 8 Isoprostane	Plasma	-do-	Competitive Enzyme Immuno Assay (EIA) Kit Method, (Cayman Chemical, USA)	Pradelles *et al.*, 1985	19
Total Antioxidant Capacity (TAOC)	-do-	Measure of aqueous and lipid soluble antioxidants (Pinchuk *et al.,* 2012)	Trolox Equivalent Antioxidant Capacity (**TEAC**),Colorimetric Kit Method (Cayman chemical, USA)	Miller *et al.*,1993	20
Ferric Reducing Antioxidant Capacity (FRAC)	-do-	Measure of aqueous antioxidant power (Pinchuk *et al.,* 2012)	Ferric Reducing Antioxidant Capacity, Colorimetric Method	Benzie and Strain, 1996	21
Clara Cell Protein (CC16)	-do-	Peripheral lung biomarker of pneumotoxicity (Hermans and Bernard, 1996) (Broeckaert *et al.*, 2006)	Sandwich ELISA Kit Method , (USCN, China)	Dierynck *et al.*, 1995	22
Leukotriene B4 (LTB -4)	-do-	Inflammatory lipid mediator (Seggev *et al.*, 1991)	Competitive EIA Kit, (Cayman Chemical, USA)	Pradelles et al., 1985	23

Table 3 (contd...)

Biomarkers assessed in the health surveillance programme among the study participants

Name of the biomarker	Biofluid*	Importance	Method of analysis	Reference	Appendix
Interleukin 6 (IL - 6)	Plasma	Inflammatory marker (Wedzicha *et al*., 2000)	Sandwich Enzyme Linked Immuno Sorbent Assay (ELISA) Kit Method, (Koma Biotech, Korea)	Baroja *et al*., 1988	24
Interleukin 8 (IL - 8)	-do-	(Higashimoto *et al*., 2009)	Sandwich Enzyme Linked Immuno Sorbent Assay (ELISA) Kit Method, (Koma Biotech, Korea)	Sticherling *et al*., 1989	25
Eosinophilic Catioinic Protein (ECP)	-do-	Biomarker of Eosinophilic inflammation (Woschnagg *et al.,* 2009)	Eosinophil Cationic Protein Sandwich ELISA Kit Method, (USCN, China)	Reimert *et al.,* 1991	26
C Reactive Protein (CRP)	-do-	Positive acute phase protein, marker of inflammation (Jain *et al.,* 2011)	Sandwich ELISA Kit Method, (Cayman Chemical, USA)	Robey *et al.,*1983	27
Total Nitrates (Nox)	-do-	Oxidative stress marker (Ridker *et al.,*2004)	Greiss Method (Cayman Chemical, USA)	Nims *et al.,* 1995	28
Myeloperoxidase (MPO)	-do-	Inflammatory protein marker (Prokopowicz *et al.,* 2012)	Sandwich ELISA Kit Method, (Enzo Life Sciences, Switzerland)	Falk and Jennette, 1988	29
Intercellular Adhesion Molecule-1 (ICAM -1)	-do-	Endothelium and epithelium inflammatory biomarker (Levitt *et al.,*2009)	Sandwich ELISA Kit Method, (USCN, China) ,	Rothlein *et al.,* 1988	30

Table 3 (contd...)

Biomarkers assessed in the health surveillance programme among the study participants

Name of the biomarker	Biofluid*	Importance	Method of analysis	Reference	Appendix
Glutathione peroxidise (GPx)	Plasma	Selenoprotein antioxidant enzyme (Tapiero *et al.,* 2003)	Glutathione peroxidase, Colorimetric Kit Method (Cayman Chemical, USA),	Paglia and Valentine, 1967	31
Thioredoxin Reductase		Selenoprotein antioxidant enzyme (Tapiero *et al.,* 2003)	Colorimetric assay kit , (Cayman Chemical, USA) ,	Luthman and Holmgren, 1982	32
Trace element analysis Selenium, Cadmium	Serum	Occupational biomarker (Sengupta, 2013)	ICPOES	Nischwitz *et al.,* 2008	33
Comet Assay	Blood	DNA Damage - biomarker of exposure to xenobiotics (Garaj-Vrhovac and Kopjar, 2003)	Single Cell Gel Electrophoresis (SCGE)	Dhawan *et al.,* 2009	34
Metabolite Marker	Urine	Occupational Non-Invasive biomarker (Vulimiri *et al.,* 2012)	^{1}H NMR Spectroscopy	Ramadan *et al.,* 2006	35

3.4 Statistical analysis

The results were analysed by SPSS 16.0 statistical software package as per Figure 14. Data was tested for normal distribution using Shapiro Wilks' test. Normal data were compared using one way ANOVA (for more than two groups) and Student's t test (for two groups). Fischer's LSD was used as post-hoc test. Non normal data were compared using Chi square test, Fischers Exact test Kruskal Wallis test. LSD and Mann Whitney test were used as post hoc test. Correlations were carried out using Pearson correlation analysis for parametric data and Spearman's rank correlation for non-parametric data. Binary logistic regression was used for the dichotomous dependent variable and categorical independent variables to calculate the odds ratio to assess the causative factor of disease.

Figure 14

Statistical analysis