2

Review of Literature

The literature pertaining to the present study **"Air quality monitoring and heath surveillance of photocopier service personnel in xerographic units"** is reviewed under the following heads:

2.1 Indoor Air Pollution

2.2 Photocopiers and their Emissions

2.3 Indoor Air Emissions and its Health Effects

2.4 Indoor Air Pollutants and Lung Function

2.5 Biomarkers and Biomonitoring

2.6 Metabolomics as a Tool to Assess Occupational Exposure

2.1 Indoor Air Pollution

Pollution has been defined as the direct or indirect human introduction of substances into the environment that harms living resources, affects human health and impairs environmental quality. Atmospheric air pollution can be defined as any gaseous or particulate matter in the air that is not a normal air constituent or is not normally present in the air in high concentration (Yang and Omaye, 2009). Although a number of physical activities (volcanoes, fire, etc.) may release different pollutants in the environment, anthropogenic activities are the major cause of environmental air pollution. Hazardous chemicals can escape to the environment by accident, but a number of air pollutants are released from industrial facilities and other activities and may cause adverse effects on human health and the environment (Kampa and Castanas, 2008).

According to World Health Organization report (WHO, 2014), around 7 million people died, i.e., one in eight of total global deaths occur as a result of air pollution exposure. This finding more than doubles previous estimates and

confirms that air pollution is now the world's largest single environmental health risk. 3.3 million death is linked to indoor air pollution whereas 2.6 million deaths occur related to outdoor air pollution. In accordance with this, Hoskins (2003) stated that the pollution levels of indoors may be higher than the outdoors. Nazaroff and Goldstein (2015) opined that indoor air atmosphere gains prominence rather than outdoor air with roughly 90% of the modern human time budget is spent indoors.

According to Zhang and Smith (2003), people usually spent longer time in residences / office set up rather than in industrial settings. Electronic equipments such as photocopier machines, printers, fax machines and laser printers are more common in an office environment (Singh *et al*., 2014). The changing workplace of office setup where we live with new sophisticated multifunctional electronic devices to ease our documentation work has drawn attention and is of prime concern as far as indoor air pollutants are considered due to their indoor air emissions. The concentration of pollutants in workplace air plays an important role in occupational safety. It depends on two basic parameters: emission of a toxic substance and the volume of air that dilutes the emitted substance (Valuntaitė and Girgždienė, 2007). Hence, health surveillance among workers in non-industrial setting is essential in health risk assessment.

. According to Tuteja, (2010) the global printing industry is estimated at around $676 billion in 2014 and is growing at a rate of 5 percent. The Indian printing industry is growing at 12.2 percent with digital printing growing at around 25 percent. India produces 150 million digital pages annually printed of which a majority is in black and white. Narita and Obinata (2011) forecasted the market for electrophotography-based printers and copiers to grow at an annual rate of approximately 8%. The main difference between the photocopier centers in India and other developed countries is that in India such centers are generally small and serve as both businesses and residences. Thus, the pollutants emitted during such processes would affect the indoor air quality and potentially have adverse health effects on the employees as well as the residents of the workplace (Massey and Taneja, 2011).

Extensive research on exposure to air pollutants in both *in vitro* and *in vivo systems* concluded that there are significant differences in biological response at the molecular, cellular and whole organism level induced by various air pollutants of different origin in addition to inter-individual variations at genomic level (Holloway *et al.*, 2012).

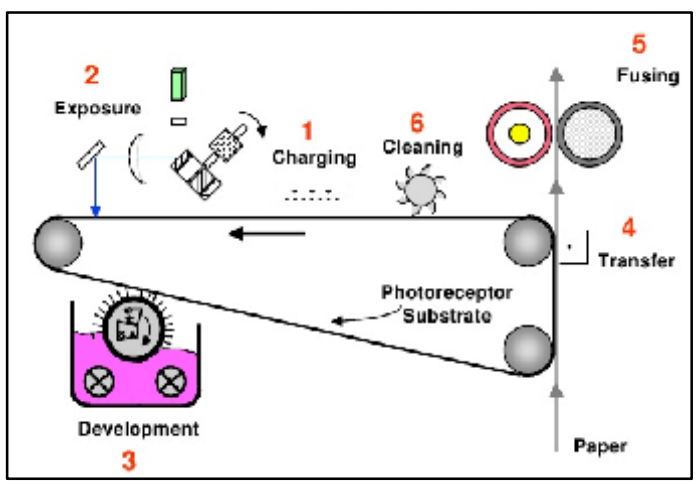
2.2 Photocopiers and their Emissions

Photocopier is the second most essential office equipment next to desktop / laptop to produce copiers of the original documents. It is based on electrostatic imaging process. Xerography, the dry ink marking process developed by the photocopy industry, has grown from nothing into a $170 billion industry worldwide. Indeed, the development of the xerographic copying and printing industry since its invention by Chester Carlson's in the year 1938 and its reality in 1959 with legendary 914 copier is one of the great applied surface science successes of all time that took dawn in modern information era in 1959 (Duke *et al.,* 2002).

2.2.1 Xerographic Process

Figure 1

Xerographic process



(Duke et al.,2002)

Xerographic process (Figure 1) consists of six process steps that begin by 1) charging a photoconductive belt or drum; 2) generating a latent image on

this photoconductor by image wise exposure to light; 3) development of this latent image with charged toner; 4) transfer of the toner image to a substrate 5) fusing of the image to that substrate and 6) the cleaning of residual charge off the photoreceptor which is needed in commercial devices to reuse the same photoreceptor for subsequent imaging (Hays, 2003).

Duke *et al.* (2002) stated that in modern digital xerographic marking engines the photoreceptor is discharged image wise by a laser beam so that the output is a latent image in which charged and discharged areas of the photoreceptor are a reflection of the image that ultimately appears on the output medium. The discharged areas correspond to black areas in a monochrome image. Gray areas are described by half tone dot patterns. Typical resolutions are 600 - 1200 dots per inch (dpi).

2.2.2 Toner

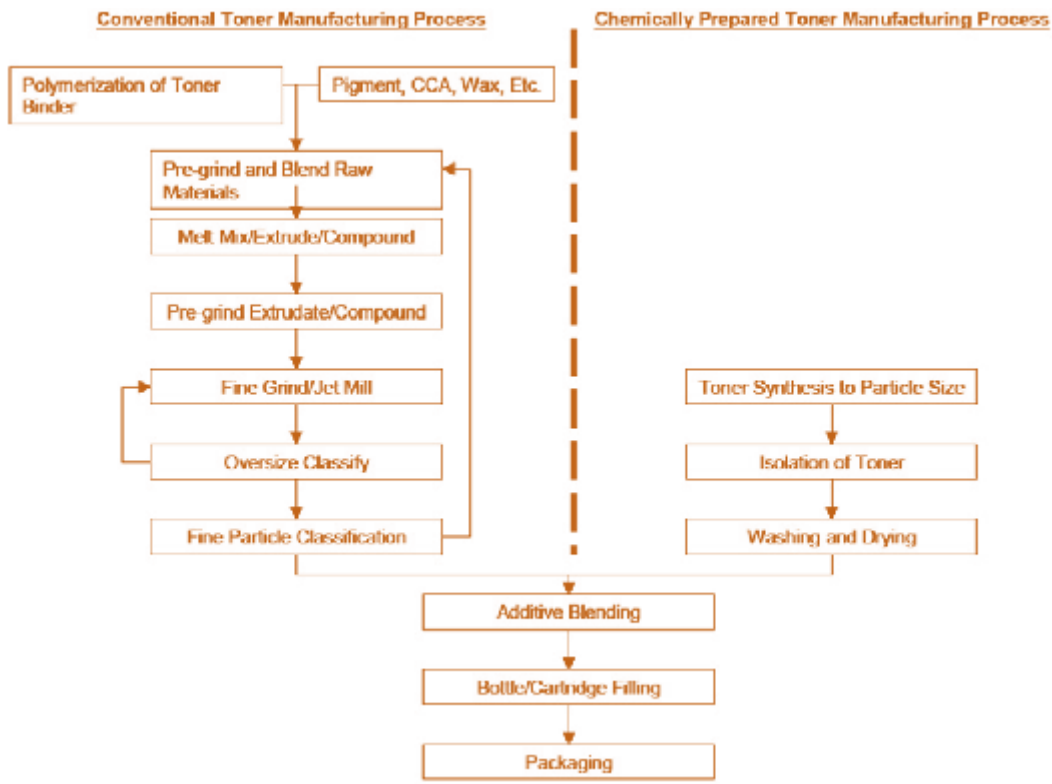
Toner is a complex multi-component composite powder, a dry ink used for xerographic printing process. Toners market was valued at USD 2.91 billion in 2013 and is likely to reach USD 4.33 billion by 2020 as per the Transparency Market Research Report (2014). Toner, as one of the largest consumables in daily office work, its demand is increasing with the popularity of printers and photocopiers. It is estimated that the global demand for toner is around 240,000–260,000 tons (Bai *et al*., 2010). There are two types of toners available in the market namely conventional toners and chemically prepared toners based on their manufacturing process outlined in Figure 2.

2.2.2.1 Toner Manufacturing Process

Toners may be produced by either top down approach or bottom-up approach or hybrid of both. According to Xerox Corporation Report (2006), the conventional method of making toners is based on a mechanical process that uses a top-down approach of physically grinding composite polymeric materials to micron-sized particles whereas chemical method utilizes sophisticated chemical design and control based nanotechnology methodology to generate nanosized particles.

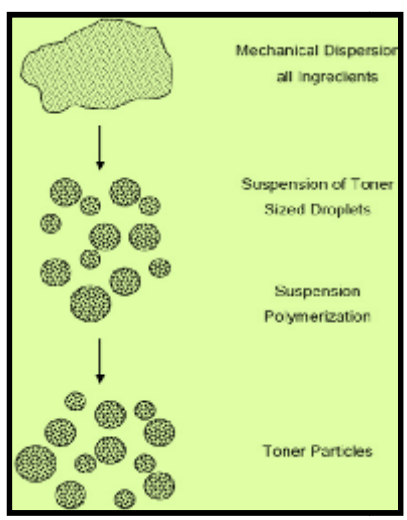
Figure 2

Outline of toner manufacturing process



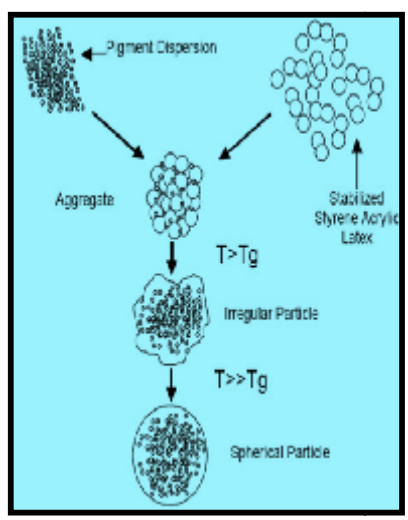
(Galliford, 2006)

Figure 3. Suspension polymerization



(Galliford, 2006)

Figure 4. Emulsion aggregation



(Galliford, 2006)

Chemically prepared manufacturing method includes suspension polymerization (Figure 3), emulsion / latex aggregation (Figure 4), polyester (elongation) polymerization and chemical milling (Galliford 2006).

Suspension Polymerization

Suspension polymerization is based on free radical polylmerization process. The polymerization is conducted at an elevated temperature and for a specific time with agitation at a specific rate. These conditions vary according to the formulation and the specification of the toner being produced. Once polymerization is complete the material is washed, filtered and dried. The dried chemcially prepared toner is then blended with extra-particulate additives such as silica.

Emulsion Polymerization / Latex Aggregation

Emulsion polymerization is a method in which, in an emulsion, monomers are diffused into micelle where free radical polymerization proceeds. The manufacturing method for toner is from two separate parts. These two parts are then mixed at elevated temperature in a low intensity mixer for a given number of hours. In this process the “Primary particles” in the form of stabilized styrene acrylic latex are formed. These non-pigmented emulsion polymerized particles are between 0.1 – 0.3 microns in size. The colorant and/or magnetite and Charge Control Agent (CCA) are added to the primary particles in their aqueous medium. Secondary particles are formed by the agglomeration of the solids in the aqueous medium. These secondary particles contain the primary particles, the pigment/magnetite and CCA. The secondary particles are 1.0 – 4.0 microns size at this stage of production. The secondary particles agglomerate further to form “associated particles” with a size range of 5.0 – 13.0 µ. Shape adjustment is conducted by control of the temperature and other conditions. The temperature of the mixed aggregate is increased to achieve glass transition temperature (Tg) which change the toner particle shape by controlling the viscosity of the heated polymer through the action of interfacial forces and surface tension. The mix is

then filtered, washed and dried yielding a pre toner ready for blending with silica as a flow and charging additive.

Polyster (Elongation) Polymerization (PxP)

Condensation Polymerization can be performed basically in two different modes. Single phase (bulk) polymerization or 2 phase (interfacial polycondensation) modes. In this process partially reacted materials called oligomers, plus other toner components are combined in a dispersing medium to prepare toner size droplets. The reaction of the materials is then completed and toner is finished by washing, filtration and drying.

Chemical Milling

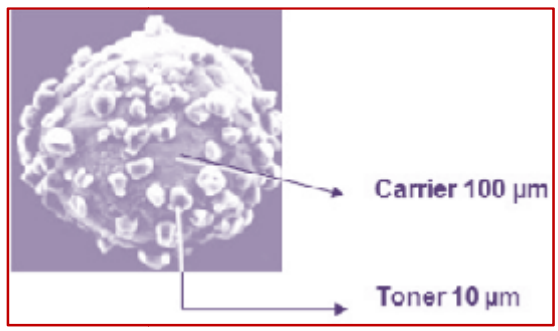
There is no polymerization step involved in this production technology. The technique is capable of using any commercially available toner resin though the preferred type would be polyester. Control of the process means that small toner of narrow particle size distribution may be produced. The particles are spheroidal and it is a feature of the process that there is the capability to control surface morphology. The degree of rough/micro-serrated texture is controllable thereby enabling the control of, amongst other parameters, charging speed and flowability.

Microencapsulation

In micro-encapsulation, the particles produced have a core/shell structure (Figure 5), with strength and thermal stability adequate to prevent attrition in any part of the filling, transportation storage or use in a print engine. The shell also controls to a great extent the tribo properties of the toner and can be manipulated to tune this property. The powder flow also depends on the shell properties in its morphology and composition. The properties derived from core include the fusing and fix properties that are determined by the melt rheologies adjusted by chemical composition and molecular weight. The core of a microencapsulated toner usually contains the colorant. The thickness of the shell or shells formed can be varied in the process giving the toner different properties.

Figure 5

Electron microscopic image of toner particle



(Duke *et al*. , 2002)

The intent is that some of the properties of the particle are derived from the core and some from the shell surrounding it. The properties derived from shell are mechanical

2.2.2.2 Toner Formulations

Toner formulations also differ between the manufacturers. Individual manufactures have their own formulae for toners while mostly they comprise around 90% thermo plastics or resins that are colored with up to 10% of pigment. Bai *et al*. (2010) opined silicon, sulphur, titanium, iron, chromium, nickel, zinc, chlorine and calcium components in toners in addition to resin component. Gminski *et al*. (2011) reported toner particles to consist of magnetite, silicon, iron, nickel, zinc, arsenic, cadmium, antimony, lead and polycyclic aromatic hydrocarbons. Barthel *et al*. (2011) reported toner as a composite of silicon, sulphur, titanium, iron, chromium, nickel, zinc, chlorine and calcium components Nelson *et al*. (2011) stated iron oxide, amorphorous silica, paraffin wax, polymethyl methacrylate, diethylene glycol and 2-pyrolidone as toner components, Whereas Bello *et al*. (2013) reported toner composition of iron, titanium, silicon, manganese, sulphur, tin, aluminium, zinc, magnesium, copper, chromium, molybdenum, nickel and long chain alkanes. Lobanov *et al*. (2009) opined that few additional components aerosil, alumina, or titanium dioxide particles in toner composition impart fluidity to toner powders. Among other

compounds that are in the bulk of the particles and determine the stability of electrostatic, magnetic and other properties of toner are magnetite nanoparticles (10 – 15 vol %).

2.2.3 Photocopiers as Source of Indoor Air Pollutants

Photocopiers are essential office equipment in office set-up. Despite their importance in document printing, reports are available that state photocopiers as potential source of indoor air pollutants (Leovic *et al*., 1998; Lee and Hsu, 2007 and Lee *et al*., 2010). Barrese *et al*. (2014) connoted that working next to a printer or copier in operation is equivalent to inhaling cigarette smoke or breathing exhaust fumes of traffic.

Photocopiers emit fine and ultrafine particulate matter (Kagi *et al*., 2007; Lee and Hsu 2007 and Lee *et al*., 2010), nano particuate matter (Khatri *et al*., 2013 and Bello *et al*., 2013), ozone (Valuntaitė and Girgždienė, 2007 ; Durga and Gokhale, 2015), VOCs (Lee *et al*., 2001; Henschel *et al*., 2001; Kowalska *et al*., 2015), UV and visible radiations (Singh *et al*., 2014), small amounts of selenium, arsenic or cadmium oxide (Bar-sela and Shoenfled, 2008).

2.2.3.1 Particulate Matter (PM)

Air pollution is a complex mixture of compounds in gaseous and particle phases, the strongest evidence from several epidemiological studies linking air pollution centers around the particulate components (Araujo and Nel, 2009).

According to Pope-III and Dockery (2006), PM is defined as an air-suspended mixture of solid and liquid particles that vary in number, size, shape, surface area, chemical composition, solubility and origin particulate matter. According to the United States Environmental Protection Agency Report (USEPA, 2004), they may be liquid, solid or solid core surrounded by liquid. It includes primary particles that are emitted directly from sources and secondary particles that are generated from gases through chemical reactions involving atmospheric oxygen (O_2), water vapor (H_2O), reactive species such as ozone (O_3), free radicals such as hydroxyl (.OH) and nitrate (.NO_3) radicals, pollutants

such as sulfur dioxide (SO_2), nitrogen oxides (NO_x) and organic gases from natural and anthropogenic sources. They are classified according to their aerodynamic diameter into size fractions such as PM_{10} ("thoracic" particles, < 10 µm), $PM_{2.5-10}$ ("coarse" particles, 2.5 to 10 µm), $PM_{2.5}$ (fine particles, < 2.5 µm) and UFP (ultrafine particles, < 0.1 µm). These particles are derived from various sources and by various mechanisms (nucleation, Aitken mode, accumulation and coarse modes).

Lee and Hsu (2007) stated that secondary organic aerosol (SOA) formation inside photocopiers might be an important source of indoor UFP during photocopying. It is formed as a result of ozone reaction with unsaturated VOCs (terpenes and styrene).

2.2.3.2 Toner Emissions

The chemical composition of airborne PM emitted by photocopiers is complex. Chamber investigations and indoor air measurements by Uhde *et al*. (2006), Kagi *et al*. (2007) and Tang *et al*. (2012) have shown that toner is one of the main factors from which fine particulate matter and submicrometer sized particles are emitted during printing process. According to Bello *et al*. (2013) PM contains major elements and classes of analytes in toner composition that includes metals namely fumed silica, iron oxide and titanium dioxide along with volatile organic compounds (VOCs), organic and elemental carbon. Barthel *et al*. (2011) found that the solid inorganic particles emitted from printers constituted about < 2% of the total number of emitted particles.

Bello *et al*. (2013) suggested that term nanoparticles could be used synonymously with the term UFP emission due to the substantial majority of photocopier emissions in three dimensions of the order of 100 nm or less. Nanoparticles emitted from printers and photocopiers are incidental nanoparticles due to their generation as unintended by-products during photocopier operation. In contrast, engineered nanoparticles are intentionally produced to fulfil desired technological functions especially toner formulations (e.g. carbon black or titanium dioxide) for high quality printing. When these toner particles become

airborne, it would result in emissions of both incidental and engineered nanoparticles.

Photocopier operations and its associated component toners add on to the risk of indoor air pollution by particulate matter emissions. Numerous other studies have also confirmed similar results (Wensing *et al*., 2008; Destalliats *et al*., 2008; Morawska *et al*., 2009; Schripp *et al*., 2009 ; He *et al*., 2010 and McGarry *et al*., 2011).

2.2.3.3 Other Associated Factors of Particulate Emissions

In addition to toner, other factors namely type of printer, paper, machine maintenance cycle and air exchange rate also affect the particulate matter concentration inside the office work area set-up as reported by Wensing *et al*. (2008); Schripp *et al*. (2009) and Tang *et al*. (2012). It was also stated by He *et al*. (2010) that fuser roller temperature variations is the strongest factor that determine the rate of particle formation in laser printer operation.

2.2.3.4 Ozone Emissions

Ozone is a colourless and odourless gas, highly unstable which presents a serious air quality problem. Barrese *et al*. (2014) opined that ozone is naturally produced in the atmosphere (the stratosphere), at ground-level, it is an air pollutant that can harm human health.

Indoor ozone is produced mostly by photocopier machines, laser printers, ozone generators and other electrical devices. It is produced during the charging and discharging of the electrostatic drum (Singh *et al*., 2014). According to Tipayarom and Tipayarom (2011), ozone is generated as by-product from the reaction of charged ions and electrons with atmospheric gases in electrophotographic process of the copier and from the corona wire (coil that serves as a positive charge in the surface of the drum of the copier). Ozone trapped in the ink destroys the charge and surface coating of the drum in the toner cartridge (Lee *et al*., 2001). However in contrast to older devices, the advanced transfer-roller-technology employed in modern laser printer and copier

for electrophotographic process instead of electrostatic charging with corona wire have reduced the amounts of ozone generation to a greater extent according to Ewers and Nowak (2006). Lee and Hsu (2007) and Destaillats *et al*. (2008) observed that even lower levels of ozone can react with other indoor air pollutants to produce secondary aerosols and ultrafine particles. Ozone is also formed when UV lamp operates during photocopying (Brown, 1999) and that ozone emission can increase between periods of routine maintenance (Leovic *et al*., 1996).

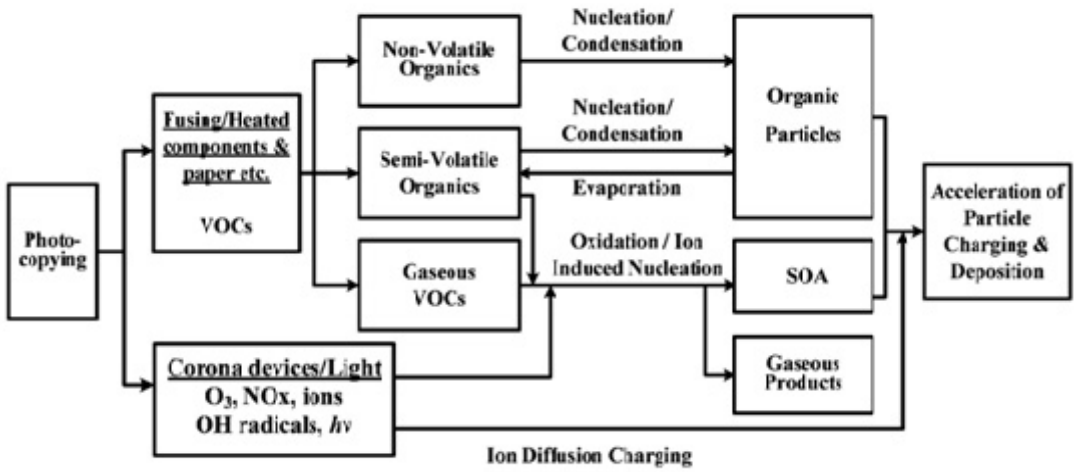
2.2.3.5 Volatile Organic Compounds (VOC) Emissions

According to Williams and Koppmann (2007), VOCs are defined as organic compounds having a vapour pressure greater than 10 Pascal, at 25°C, a boiling point of up to 260°C at atmospheric pressure and 15 or less carbon atoms. They are photochemically reactive and form a part of large family that consists of vast array of aliphatic, aromatic hydrocarbons, their halogenated derivatives, alcohols, ketones and aldehydes (Srivatsava and Mazumdar, 2011).

VOCs emission from photocopiers includes both primary and secondary origin. A conceptual model of indoor air chemistry and particle formation and removal during photocopying is presented in Figure 6. VOCs emissions of primary origin are generated as part of physical process of nucleation and condensation. They are generated by toners and inks that are subject to heating during the printing process, as well as particles of paper (Kowalska *et al*., 2015). Based on the characteristics of the toner and the fuser materials, the toners at the fuser temperature of around 200° C consistently emit VOCs such as benzene, toluene, styrene, ethylbenzene, xylenes, acetophenone, alkanes and aldehydes (Leovic *et al*., 1998; Brown, 1999; Stefaniak *et al*., 2000; Lee *et al*., 2006 and Hsu *et al.,* 2006). Volatile chlorinated compounds trichloroethylene and tetrachlorethylene possibly carcinogenic to humans have also been identified in the office indoor air with printers and copiers (Kowalska *et al*., 2015).

Figure 6

Conceptual model of indoor air chemistry and particle formation and removal during photocopying process



(**Lee and Hsu, 2007)**

VOCs of secondary origin are generated as by-product of oxidation reactions and ion-induced nucleation according to Lee and Hsu (2007). In oxidation reactions of the by-products of corona charging in photocopying process, such as ozone, NO_x and OH-radicals, act as strong oxidants for the oxidations of emitted VOCs. In ion induced nucleation reaction, (transition from gas to particle process-super saturated vapours condense on ions), caused mainly by UV irradiation inside photocopiers spaces. It initiates ion induced nucleation of the organic vapors leading to the formation of UFP and in-turn the SOA formation. Fluorescent lamp of photocopier acts as the source of UV radiation according to Brown *et al*. (2000).

According to Lee and Hsu (2007), a variety of other materials in photocopy centres such as printed documents, cleaning solvent, office furniture, building and flooring materials in addition to other office equipments might also emit chemicals into the indoor environment. The toner manufacturing process may also generate compound oxidation side products such as acetophenone, benzaldehyde, benzoic acid and phenol (Henschel *et al*., 2001). According to Lee *et al*. (2001) their concentrations of BTEXS (Benzene, Toluene,

Ethylbenzene, Xylenes and Styrene) were found to be well below their occupational exposure threshold limit guidelines in well ventilated office room with copiers. In contrast, Lee *et al*. (2006) demonstrated high exposure of BTEXS among photocopier workers in photocopy centers with a lifetime cancer risk exceeding 1 $X10^{-6}$. Regarding noncancer risk, the hazard index for all estimated cases exceeded 1.0 and benzene contributed most to the overall total risks. Hence the chronic exposures to BTEXS in typical Taiwanese photocopy centers may have cancer and noncancer health risks

2.2.3.6 Semi Volatile Organic Compounds Emissions

Semi Volatile Organic Compounds Emissions (SVOCs) are class of organic compounds with vapor pressures of 10^{-9} to 10 Pa at 25°C (77°F) which are low in comparison to VOCs (10 to 10^4 Pa).They are commonly found in gas and condensed phases, redistributing from their original source to indoor air and interior surfaces. They include alkylphenols, organochlorines, organophosphorus, compounds, phthalate esters, polybrominated diphenyl ethers, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, non-ionic surfactants and pesticides as reported by Xu and Zhang (2011).

SVOCs like the flame-retardant tri-xylyl phosphate, naphthalenes and siloxanes were identified during test chamber experiments as possible constituents of the emitted printer aerosol (Wensing *et al*., 2008). Maddalena *et al*. (2011) have extensively reviewed about indoor emission of SVOC compounds namely phthalate esters, perfluoroalkyl sulfonamaides and diethylhexaphthalate from office equipments.

2.2.3.7 Selenium Emissions

The photoreceptor is the central element of the xerographic process. This device's fundamental responsibility is the transport of the page images of a document in their various forms through the various steps of the xerographic process. It may be present either as a rigid drum or as a belt (Duke *et al*., 2002). It is basically a metal roller covered by a layer of photoconductive material which is made up of a semiconductor (O'Connell, 2001). Selenium (As_2Se_3), is a

chalcogen semiconductor which is a good photoconductor adopted in photocopying (xerographic process). Photocopier drum acts as a screen which is smeared with finely powdered selenium (Palanna, 2009). A small amount of selenium, arsenic and cadmium oxide were found in indoor air emissions of photocopier unit (Bar-sela and Shoenfled, 2008).

2.3 Indoor Emissions and its Health Effects

Air pollution has been associated with several adverse health effects that depend on the physical and chemical properties of contaminants, time and frequency of exposure. However, most information is about acute health effects of air pollution, but health effects due to chronic exposure are not well known (Linares *et al*., 2010). Accumulating epidemiological studies suggest that indoor air pollutants cause acute effect that includes respiratory symptoms, cardiovascular events, hospital admissions and mortality. Short-term effects on lung function are well documented in contrast to long-term exposures due to air pollution that have been associated with chronic bronchitis, markers of atherosclerosis, lung cancer and mortality (Götschi *et al*., 2008).

Office exposure usually involves a complex mixture of indoor air pollutants. Hence, assessment of office exposure is more essential among those individuals who are in close proximity and more susceptible to such complex mixture of indoor office air pollutants (Zhang and Smith, 2003).

According to Bernstein *et al*. (2008), in the non-occupational indoor setting, environmental exposures are often more subtle and not readily recognized. In the most extreme cases, controversial terms like sick building syndrome (SBS), toxic mold syndrome and multiple chemical sensitivity have been coined for lack of a better way to characterize unexplained constellation of symptoms that are attributed to some exposure in the home or nonindustrial occupational setting. Furthermore, very little information is available regarding permissible exposure levels for the nonindustrial workplace for known indoor air pollutants.

As far as photocopier emissions are considered, investigations by Nakadate *et al*. (2006) showed that the current exposure levels in photocopy centers may be sufficiently safe in well controlled work environments, especially if the photocopier is handled carefully. Yang and Haung (2008) supplemented this view stating that occupational exposure to pollutants emitted from photocopiers was not significantly associated with an excess of chronic respiratory symptoms and acute irritative symptoms in photocopy employees in well controlled work environments.

2.3.1 Particulate Matter

Air pollution is due to a complex mixture of compounds in gaseous (ozone, CO and nitrogen oxides) and particle phases though among them, the strongest evidence among several hundred epidemiological studies linking air pollution with human health effects, centers around the particulate components (Araujo and Nel, 2009). This is because particles contain a broad range of toxic substances and are considered reliable indicators of other pollutants (such as nitric oxides) and hence of the global adverse effect of air pollution (Coccini *et al.*, 2012).

Worldwide epidemiological studies show a consistent increase in cardiac and respiratory morbidity and mortality from exposure to particulate matter. PM is a key ingredient of polluted air and is estimated to kill more than 500,000 people each year (Nel *et al*., 2006). This also corroborates with the burden of disease data report by WHO, 2014

Exposure to fine particulate matter is mainly through inhalation (Li and Nel 2011; Jackson *et al*., 2012). However, few studies claim their entry into human systems especially of engineered nano particulates through routes other than inhalation that includes ingestion, skin and injection (Adetunji *et al*., 2009).

According to the International Commission on Radiological Protection (IRCP) (1994), model for particle deposition in the respiratory tract inhaled particles deposit in various segments of the human respiratory tract that can be grossly divided into three anatomical regions: the nasopharyngeal,

tracheobronchial and alveolar regions. In accordance with this, Pope-III and Dockery (2006) stated that PM_{10} deposits in the extrathoracic and upper tracheobronchial regions whereas ultrafine PM with aerodynamic diameter < 0.1 µm, may translocate from the alveoli to the circulatory system. $PM_{2.5}$ can be inhaled more deeply into the lungs, with a portion depositing in the alveoli and entering the pulmonary circulation and presumably the systemic circulation according to Sun *et al*. (2010).

Araujo and Nel (2009) reports that UFP PM are more toxic and dangerous in exertion of prooxidative effects and activation of molecular pathways leading to inflammation in comparison to other particles of PM due to their smaller size, higher particle number concentration, greater bioavailability due to large surface area / volume ratio, larger content of redox cycling organic chemicals and greater lung penetration and higher airway deposition with more pro-oxidant and pro-antherogenic effect. Studies by Nel *et al*. (2006) and Xia *et al*. (2009) also concurred with this report.

There are various reports available that outline the different mechanisms of disease pathogenesis on exposure to UFP PM. All these epidemiological studies in common link oxidative stress and inflammation (local and systemic) to PM exposure.

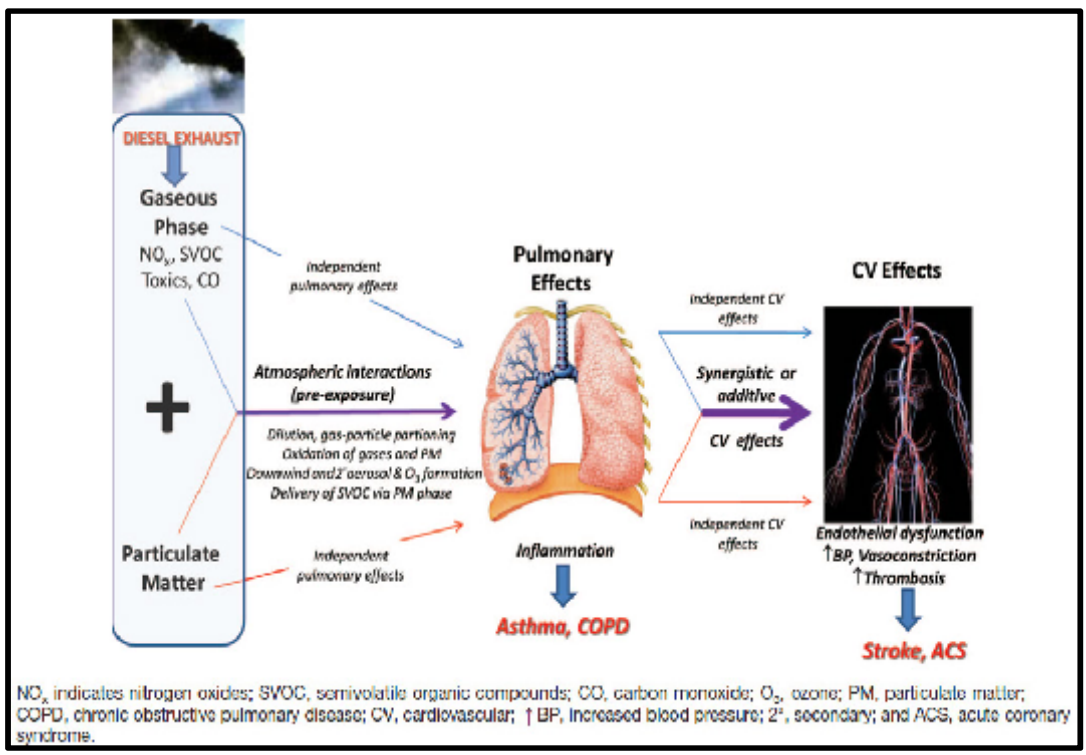
Jackson *et al*. (2012) concluded that inflammation is the prime reason for biological and pathological consequences that results in oxidative stress, DNA damage, fibrosis, cardiovascular events and lung diseases on pulmonary exposure to nanoparticulate matter where as Emmerechts and Hoylaerts (2012) postulated that three pathways lead to cardiovascular effects primary of which is interference with the autonomic nervous system followed by translocation of UFP into systemic circulation with latter pulmonary inflammation associated oxidative stress.

It was proposed by Xia *et al*. (2009) that induction of oxidative stress might be the key injury disease pathogenesis mechanism that relates to pro-inflammatory and pro-atherogenic effects of UFPs in respiratory and

cardiovascular tract. They postulated three possible modes of extra pulmonary effects in accordance with Nemmar *et al*. (2002) which includes the following: 1) inhaled particles may release organic chemicals and transition metals from the lung to the systemic circulation, 2) pulmonary inflammation could lead to the release of reactive oxygen species, cytokines and chemokines to the systemic circulation 3) UFPs could also gain access to the systemic circulation by directly penetrating the alveolar/ capillary barrier in the lung. Hoffmann *et al*. (2009) also confirmed the translocation of particles from lung into systemic circulation and later to extra-pulmonary organs. The health effects due to composite air pollutant particulate matter mixture are depicted in Figure 7.

Figure 7

Health effects due to composite air pollutant particulate matter mixture



NO_x indicates nitrogen oxides; SVOC, semivolatile organic compounds; CO, carbon monoxide; O_3, ozone; PM, particulate matter; COPD, chronic obstructive pulmonary disease; CV, cardiovascular; ↑ BP, increased blood pressure; 2°, secondary; and ACS, acute coronary syndrome.

(Brook and Brook, 2011)

Coccini *et al*. (2012) proposed potential markers of inflammation and coagulation mechanism on exposure to UFP PM that embrace the following: airway injury or activation of blood cells, such as monocytes, caused by particles

deposited in the alveoli, leads to a release of pro-inflammatory cytokines interleukins (IL); IL-6 and IL-8. Increased production of IL-6 and IL-8 activates mononuclear as well as endothelial cells, initiating the hepatic synthesis of acute-phase proteins, such as C-reactive protein (CRP) and serum amyloid A (SAA), followed by an up regulation of adhesion molecules expression (e.g. E-selectin, von Willebrand factor antigen,ICAM-1) as markers of endothelial dysfunction. The enhanced acute-phase response as well as endothelial cell activation in turn increase the procoagulant activity, indicated by a rise in coagulation proteins (e.g. fibrinogen, factor VII, prothrombin fragment 1+2, D-dimer) evident of clotting cascade activation. These changes in blood parameters, together with plaque instability, may ultimately lead to a coronary event in susceptible patients.

Kleinsorge *et al*. (2011) reported oxidative stress and significant DNA damage among photocopier workers. Similarly, Khatri *et al*. (2013) also noticed higher oxidative stress and local air way inflammation on short term exposure to photocopier emissions.

2.3.2 Ozone

Singh *et al*. (2014) reported that ozone has much stronger effect on human nostril, throat and eyes than that of other pollutants. Sundell (2000) categorized the human disorder symptoms for the ozone concentrations. According to his classification, when concentration attains 50.9 ppb to 152.8 ppb people may have headache and eyes irritation, respectively.

According to Mortimer *et al*. (2002), daily exposure to high levels of ozone may cause pulmonary damage and frequent asthma attacks, even at very low concentration of ozone. Schegele *et al*. (2009) reported that with repeated ozone exposure, the decline in lung function were attenuated, but markers of airway inflammation and injury persisited or increased. Kim *et al*. (2011a) indicated that ozone exposure initiate and exacerbate pulmonary disease that might lead to lung inflammation and significant decline in lung function. Chuang *et al*. (2009) reported that ozone exposure might significantly alter blood pressure, vascular

function, heart rate, endothelial dependent vascular function, oxidative stress, mitochondrial damage and atherogenesis. It was reported by Palli *et al*. (2009) to modulate oxidative DNA damage in lymphocytes.

Extensive report by Maddalena *et al*. (2011) indicated ozone emission in office area with printers and copiers. Singh *et al*. (2014) reported ozone emissions of 1.8 - 10.0 ppb in basement and 5.3 - 45.8 ppb levels in ground floor photocopier centres.

Ozone being a strong oxidant reacts with other pollutants to form secondary products that are known to have diverse adverse health effects in xerographic centres. Nel *et al*. (2006) reported SOA formation from a styrene−ozone system and proposed 3,5-diphenyl-1,2,4-trioxolane and a hydroxyl-substituted ester as the major secondary aerosol forming products.

2.3.3 Volatile Organic Compounds

VOCs produce negative health effects if humans are exposed to high concentrations. Long-term exposure to low concentrations of VOCs at or above regulatory standards may result in liver and kidney damage, resulting in elevation of liver enzyme activities and changes in lipid metabolism (Tanyanont and Vichit-Vadakan, 2012).

Occupational exposure to VOC namely propane, iso-butane and benzene is associated with increased cardiovascular risk (Tsai *et al*., 2010). Ma *et al*. (2015) reported decreased heart rate variability and inflammatory blood markers due to occupational exposure. Delfino *et al*. (2003) reported that exposure to outdoor VOCs namely benzene, m-xylene, p-xylene, ethylbenzene and tetrachloroethylene resulted in higher respiratory symptoms. But, they did not notice any stronger association between VOC and lung function. In tune with this, Weichenthal *et al*. (2012) also indicated no association between VOC and acute cardiorespiratory effect.

Lee *et al*. (2006) demonstrated high exposure of VOC's especially benzene, toluene, ethylbenzene and xylene among photocopier workers in

photocopy centers with a lifetime cancer risk exceeding 1 $X10^{-6}$. Barro *et al*. (2009) reported that some VOCs induce cancer in animals and some of them are suspected or known to cause cancer in humans, even at very low concentrations. Key signs or symptoms associated with exposure to VOCs include eye irritation, nose and throat discomfort, headache, allergic skin reaction, nausea, fatigue, or dizziness. Andris-Jr (2015) opined VOCs as one of the causative agents of sick building syndrome.

2.3.4 Semi Volatile Organic Compounds

Xu and Zhang (2011) reported that SVOCs are linked to serious adverse health effects. Alkyl phenols are endocrine-disrupting chemicals that block endogenous hormones. Exposure to some phthalates results in profound and irreversible changes in the development of the reproductive tract in males; exposure to poly brominated diphenyl ethers causes impairment of brain and nerve tissues. Poly chlorinated biphenyls are potent neurotoxicants. Poly cyclic aromatic hydrocarbons lead to lung, bladder and gastrointenstinal cancer and organ damage.

2.3.5 Toners

Toner, the most important component in xerographic process is associated with many health effects due to its ultra fine particle size and their composition.

Bai *et al*. (2010) showed that intratracheal instillation of toner particles inhibited the normal growth and lead to inflammatory responses and lesions in the lung tissues. Morimoto *et al*. (2013) reported pulmonary toxicity of toner following inhalation and intratracheal instillation among rats. Chronic inhalation of toner by rats was found to increase in inflammatory response (Slesinski and Turnbull, 2008).

In humans, toner emissions are associated with sick building syndrome (Jakkola *et al*., 2007). Their exposure might occur through direct skin contact, or by inhalation or ingestion (Gminski *et al*., 2011). Balakrishnan and Das (2010)

reported that toner dust might irritate respiratory tract that results in cough and sneezing.

There are human case studies available for health effects of toner powder. Gallardo *et al*. (1994) reported siderosilicosis. Ambruster *et al* (1996) reported granulomatous pneumonitis. Rybicki *et al*. (2004) reported sarcoidosis and Theegarten *et al*. (2010) reported sub mesothelial deposition of carbon nanoparticles in the peritoneum of photocopier exposed worker.

Kitamura *et al*. (2009) observed a significant partial influence of toner exposure on pulmonary function as indicated by the peripheral respiratory tract indices. Elango *et al*. (2013) reported that chronic exposure to photocopier emission is not only associated with decline in lung function, but also oxidative stress and systemic inflammation and highly prone to cardiovascular disease. In contrast, Nakadate *et al*. (2006), Terunuma *et al*. (2009) and Yanagi *et al*. (2014) reported no significant toner exposure effects on respiratory function in epidemiological studies among toner handling group of workers.

2.3.6 Selenium

Selenium is of fundamental importance to human health. It is an essential component of several major metabolic pathways, including thyroid hormone metabolism, antioxidant defence system and immune function (Brown and Arthur, 2001). Serum levels of selenium provide useful information in the clinical categorization of deficiency and toxicity status (Arnaud *et al*., 2008). According to Hamilton (2004), selenium has three levels of biological activity: (1) trace concentrations are required for normal growth and development; (2) moderate concentrations can be stored and homeostatic functions maintained; and (3) elevated concentrations can result in toxic effects.

Au *et al*. (2005) observed dizziness, fatigue and irritation of mucous membranes in people exposed to selenium in workplace air. In extreme cases it leads to pulmonary oedema and severe bronchitis. Rischer *et al*. (2003) indicated that primary target organ for selenium exposure in humans is the lung in comparison to other organs / organ systems. Workers highly exposed to

selenium dust reported stomach pain and headaches whereas briefly exposed workers reported respiratory symptoms such as pulmonary oedema, bronchial symptoms, asphyxia, persistent bronchitis, elevated pulse rates, lowered blood pressure, vomiting, nausea and irritability.

2.4 Indoor Air Pollutants and Lung Function

Lung function is an excellent operative marker of the effects of air pollution in the general population. It is objective and quantitative, an early predictor of cardiorespiratory morbidity and mortality, able to describe trajectories to the occurrence of chronic obstructive pulmonary disease (COPD) and coherent with experimental data on deposition and accumulation of pollutants in airways and lungs and the resulting systemic inflammation and oxidative stress (Sunyer, 2009).

Spirometry, the most frequently performed lung function test, is the cornerstone of occupational respiratory evaluation programs. In the occupational health setting, spirometry plays a critical role in the primary, secondary and tertiary prevention of workplace-related lung disease. Used for both screening and clinical evaluations, spirometry tests are performed in a variety of venues ranging from small clinical practices to large testing facilities and multiple plant medical departments within an industry (Townsend, 2011). According to Soriano *et al*. (2009), several reference values have been published that take into account age, sex and height but with rather large differences between them. It is therefore recommended that in epidemiological studies, examinations be carried out also on control groups to avoid total dependence on standard reference values. The procedure for forced expiratory manoeuvres and their derived parameters have been standardised separately by the American Thoracic Society (ATS) and the European Respiratory Society (ERS) according to Miller *et al*. (2005) and now also jointly according to Pellegrino *et al*. (2005).

Table 1 presents the list of lung function parameters assessed by spirometry and Figure 8 depicts the spirogram (flow-volume loop) obtained by spirometry.

Table 1

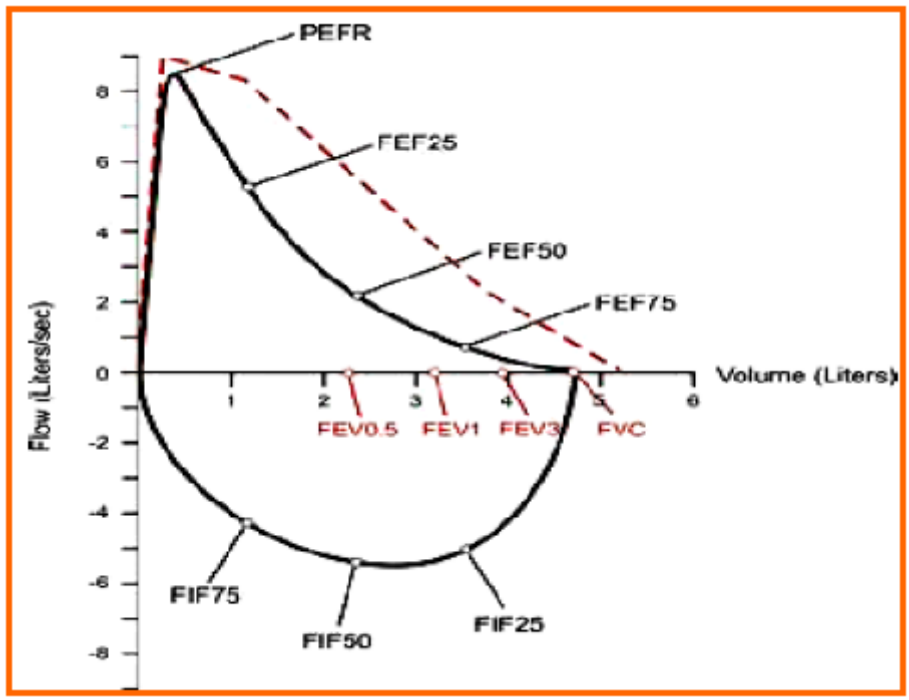
Lung function parameters assessed by Spirometry

Lung function Parameter	Description
Forced Vital Capacity (FVC)	The total volume of air that can be exhaled during a maximal forced expiration effort.
Forced Expiratory Volume in 1 Second (FEV_1)	Forced expiratory volume in one second; the volume of air exhaled in the first second under force after a maximal inhalation.
Force Expiratory Volume in 6 seconds (FEV_6)	The amount of air exhaled with maximum effort in the first six seconds
FEV1/ FVC	FEV1/ FVC ratio
Peak Expiratory Flow (PEF)	PEF is the highest flow achieved from a maximum forced expiratory manoeuvre started without hesitation from a position of maximal lung inflation
Forced Expiratory Flow (FEF_{25-75}) or Maximum Mid-Expiratory Flow (MMEF)	Forced expiratory flow over the middle one half of the FVC manoeuvre
Forced Expiratory Flow 25% (FEF_{25})	The amount of air that was forcibly expelled in the first 25% of FVC manoeuvre
Forced Expiratory Flow 50% (FEF_{50})	The amount of air that was forcibly expelled in the first half of FVC manoeuvre
Forced Expiratory Flow 75% (FEF_{75})	The amount of air that was forcibly expelled in the first 75% of FVC manoeuvre
Peak Inspiratory Flow (PIF)	The fastest flow rate achieved during inspiration
Peak Expiratory Flow (PEF)	The maximum flow rate achieved during FVC manoeuvre
Maximal Ventilatory Volume (MVV)	The MVV is the maximum volume of air a subject can breathe over a specified period of time (12 s for normal subjects).

Pellegrino *et al*. (2005)

Figure 8

Spirogram obtained by spirometry



(www.morgansci.com, 2012)

2.4.1 Interpretation of Ventilatory Function Test

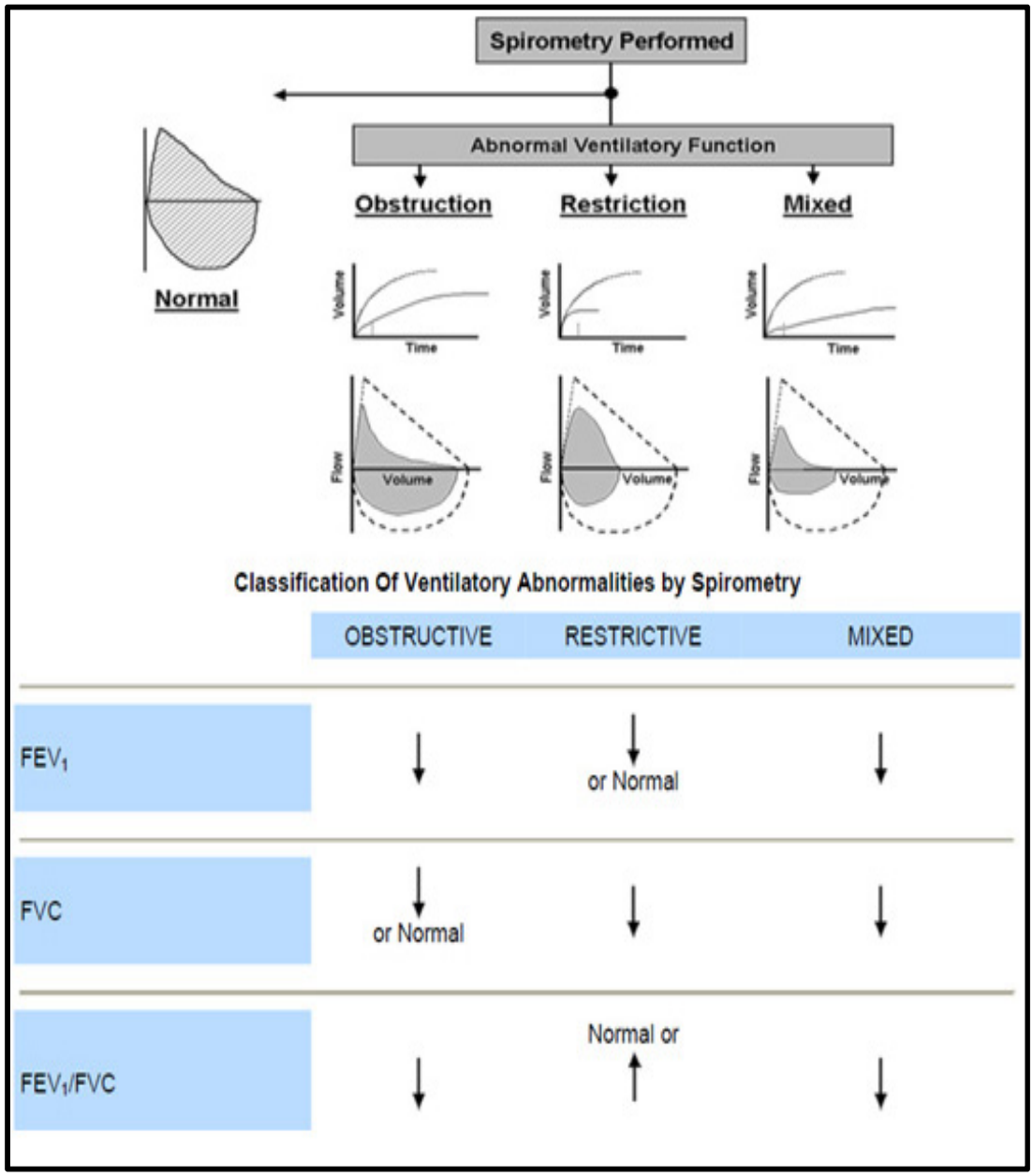
Lung function assessment is the main non-invasive procedure to evaluate respiratory health, identifying ventilation alterations, such as restrictive or obstructive pathologies (Falcon-Rodriguez *et al*., 2016). According to Pierce and Johns (2008) measurements of ventilatory function may be very useful in a diagnostic sense but they are also useful in following the natural history of disease over a period of time, assessing preoperative risk and in quantifying the effects of treatment. The presence of ventilatory abnormality can be inferred if any of FEV1, VC, PEF or FEV1/FVC value is outside the normal range.

2.4.2 Classification of Abnormal Ventilatory Function

Abnormal ventilatory function is classified into three types. Their categorization as defined by Pierce and Johns (2008) in spirometry hand book is as follows and they are depicted in Figure 9:

Figure 9

Schematic diagrams of different types of ventilatory patterns



(Pierce and Johns, 2008)

Schematic diagram illustrating spirograms for obstructive, restrictive and mixed ventilatory defects. The shape of the expiratory flow-volume curve varies between **obstructive ventilatory defects** where maximal flow rates are diminished and the expiratory curve is scooped out or concave to the x-axis **restrictive diseases** where flows may be increased in relation to lung volume (convex).

1. A reduction of FEV1 in relation to the forced vital capacity will result in a low FEV1/FVC and is typical of obstructive ventilatory defects. The lower limit of normal for FEV1/FVC is around 70-75%. In obstructive lung disease the FVC may be less than the slow VC because of earlier airway closure during the forced manoeuvre. This may lead to an overestimation of the FEV1/FVC. Thus, the FEV1/VC may be a more sensitive index of airflow obstruction.

2. The FEV1/FVC ratio remains normal or high (typically > 80%) with a reduction in both FEV1 and FVC in restrictive ventilatory defects (e.g. interstitial lung disease, respiratory muscle weakness and thoracic cage deformities such as kypho-scoliosis).

3. A reduced FVC together with a low FEV1/FVC ratio is a feature of a mixed ventilatory defect in which a combination of both obstruction and restriction appear to be present, or alternatively may occur in airflow obstruction as a consequence of airway closure resulting in gas trapping, rather than as a result of small lungs

2.4.3 Exposure to Pollutants and Obstructive Lung Disease

Airflow obstruction in lungs arises due to emphysema, chronic obstructive bronchitis, bronchiectasis and asthma. Chronic Obstructive Pulmonary Disease (COPD) is characterised by physiological abnormalities, including airflow limitation, abnormalities in gas exchange and lung hyperinflation, associated with multiple markers of systemic inflammation (Cazzola *et al*., 2008). It comprises of the pulmonary emphysema (characterized by enlargement and destruction of pulmonary alveoli), chronic bronchitis (characterized by chronic cough and expectorations) and small airway disease (characterized by distortions, tortuosities, fibrosis and smooth muscle hyperplasia of the small airways). Bakke *et al*. (2011) highlighted that COPD is a preventable and treatable disease with some extra pulmonary effects and also noted that airflow limitation is usually progressive and associated with abnormal inflammatory response of the lung to noxious particle and chemicals. According

to Balmes *et al*. (2003), approximately 15–20% of the population burden of COPD is attributable to occupational exposures.

COPD is a disease characterized by inflammation both in its stable phase and during exacerbations. Inflammation is present in the respiratory compartment, the inflammatory cells and different mediators of inflammation are present. Studies have shown that some mediators of inflammation have a high level at systemic level also, inducing a certain grade of systemic inflammation, mainly responsible for the systemic manifestation of the disease. It seems that both the local and the systemic inflammation are amplified during exacerbations (Barbu *et al*., 2011). Agusti *et al*. (2008) reported elevated circulating levels of white blood cells, C-reactive protein, interleukins (IL-6 and IL-8), fibrinogen and tumour necrosis factor (TNFα) with persistent low grade systemic inflammation. Exacerbation of COPD could be caused by bacteria, viruses, cigarette smoking and exposure to indoor and outdoor air pollution (Falcon-Rodriguez *et al*., 2016).

2.4.4 Exposure to Pollutants and Restrictive Lung Disease

Schwartz (1989) reported that chronic exposure to ozone resulted in restrictive lung disease. Biomass fuel has deleterious effects on pulmonary function and structure leading to both obstructive and restrictive pathologies according to Özbay *et al*. (2001). World trade centre disaster workers experienced low FVC. There are several possible explanations suggested by Herbert *et al*. (2006) for the high rates of low FVC observed in this group: 1) true restriction due to parenchymal lung disease (e.g., interstitial lung diseases such as sarcoidosis, idiopathic pulmonary fibrosis, pneumoconiosis); 2) true restriction due to physical factors such as obesity or chest wall abnormalities or 3) “pseudo restriction” due to air trapping (e.g., airways obstruction) or sub-maximal inspiratory and/or expiratory effort (typically the result of chest pain/tightness or in an attempt to reduce coughing during the test), A Swiss study concluded that long term exposure to PM_{10} reduced FVC by 3.4% per year increament of 10 µg / m^3 of PM_{10} (Ackermann-Liebrich *et al*., 1997).

Pulmonary fibrosis is a restrictive disease that presents an irreversible decrement in the vital capacity. Furthermore, some cells produce an excess of extracellular matrix components and the pulmonary remodeling like an irreversible distortion of the lung's architecture Exposure to ambient particles could lead to pulmonary fibrosis especially the exposure to elements or chemicals such as aluminium, silicon, silicon oxide, carbon black, titanium dioxide, talcum powder, asbestos and other fibers can cause epithelial damage and rise the levels of interleukins especially IL- 8 and IL-13 (Falcon-Rodriguez *et al*., 2016).

Sarcoidosis is associated with exposure to inorganic particles, organic particles and environments with reported moulds. Occupational studies have shown positive associations to sarcoidosis with service in the U.S. Navy, metalworking, fire fighting and the handling of building supplies. About 65% of sarcoidosis patients have airflow limitation which indicates restrictive ventilatory dysfunction, with reduced forced vital capacity (FVC) and reduced forced expiratory volume in 1 second (FEV1). At least 50% of patients also have concurrent obstructive airway disease, with a reduced ratio of FEV1 to FVC. Airway hyper reactivity occurs in 5 to 83% of patients. In 80% of patients presenting with abnormal spirometric findings, the values return to the normal range within 2 years (Lannuzzi *et al*., 2007).

Exposure to occupational dusts, mists, or residential exposure to pet birds causes another kind of restrictive lung disease, hypersensitivity pneumonitis (extrinsic allergic alveolitis) caused by inhaled allergens which can progress to disabling or even fatal end-stage lung disease. The symptoms and physical findings are nonspecific. Lung function tests show restrictive and diffusion defects with hypoxemia, especially after exercise. Although patients produce antibody exuberantly, the immunopathogenesis involves cellular immunity notably, $CD8^+$ cytotoxic lymphocytes, multinucleate giant cell granulomas and ultimately interstitial fibrosis (Patel *et al*., 2001).

2.5 Biomarkers and Biomonitoring

Human biomonitoring is acquiring exposure and biological effect data through the analysis of cells, tissues, or body fluids. The biochemical or biological variable measured for the purpose of biomonitoring is designated as biomarker. Biomarkers can detect the exposure, effect of exposure or the individual susceptibility to an exposure. Therefore they are of prime interest for the assessment of the exposure and early biological effects in epidemiology as well as occupational and environmental medicine. They are observable endpoints in the continuum of events ranging from exposure to diseases. The chain of events includes: external dose', 'internal dose' (absorbed dose), 'biologically effective dose' (target dose), 'early biological effects' and 'health effects (Scherer, 2006).

In air pollution research, various biomarkers linking air pollution exposure to their adverse effects in the respiratory and cardiovascular systems have been identified in human studies and most of these biomarkers are associated with two major toxicological response pathways, oxidative stress and inflammation. Nano particulate matter exposure-associated biological responses at biochemical, molecular and cellular levels, a process known as biomarker studies. The ideal biomarkers for assessing environmental and occupational exposures should be able to provide strong mechanistic, molecular, or biochemical basis for the diseases, be exposure specific, reflect early adverse health effects, have clinical relevance and easy to use (Li and Nel, 2011).

2.5.1 Oxidative Stress Markers

Oxidative stress is defined as a serious imbalance between reactive species production and antioxidant defenses. A disturbance in the balance between pro-oxidants and antioxidants in favour of the former, leading to oxidative damage, gives rise to an increase in oxidative stress as suggested by Halliwell (2007). There is abundant evidence of alterations in the oxidative status of subjects occupationally exposed to pollutants in office environments. The oxidant potential of the air pollutants in the workplace triggers oxidative stress

and inflammation which may reflect the increased oxidant burden in the living system (Lin and Thomas, 2010).

Oxidative stress and inflammation are the two primary mechanisms implicated in pulmonary cell injury and tissue damage following exposure to air pollution in general. Although pollutant specific mechanisms remain unclear, common patterns of immune response includes activation of phagocytic cells, clearance of foreign agents, antibody mediated immunity, cellular influx and increase in pulmonary cell permeability (Williams *et al*., 2011). Figure 10 represents the oxidative stress caused by air pollution.

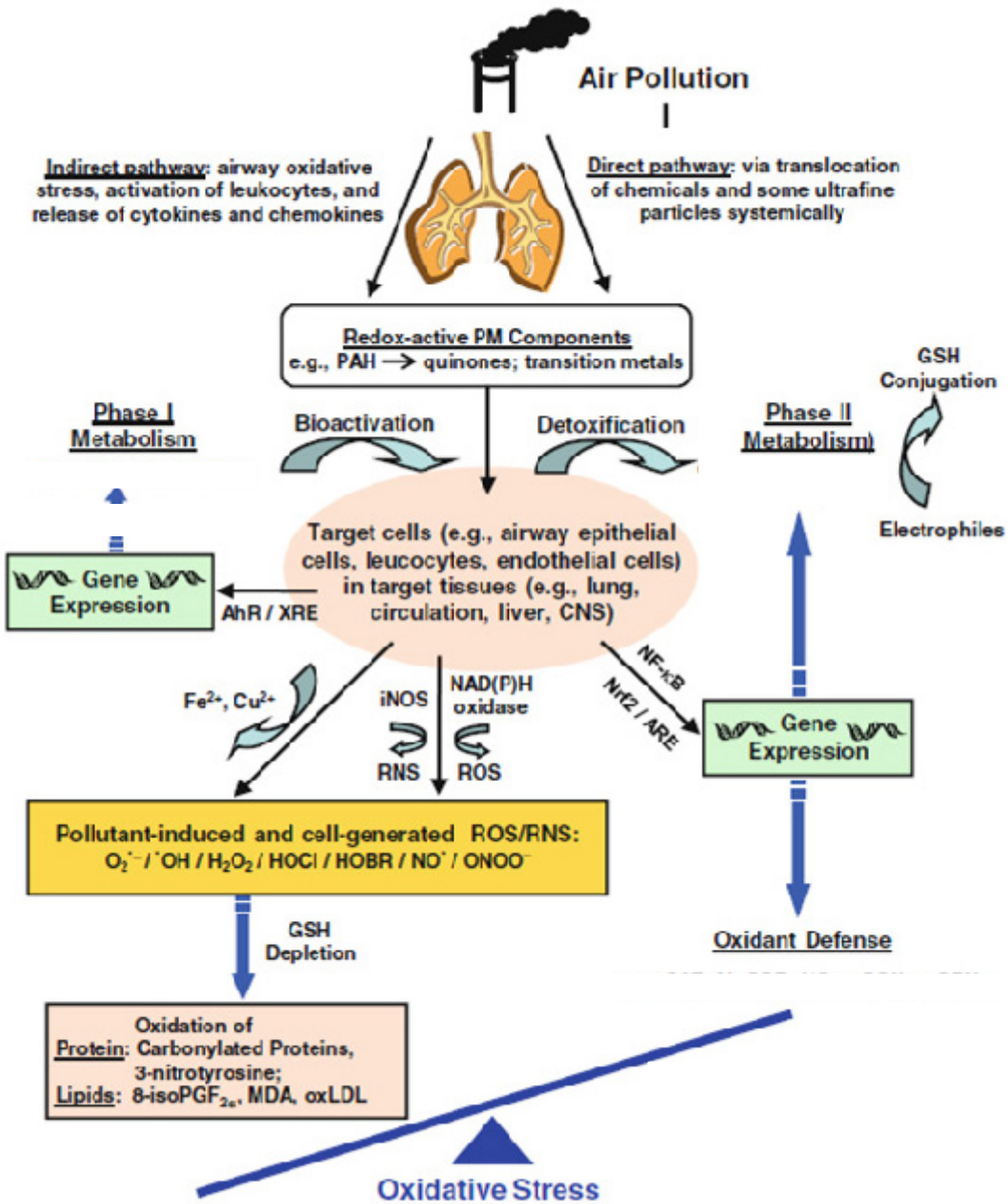
Biomarkers of oxidative stress include macromolecules that are modified by interaction with reactive oxygen species (ROS) in the microenvironment and the molecules of the antioxidant system that change in response to increased redox stress (Ho *et al.*, 2013).

Lipids are the prime targets of oxidation because of their molecular structure with abundant double bonds (Porter *et al*., 1995). Two of the most well studied oxidative stress biomarkers includes lipid peroxidation products namely malondialdehyde and isoprostanes. 8- Isoprostane is a relatively stable oxidative stress marker at physiological temperature. It is an analog of prostaglandin produced from arachidonic acid, a polyunsaturated fatty acid present in the phospholipid of cell membrane by free radical catalyzed peroxidation reaction (Morrow *et al*., 1992).

Halliwell (1997) defined antioxidants as a substance that, when present at low concentrations compared to those of the oxidizable substrate, significantly delays, or inhibits, oxidation of that substrate. They may be small molecules or proteins or enzymes (Niki *et al*., 1995).

Figure 10

Oxidative stress caused by air pollution



(Delfino *et al*., 2011)

8-isoPGF2α - 8-isoprostane ;

MDA - Malondialdehyde ;

oxLDL - Oxidised low density lipoprotein

GSH - Glutathione

Niki, (2010) envisaged four lines of antioxidant defense mechanisms that include: 1) preventing antioxidants 2) scavenging antioxidants 3) repair and 4) denova antioxidants. The preventing antioxidants function as the first line of defense by suppressing the formation of reactive oxygen and nitrogen species (ROS/RNS). The scavenging antioxidants remove active species rapidly before the active species attack biologically essential molecules. These scavenging antioxidants act as the second line defense in vivo. Various enzymes function in the third line defense by repairing damages, clearing the wastes and reconstituting the lost function. In addition, the adaptation mechanism functions as the fourth line defense, in which appropriate antioxidants are generated at the right time and transferred to the right position in right concentration. Furthermore, there is now increasing evidence showing that some antioxidants act as a cellular signaling messenger to regulate the level of antioxidant compounds and enzymes.

Total antioxidant capacity is evaluated either by determining the rate of oxidation of the antioxidant or by measuring the protection of an easily determined indicator against oxidation by the antioxidants (Pinchuk *et al*., 2012).

The oxidation system of 2,2'-azino-di(3-ethylbenzthiazoline-6-sulfonic acid (ABTS) by myoglobin with hydrogen peroxide (H_2O_2) has been used for Total Antioxidant Capacity (TAC) assay as Trolox Equivalent Antioxidant Capacity (TEAC). Myoglobin reacts with H_2O_2 and ferrylmyoglobin is formed. Ferrylmyoglobin oxidizes ABTS to ABTS cation radical (ABTS•+) and its formation can be monitored at 600 nm or 734 nm. The oxidation of ABTS is inhibited via the reaction of Trolox or antioxidants with ferrylmyoglobin (Kambayashi *et al*., 2009).

Total antioxidant capacity is based on the capacity of antioxidants to reduce the ferric complex of 2,4,6-tripyridyl-s-triazine (Fe^{3+} TPTZ) to the colored ferrous complex Fe^{2+} TPTZ at pH 3.6 (Benzie and Strain, 1996). Interpretation of the results is based on the hypothesis that the capability of water soluble antioxidants to reduce ferric ions reflects their ability to reduce ROS. According to

the problem with this test is that it does Pinchuk *et al.* (2012), not take into consideration important antioxidants, including thiols (glutathione) and albumin but does include reductants that do not act as antioxidants.

2.5.2 Inflammatory Markers

Inflammation is a complex defence mechanism in which leukocytes migrate from vasculature into damaged tissues to destroy the agents that potentially cause tissue injury (Gabay, 2006). Inflammatory markers / chemokines / cytokines reflect the level of inflammation. They are generally molecules that directly promote local inflammation or indirectly recruit and attract inflammatory cells (Lin and Thomas, 2010). A clue to link between indoor air pollutant exposure in occupational settings and systemic inflammation arises from studies that associate the change in the levels of inflammatory markers (Kim *et al*., 2005 and Fang *et al*., 2010).

2.5.3 Leukotriene B4

Leukotriene B4 is an arachidonic acid metabolite synthesised by the combined action of 5-lipoxygenase and LTA4 hydrolase (Samuelsson and Funck, 1989). It is predominantly made by inflammatory cells like neutrophils, macrophages and mast cells (Funck, 2001). It has long been recognized as potent lipid mediator of inflammation. It stimulates a range of functions that include aggregation, stimulation of ion fluxes and enhancement of lysosomal enzyme release, superoxide anion production, chemotaxis and chemokinesis (Ford-Hutchinson, 1989; Mayatepek and Hoffmann, 1995). It is found to promote neutrophils migration to the site of tissue inflammation. It plays an important role in the immune response to antigenic stimulation. It also stimulates cytotoxic T cell, natural killer cell and suppressor T cell activity (Comandini *et al*., 2009)

2.5.4 C-Reactive Protein (CRP)

C-reactive protein is a non specific acute phase protein. It is named for its capacity to precipitate the somatic C-polysaccharide of Streptococcus pneumoniae. It is a sensitive marker of inflammation and tissue damage. It is produced by the liver in response to interleukin-6 (Pepys and Hirschfield, 2003).

CRP reflects total systemic burden of inflammation in several disorders and has been shown to up regulate the production of proinflammatory cytokines (Karadag *et al*., 2008). C-reactive protein is a predictor of prognosis in Chronic Obstructive Pulmonary Disease (Dahl *et al*., 2007). Higher levels of C-reactive protein in young adults are associated with subsequent decline in lung function, suggesting low-grade systemic inflammation (Rasmussen *et al*., 2009). CRP is a best studied biomarker to assess the risk of cardiovacular disease as opined by Samuelsson *et al*. (2015).

Mannio *et al*. (2003) proved an association between both lung disease (both obstructive and restrictive) and elevated levels of biomarkers fibrinogen and C-reactive protein as indicators of inflammation.

2.5.5 Interleukins

Interleukins are cytokines that act on leukocytes. They are produced in large amounts during all kinds of immune responses and the outcome of an inflammatory reaction is determined by the balance of cytokines produced. Interleukins can have pro- and anti-inflammatory functions. Pro-inflammatory interleukins are induced by a variety of stimuli, including infections with pathogens and also sterile organ damage. Their main function is to stimulate immune responses that result in the elimination of invading pathogens or damaged and dying cells. At the same time, anti-inflammatory mediators are produced to protect the host's body from exaggerated immune responses and to limit organ damage (Hammerich and Tacke, 2014).

IL-6 is both a pro and anti-inflammatory cytokine that is elevated in most, if not all, inflammatory states and infectious responses. It is also involved in the regulation of metabolic, regenerative and neural process (Scheller *et al*., 2011). It plays a key role in acute phase response as defined by a variety of clinical and biological features such as the production of acute phase proteins. It has stimulatory effect on B and T cells favouring chronic inflammatory response (Gabay, 2006). Brook and Brook, (2011) observed that IL-6 is directly involved in the stimulation of CRP from the liver and is linked to cardiovascular disease.

IL-8 is a powerful cytokine and activator of neutrophils. In lungs, it seems to be the most potent chemoattractant for these cells. Although IL-8 can be produced by several cell types, it can also be synthesised by neutrophils in response to inflammatory mediators (Monteseiri'n, 2009). IL-8 is primarily responsible for the recruitment and activation of monocytes and neutrophils, the signature cells of acute inflammatory response. In addition they also play an important role in various pathological conditions such as chronic inflammation (Pourfarzam *et al*., 2009).

2.5.6 Clara Cell Protein (CC16)

The lung epithelium secretes several specific proteins into the airspaces of the respiratory tract. These include the 16-kDa bronchiolar Clara cell secretory protein (CC16), the main secretory product of bronchiolar Clara cells, as well as surfactant-associated proteins (SP)-A and -B, secreted primarily by alveolar type II cells. These proteins occur physiologically in small amounts in blood and because they are secreted into the respiratory tract, their occurrence in serum can only be explained by leakage into the vascular compartment. Although the exact mechanisms by which these proteins enter the blood remains poorly understood, their concentrations in serum, referred to as pneumoproteinaemia can be used to assess the leakage of the lung epithelial barrier and/or the integrity of the epithelial cells secreting them. Indeed, their intravascular leakage increases in conditions characterised by pulmonary inflammation and/or pulmonary epithelial injury (Robin *et al.*, 2002). It is an immune suppressive and anti inflammatory protein secreted by the Clara cells of the distal respiratory epithelium (Broeckaert and Bernard, 2000).

CC16 also plays an important role in the sequestration or clearance of harmful substances deposited in the respiratory tract (Hermans and Bernard, 1999). It has been proved to be a potential biomarker of lung epithelial injury in numerous diseases that include idiopathy pulmonary fibrosis (Ye *et al*., 2004), COPD (Lomas *et al*., 2008), sarcoidosis (Hermans *et al*., 2001), asthma (Shijubo *et al*., 1999), acute respiratory distress syndrome (Lesur *et al*., 2006)

and occupational exposures (Ulvestad *et al*., 2007).Novel approaches have been developed to diagnose the airway damage and inflammation by detecting CC16 levels in urine and EBC samples (Mirowsky and Gordon, 2015).

CC16 levels were found to be increased transiently on exposure to smoke (Bernard *et al.,* 1997). Acute environmental exposures can cause a transient increase in CC16 level, but repeated exposures to cigarette smoke can result in chronically decreased serum CC16 levels (Zhu *et al*., 2015).

2.5.7 Eosinophilic Cationic Protein (ECP)

Eosinophilic Cationic Protein (ECP) is a small polypeptide released from activated eosinophils (Chihara, 2005), It is one of the important mediators of allergic inflammation in respiratory tract mucosa (Bousquet *et al*., 2000). ECP also known as human RNase 3, belongs to the mammalian RNase A superfamily and its RNase activity is required for some of its biological properties. It is involved in immune host defense, with a cytotoxic activity against a wide range of pathogens. During inflammation and eosinophilia disorders, it is secreted to the inflammation area, where it would contribute to the immune response. Its secretion causes also severe damage tf o the host own tissues. ECP presents a high affinity for heparin and this property might be crucial for its immunomodulating properties, antipathogen action and its toxicity against eukaryotic cells (Torrent *et al*., 2011). It is also found to be closely related to the presence or activation of neutrophils rather than to their involvement in allergic process (Azevedo *et al*., 2001).

Bystrom *et al*. (2011) proved that several types of inflammatory stimulus caused ECP degranualtion that includes: interaction with adhesion molecules, stimulation by leukotriene B_4, platelet activating factor, interleukin- 5, immunoglobulins and complement factors C5a and C3. All seemed to cause ECP release.

ECP is categorized as an upper air way inflammatory biomarker, whose levels were found to increase in preschool children exposed to indoor air

pollutants (Wesley and Jalaludin, 2015). Serum ECP levels were found to be increased during exacerbations of chronic bronchitis with airway obstruction (Fiorini *et al*., 2000). Elango *et al*. (2013) reported increased levels of ECP in photocopier operators due to photocopier exposure.

2.5.8 Myeloperoxidase (MPO)

Myeloperoxidase is a cationic heme containing protein with bactericidal and pro-inflammatory properties that is released by recruited neutrophils and accumulates on endothelial cells and in matrix proteoglycans in the albuminal space (Williams *et al*., 2011).

MPO catalyses a number of reactant oxidant species and has impacts on nitric oxide formation nitric oxide through complex mechanisms, including direct catalytic consumption resulting in endothelial dysfunction (Tang *et al*., 2009).

According to Comandini *et al*. (2009), MPO-derived diffusible radical species are capable of initiating lipid peroxidation as well as promoting an array of post-translational modifications in target proteins, including halogenation, nitration and oxidative cross-linking. It amplifies the oxidative potential of H_2O_2 derived from leukocyte NADPH oxidases, xanthine oxidase, uncoupled nitric oxide synthase and various isoenzymes by forming potent oxidants capable of chlorinating and nitrating phenolic compounds.

van-der-Veen *et al*. (2009), opined that MPO derived oxidants are linked to tissue damage in many diseases, especially those characterized by acute or chronic inflammation. These effects are beyond its oxidative properties which were observed to be independent of its catalytic activity affecting various processes involved in cell signalling and cell–cell interactions and were capable of modulating inflammatory response.

2.5.9 Intercellular Adhesion Molecule (ICAM1)

Intercellular Adhesion Molecule 1 (ICAM-1) is a 90 Kilo Dalton inducible surface glycoprotein that promotes adhesion in immunological and inflammatory reactions. ICAM-1 is a ligand of lymphocyte function-associated antigen-1

(LFA-1), a αβ complex that is a member of the integrin family of cell-cell and cell-matrix receptors. ICAM-1 is encoded by an inducible 3.3 kb mRNA (Staunton *et al*., 1988). The amino acid sequence specifies an integral membrane protein with an extracellular domain of 453 residues containing five immunoglobulin-like domains. ICAM-1 is a transmembrane protein that mediates endothelial transmigration of leukocytes in inflammatory response and in the regulation of vascular permeability (Frank and Lisanti, 2008).

ICAM-1 is constitutively present on endothelial cells, but its expression is increased by pro-inflammatory cytokines, in atherosclerotic tissues and the progression of autoimmune diseases. The ligation of ICAM-1 on the surface of endothelial or smooth muscle cells was observed to lead to the activation of several pro-inflammatory signalling cascades. A circulating or soluble form of ICAM-1 (sICAM-1) has been measured in various body fluids, with elevated levels being observed in patients with atherosclerosis, heart failure, coronary artery disease and transplant vasculopathy (Lawson and Wolf, 2009).

Ambient air particulates stimulate enhanced expression of intercellular adhesion molecule-1 (ICAM-1) in cultured epithelial cells according to Kennedy *et al*. (1998). Pollutant-related cardiovascular events involve emigration of inflammatory cells from blood to tissue sites which in turn involves up regulation of adhesion molecules on vascular and on circulating leukocytes as envisaged by Frampton (2001).

2.5.10 Nitric oxide (NO)

NO is an extremely volatile compound that is difficult to determine in serum. It is quickly converted to nitrite (NO_2^{-}) and nitrate (NO_3^{-}) (Moshage *et al*., 1995). It is estimated that more than 95% of whole blood nitric oxide gets converted to nitrate and nitrites within one hour (Groeneveld *et al*., 1997). Thus levels of these in serum are considered surrogate markers for nitric oxide (Kalugalage *et al*., 2013). Thus the best indicator of total nitric oxide production is the sum of both nitrite and nitrate.

Nitric oxide is converted to a highly reactive radical formed from the semi essential amino acid L- arginine by the action of the stereo specific enzyme nitric oxide synthase (NOS) (Palmer *et al*., 1988). Enhanced nitric oxide production due to induced expression of iNOS by proinflammmatory cytokines is instrumental in the pathophysiology of inflammation (Barnes and Belvisi, 1993). The measurement of total nitrates and nitrites is often used as a surrogate marker for nitric oxide, in specific to vaso-relaxation (Bloomer *et al*., 2011).

In addition to its discovery as a vasodilator, NO was found to mediate several protective functions of the endothelium (Bian *et al*., 2008). It does these protective functions by inhibiting (1) neutrophil activation and adhesion, (2) platelet adhesion and aggregation, (3) vascular smooth muscle proliferation and (4) expression of proinflammatory cytokines.

2.5.11 Selenium and Selenoproteins

Selenium is an essential trace element that exerts its biological effect through several selenoproteins of which there may be more than 30 in mammalian systems. Among them, few important selenoproteins includes glutathione peroxidases (GPx) and thioredoxin reductase. Thus, selenium functions in the body as an antioxidant, in thyroid hormone metabolism, redox reactions, reproduction and immune function. Plasma selenium is an important biomarker. It comprises of selenium in the form of selenocysteine in the two selenoproteins and selenomethionine present at methionine positions in all proteins plus small-molecule forms that contribute <3% of the total selenium content. Selenium is also an exposure biomarker studied in biomonitoring studies (Marie-Desvergne *et al*., 2015).

2.5.12 Glutathione peroxidase (GPx)

Glutathione peroxidase catalyses the reduction of hydroperoxides, including hydrogen peroxide, by reduced glutathione thereby, protecting the cell from oxidative damage. GPx enzymes are tetramer of four identical subunits. Each subunit contains a selenocysteine in the active site which participates directly in the two electron reduction of the peroxide substrate. The enzyme uses

gulathione as the ultimate electron donor to regenerate the reduced form of the selenocysteine (Ursini *et al*., 1985 and Forstrom *et al*., 1978). It is an intracellular enzyme and in general, their activity outside cells is very low as compared to the intracellular ones. They are released into extracellular environment during cell damage (Halliwell and Gutteridge, 1999). Glutathione peroxidase is an important plasma biomarker to assess selenium status (Combs *et al*., 2011).

2.5.13 Thioredoxin reductases (TrxR)

Thioredoxin reductases (TrxR) are a family of selenium containing pyridine nucleotide-disulfide oxidoreductases. It transfers electrons from NADPH to thioredoxin, which in turn reduces thioredoxin reductase, methionine sufoxide reductase, ribonucleotide reducase and other important redox proteins. It also reduces other substrates that include selenite, lipid hydroperoxide, vitamin K and hydrogen peroxide (Mustacich and Powis, 2000). Arnér (2009) established that it's an important selenoprotein with versatile function that supports several crucial processes of cell function, cell proliferation, antioxidant defense and redox-regulated signaling cascades. Their activity is required for normal thioredoxin function. Nordberg and Arnér (2001) stated that TrxR with its highly reactive active site containing selenocysteine residue has profound reductive capacity, reducing several substrates in addition to Trx. Due to the reactivity of TrxR, the enzyme is inhibited by many clinically used electrophilic compounds including nitrosoureas, aurothioglucose, platinum compounds and retinoic acid derivatives and hence could serve as potential target as suggested by Conterato *et al*. (2011) to study inhibition by metals and electrophilic agents that lead to its disruption in function and progress in oxidative events.

2.5.14 Genotoxicity

Genotoxicity is defined as any toxic modification of the structure or function of the genetic material, DNA (Vineis and Brandt-Rauf, 1993). The single cell gel electrophoresis (comet) assay is a technically simple and fast method that detects genotoxicity in virtually any mammalian cell type without requirement for cell culture (Møller, 2006). It is considered a suitable test for DNA-damaging

potential in biomonitoring studies (Møller *et al.*, 2000). It is the method of choice for population-based studies of environmental and occupational exposure to air pollutants, metals, pesticides, radiation and other xenobiotics according to (Valverde and Rojas, 2009). Comet assay is a useful exposure biomarker for human biomonitoring to identify the environmental genotoxins (Dhawan, 2012). Exposure biomarkers encompass: (i) chemicals or metabolites thereof, (ii) protein and DNA adducts, including types of DNA lesions detected by the comet assay (Møller, 2006).

Genotoxic effects attributed to particulate matter may relate to the content of organic compounds and to the oxidative DNA damage generated by transition metals like iron according to (El-Assouli, 2011). Schins and Knaapen (2007) reported that two principle modes of genotoxic action can be considered for poorly soluble particles particles such as titanium dioxide, carbon black and diesel exhaust particles, referred to as primary and secondary genotoxicity. Primary genotoxicity is defined as genetic damage elicited by particles in the absence of pulmonary inflammation, whereas secondary genotoxicity implies a pathway of genetic damage resulting from the oxidative DNA attack by reactive oxygen/nitrogen species (ROS/RNS), generated during particle-elicited inflammation. Conceptually, primary genotoxicity might operate via various mechanisms, such as the actions of ROS (e.g., as generated from reactive particle surfaces), or DNA–adduct formation by reactive metabolites of particle-associated organic compounds (e.g., polycyclic aromatic hydrocarbons).

2.6 Metabolomics as a tool to assess occupational exposure

Metabolomics is defined as the analysis and interpretation of the global metabolic data expressing the multiparametric metabolic response of living systems to genetic modification, pathophysiological stimuli and environmental influences (Serkova and Niemann 2006). Metabolomic analysis can be applied to the study of biological fluids collected in non-invasive or minimally-invasive ways (Baraldi *et al*., 2009).

Sofia *et al*. (2011) reported about the methods used in metabolomic analysis with their pros and cons. They are mass spectrometry (MS) or nuclear magnetic resonance (NMR)-based spectroscopy, since these techniques can handle complex biological samples with a high sensitivity, selectivity and throughput. MS is a powerful method for identifying and quantifying metabolites and it is considered more sensitive than 1H-NMR. MS works in the range of femtomoles while ^{1}H-NMR works in the range of micromoles, if provided with cryoprobe. ^{1}H-NMR spectroscopy enables the detection of almost all proton-containing metabolites in a sample. Some of the advantages of this technique are that it is non-selective, fast, non-destructive and usually demands no sample preparation. Recently, NMR has been applied to biofluids to probe the metabolic status and to investigate different diseases.

Different approaches can be applied in the study of metabolites. Metabolite targeting is the most direct approach, aiming to identify and quantify a specific metabolite, the product of an enzymatic pathway or the degradation product of a drug or toxic agent. Metabolite fingerprinting, however, is a truly comprehensive methodology that aims to classify samples without any apriori hypothesis to identify metabolite patterns associated with a given pathological condition, with an environmental or genetic change, or with exposure to a given toxic agent or drug. Metabolic fingerprinting does not necessarily involve identifying each metabolite, but tries to detect the metabolic characteristics that discriminate between groups of subjects. The search for these metabolic patterns is open to new findings (Carraro *et al*., 2010).

NMR and MS spectra are highly complex and the biological information they contain can only be extracted by applying bioinformatics tools, such as pattern recognition methods. These are computer-based procedures that can be classified as unsupervised or supervised. Once a metabolic pattern typical of a given condition has been characterized, the analysis may go on to identify single biomarkers relevant to sample clustering. Fundamental support for molecular identification comes from various on-line databases, the most comprehensive of which is the Human Metabolomic Database (HMDB) (Baraldi *et al*., 2009).

Bouatra *et al*. (2013) reported that for urinary metabolomics study, an online database central repository platform containing the complete set of 2651 confirmed human urine metabolite species, their structures (3079 in total), concentrations, related literature references and links to their known disease associations are freely available at (http://www.urinemetabolome.ca.) called as Urine Metabolome Data Base (UMDB). The UMDB through its MetaboCard / HMDB links includes fully hyperlinked human metabolic pathway maps (http://www.smpdb.ca/). These maps are intended to help users visualize the chemical structures on metabolic pathways and to get detailed information about metabolic processes. These UMDB pathway maps are quite specific to human metabolism and explicitly show the subcellular compartments where specific reactions are known to take place.