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REVIEW ARTICLE

Nanoparticle exposures from nano-enabled toner-based printing equipment and human health: state of science and future research needs

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ABSTRACT

Toner formulations used by laser printers (LP) and photocopiers (PC), collectively called "toner-based printing equipment" (TPE), are nano-enabled products (NEP) because they contain several engineered nanomaterials (ENM) that improve toner performance. It has been shown that during consumer use (printing), these ENM are released in the air, together with other semi-volatile organic nanoparticles, and newly formed gaseous co-pollutants such as volatile organic compounds (VOC). The aim of this review is to detail and analyze physico-chemical and morphological (PCM), as well as the toxicological properties of particulate matter (PM) emissions from TPE. The review covers evolution of science since the early 2000, when this printing technology first became a subject of public interest, as well as the lagging regulatory framework around it. Important studies that have significantly changed our understanding of these exposures are also highlighted. The review continues with a critical appraisal of the most up-to-date cellular, animal and human toxicological evidence on the potential adverse human health effects of PM emitted from TPE. We highlight several limitations of existing studies, including (i) use of high and often unrealistic doses *in vitro* or *in vivo*; (ii) unrealistically high-dose rates in intratracheal instillation studies; (iii) improper use of toners as surrogate for emitted nanoparticles; (iv) lack of or inadequate PCM characterization of exposures; and (v) lack of dosimetry considerations in *in vitro* studies. Presently, there is compelling evidence that the $PM_{0.1}$ from TPE are biologically active and capable of inducing oxidative stress *in vitro* and *in vivo*, respiratory tract inflammation *in vivo* (in rats) and in humans, several endpoints of cellular injury in monocultures and co-cultures, including moderate epigenetic modifications *in vitro*. In humans, limited epidemiological studies report typically 2–3 times higher prevalence of chronic cough, wheezing, nasal blockage, excessive sputum production, breathing difficulties, and shortness of breath, in copier operators relative to controls. Such symptoms can be exacerbated during chronic exposures, and in individuals susceptible to inhaled pollutants. Thus respiratory, immunological, cardiovascular, and other disorders may be developed following such exposures; however, further toxicological and larger scale molecular epidemiological studies must be done to fully understand the mechanism of action of these TPE emitted nanoparticles. Major research gaps have also been identified. Among them, a methodical risk assessment based on "real world" exposures rather than on the toner particles alone needs to be performed to provide the much-needed data to establish regulatory guidelines protective of individuals exposed to TPE emissions at both the occupational and consumer level. Industry-wide molecular epidemiology as well as mechanistic animal and human studies are also urgently needed.

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Abbreviations: A549: adenocarcinomic human alveolar basal epithelial cells; ACH: air changes per hour; APS: aerodynamic particle sizer; ASHRAE: American Society of Heating, Refrigerating, and Air-Conditioning Engineers; BCMN: buccal cells with micronuclei; bw: body weight; CAS8: clustered Regularly Interspaced Short Palindromic Repeat-associated protein 8; CAT: catalase activity; CB: carbon black; CC-16: Clara cell protein 16; CMD: count median diameter; CPC: condensation particle counter; Cpd: compound; Cr: chromium; CRP: C-reactive protein; DICA: damage index by Comet assay; Dnmt3a: DNA methylation machinery; EC: elemental carbon; ECP: eosinophilic cationic protein; EDX: energy dispersive X-ray spectroscopy; EGF: epidermal growth factor; ELCR: excess lifetime cancer risk; ENM: engineered nanomaterial; Fe: iron; FEV1: forced expiratory volume in 1 s; FGF: fibroblast growth factor; FRAC: ferric reducing antioxidant capacity of serum; FVC: forced vital capacity; GM-CSF: granulocyte-macrophage colony-stimulating factor; GSH/GSSG: reduced versus oxidized glutathione ratio; GPX1: glutathione peroxidase 1; HO1: heme oxygenase 1; IAQ: indoor air quality; ICAM-1: intercellular adhesion molecule 1; IFN: interferon; IL: interleukin; IP: interferon γ-induced protein; LDH: lactate dehydrogenase; LINE: long interspersed nuclear element; LP: laser printer; LTB4: leukotriene B4; MCP: monocyte chemoattractant protein; MCR: medical case report; MFP: multifunction printer; MIP: macrophage inflammatory protein; Mn: manganese; MPPD: multiple particle path dosimetry; NEP: nano-enabled product; Ni: nickel; NP: nanoparticle; O3: ozone; OC: organic carbon; OR: odds ratio; PAH: polycyclic aromatic hydrocarbons; PC: photocopier; PCM: physico-chemical and morphological; PEPs: laser printer-emitted particles; PM:

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particulate matter; PM0.1: particulate matter <0.1 μm in diameter; RANTES: regulated on activation, normal T cell expressed and secreted; RR: risk ratio; SDS: safety data sheets; Si: silicon; SOA: secondary organic aerosols; SOD1: superoxide dismutase 1; STEM: scanning and transmission electron microscopy; SVOC: semi-volatile organic compounds; TBARS: thiobarbituric acid reactive substances; TD: thermodenuder; TE: transposable element; THP-1: human acute monocytic leukemia cell line; Ti: titanium; TNC: total number concentration; TNF: tumor necrosis factor; TPE: toner-based printing equipment; tVOC: total volatile organic compound; UFP: ultrafine particle (<0.1 μm in diameter); USEPA: United States Environmental Protection Agency; VEGF: vascular endothelial growth factor; VOC: volatile organic compound; WHO: World Health Organization; Zn: zinc

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Introduction

In virtually every commercial and home office, retail business, and academic institutions, workers and the public rely on laser printers (LP) and/or photocopiers (PC) for document production in some phase of daily operations. PC have been in commercial use since they were developed in 1937 by Xerox engineers searching for a means to accelerate the in-house document duplicating process with their work turned into a corporate venture in the 1950s. In 1969, engineers at Xerox created the LP (Reilly 2003). During the unprecedented growth of computing and business machines, LP and PC have become faster, more reliable, affordable, and produce higher quality images. LP and PC rely on very similar, if not the same, technology and materials to produce an image onto a sheet of paper. In more detail, irrespective of color or monochrome printing, toner-based printing requires a photosensitive drum to attract the toner powder and fuse it on the paper using elevated levels of pressure and heat (Pettersson & Fogden 2006).

Dry toner formulations are proprietary and generally little is known beyond information reported in Safety Data Sheets (SDS). Universal components of toner include carbon black (CB), organic resin or waxes, styrene, and iron (III) oxide. However, both the exact composition and the manufacturing process vary slightly from manufacturer to manufacturer. Toner is generally made through (1) compounding toner ingredients into a slurry that once dried is mechanically pulverized into a fine powder consisting of irregularly shaped particles with approximate diameters ranging from 5 to 10 μm or (2) emulsion aggregation, which involves extrusion and additional processing that results in uniform spheres with diameters starting at approximately 5 μm to considerably larger sizes (Burns et al. 2002; O'Rourke et al. 2003).

Studies investigating the impact of toner-based printing equipment (TPE) use on indoor air quality (IAQ) date back to the early 1990s in early Sick Building Syndrome investigations. Many of these studies focused on gaseous pollutants such as volatile organic compounds (VOC), ozone (O_3), and to a lesser extent, particulates from a variety of sources (i.e. indoor cooking, microbial spores, and printing and copying) (Harrison et al. 1992; Jones 1999; USEPA 2013). Over the past decade, a considerable body of literature has documented that LP and PC emit significant numbers of nanoparticles during and following their operation.

For clarity, particulate matter (PM) less than 100 nm ($PM_{0.1}$), ultrafine particles (UFP), and nanoparticles (NP) are defined as particles having one or more dimensions of less than 100 nm (OSHA 2011; WHO 2011). Further distinctions are made in the literature between incidental and engineered nanoparticles or nanomaterials (ENM), reflecting their origin of formation (incidental by-products of combustion or other industrial processes versus intentional industrial manufacturing, respectively), formation mechanisms, and whether nanoparticle generation/production was intentional or not. With large-scale commercialization of nanotechnology and widespread market penetration of nano-enabled products, new mixed incidental and engineered nanoparticle exposure scenarios have emerged at various stages of a product life cycle, from the production of raw materials to end-of life recycling and disposal (Pal et al. 2015b; Sotiriou et al. 2015, 2016). Addition of ENM in this mix creates new technical and conceptual challenges related to assessing the chemical and toxicological properties of emissions, untangling the role of ENM form that of incidental nanoparticles, and eventually assessing the risk (Watson-Wright et al. 2017).

In the context of TPE emissions, as we will discuss in detail in later sections, several metal oxides ENM (iron oxide, manganese oxide, copper oxide, titania, alumina, etc.) that are used in toner formulations, become airborne. In general, ENM, are characterized by larger surface area, more active and often catalytic surface chemistry, slower physiological clearance, longer retention in the lungs, and translocation to

extra-pulmonary organs, with subsequent accumulation in the liver and spleen compared with their respective microscopic counterparts (Takenaka et al. 2006; Semmler-Behnke et al. 2007; Kreyling et al. 2007; Semmler-Behnke et al. 2008; Kreyling et al. 2009; Hsieh et al. 2013; Cohen et al. 2014; Konduru et al. 2014; Yokel et al. 2014; Zhou et al. 2014; Konduru et al. 2015; Grassian et al. 2016; Konduru et al. 2016). These physico-chemical attributes of ENM often translate to higher toxicity per unit mass (Demokritou et al. 2013).

Over the past few years, several papers have been published on the exposure characterization and toxicology of PM emitted from TPE, the overall outcome of which is a qualitative shift in our understanding of these exposures and their subsequent human and environmental health implications.

The purpose of this manuscript is to provide a comprehensive and critical appraisal of the existing, and more importantly, emerging literature on the toxicological, epidemiological, and physico-chemical and morphological (PCM) characterization of emissions from TPE. Furthermore, we identify current knowledge gaps and future research needs (e.g. the need to develop better exposure controls and to assessing the disease burden in consumers and workers exposed to this technology), as well as develop the necessary regulatory framework to minimize current exposures and risks.

Methods

Online searches were performed using Google Scholar (https://scholar.google.com), Science.gov (www.science.gov), PubMed, EMBASE, and Web of Science search engines with the search phrases "ultrafine particles and laser printers", "nanoparticles and laser printers", "ultrafine particles from photocopiers", "nanoparticles from photocopiers", "toners", and "toners and patents". The literature was also searched for regulations related to $PM_{0.1}$ in the occupational environment. This search included the United States Occupational Safety and Health Administration (OSHA), the United States Environmental Protection Agency (US EPA), and the World Health Organization (WHO). In addition to online searches, our group has actively investigated TPE PM emissions for several years and has amassed a reasonably thorough library of research publications, from which we draw a great deal of reference material. The search was restricted to peer-reviewed publications in English from the years of 2000–2016.

Results

We included 54 peer-reviewed papers specifically related to toners, emissions, and exposures from TPE. Thirty-two articles focused on emissions and their chemistry, while 22 pertained solely to toxicological characterization. As mentioned earlier, papers that dealt with toxicological assessment of toners, as well as epidemiological studies on workers engaged in toner manufacturing were excluded as being outside the scope of this review. The main reason is that TPE-emitted nanoparticles represent an exposure scenario that is distinct from that of toner manufacturing.

Exposures

A chronological summary of main studies related to exposures is presented in the top part of Figure 1. It is worth noting that other important papers were published but could not been included for the sake of space limitations. In this timeline, only breakthrough discoveries/papers in this research area were included based on authors' personal assessment of events. The topic of exposures from TPE has been studied continuously over the past 15 years. Earlier studies focused on emissions of VOC and O_3, then PM_{10}, $PM_{2.5}$, and only recently the attention has shifted to $PM_{0.1}$ and ENM in toners and emitted PM. Thirty-two studies have been published on exposures from the years 2000 to 2015. Table 1 provides a summary of the exposure and emission studies from TPE with a primary focus on particle and gaseous co-pollutant emissions.

It is important at this stage to make some important distinctions between three concepts related to exposures to nanoparticles in general and, more specifically, the TPE-emitted nanoparticles: emission measurements, area measurements in lieu of surrogate personal exposures, and personal exposure measurements. Briefly, emission measurements refer to measurement at the point of release or emission of a pollutant. Area measurements refer to collection of information in affixed location inside a room or building floor. Personal measurements refer to use of a sampler in the breathing zone (lapel) of a person (often a worker over the duration of the workday or activity of interest. In the context of TPE-emitted nanoparticles, emission measurements are taken next to the printer or he exhaust point of the photocopier; area measurements are taken at some distance in the room (often in the center of the room or between the photocopier and the desk). In personal sampling, nanoparticle monitors are worn by photocopier operators for the whole day.

None of the studies discussed here conducted true personal exposure assessment to such nanoparticles, the primary reason being lack of personal nanoparticle monitors, which have become commercially available only recently. Although advantages of personal sampling are well understood and documented in the quantitative exposure assessment literature, this is a technologically driven limitation. All studies discussed here fall in the emissions or area measurements. Area measurements are used as surrogates of average personal exposures and, for this reason, it is important to understand how they compare to personal exposures. The issues has been studies extensively in the quantitative exposure assessment literature in occupational settings and personal monitoring almost always results in higher exposure estimates than area measurements (Demokritou et al. 2001; Rappaport & Kupper 2008). Emission measurements typically would result in higher exposure estimates than personal exposures. This is because of two primary factors: (i) that airborne concentration of a pollutant (including nanoparticles) decreases with distance from the source (due to dilution in the air and removal of contaminant from the air, e.g. nanoparticles agglomerate and/or attach to larger particles and settle); and (ii) that because workers (or consumers) move around during the day, their daily personal exposures reflect not only

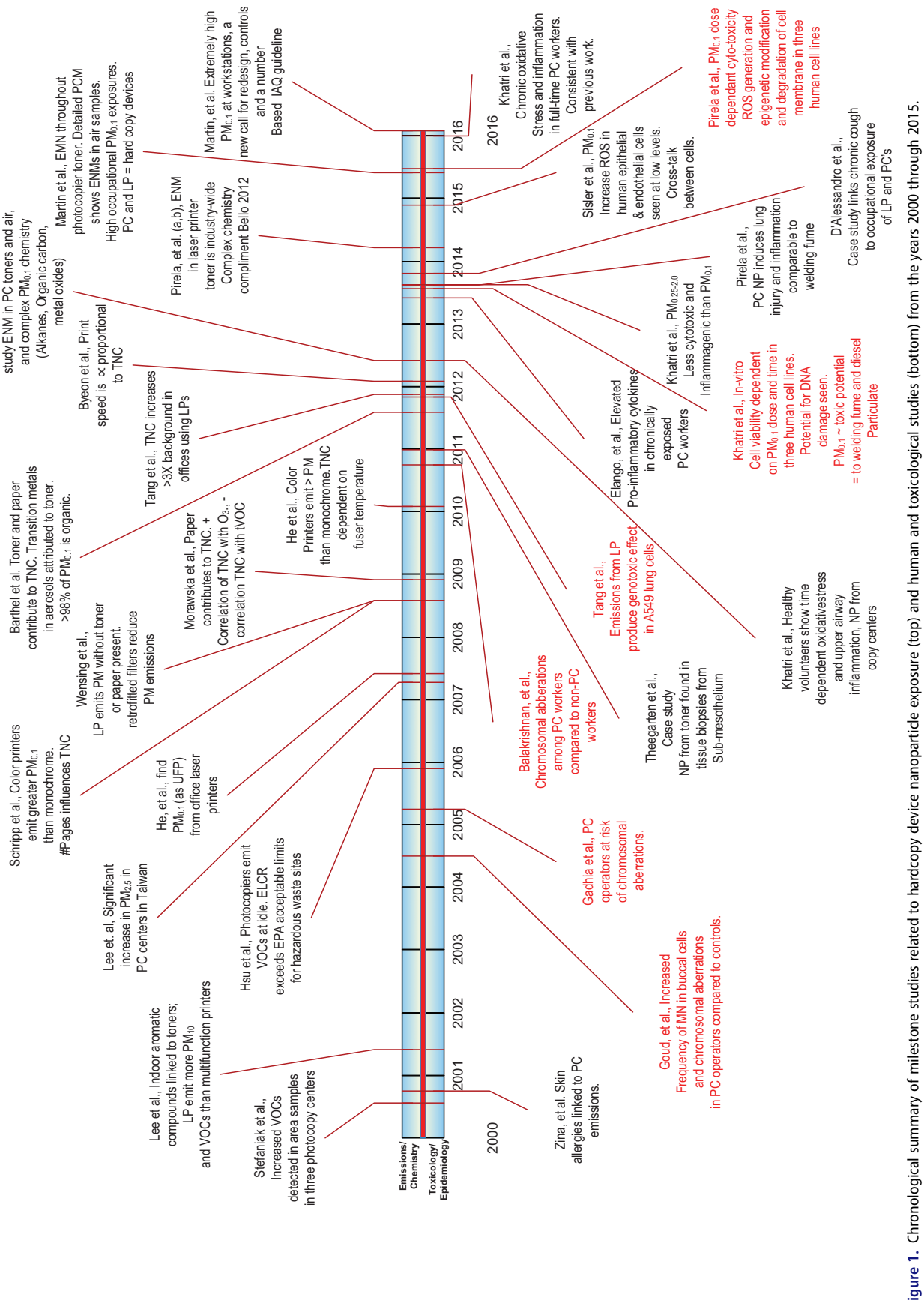

Figure 1. Chronological summary of milestone studies related to hardcopy device nanoparticle exposure (top) and human and toxicological studies (bottom) from the years 2000 through 2015.

proximity to the source but also the time spent at different locations.

Martin et al. (2017) monitored the activity in 15 photocopy centers in Massachusetts and found that photocopier operators often spent on average 63% of their time at the desk (range 35–90%), which was located on average 1.7 m away from the nearest photocopier (range 0.7–3 m).

Exposure data collected in chamber studies that employ small volume chambers (1 m^3 or less) would be considered source/emission studies. If chambers are designed to mimic a room, then depending on the location of instruments, the data can be considered as area or source measurements.

It is also important to note that a variety of real-time aerosol measurements instruments have been used over time. Their specifications, such as size range of nano/particle and time to generate a full-size distribution, vary, and thus global nanoaerosol measurement metrics, such as total number concentration and size distributions, vary somehow between instruments. A detailed discussion of this issue is outside the scope of this review and the interested reader is encouraged to consult available reviews on this topic (e.g. Table in Amaral et al. 2015).

In subsequent sections, we provide an analysis of the aforementioned studies, distinguishing, where appropriate, between emission studies and area-based exposure studies.

Gaseous pollutants

In one of the first investigations in commercial photocopy centers with a focus on VOCs, Stefaniak et al. (2000) documented conditions inside three photocopy centers that included a total of seven high-volume PC. Measureable concentrations of short-chain alkanes (C5-C11) ranging from 0.1 to 21,300 ppb, as well as aromatic solvents including benzene, ethylbenzene, toluene, and xylenes were found in area and personal air samples collected from each center. The authors concluded that the reported chemical composition of indoor air samples was attributed to the toner formulations and PC use. In a side-by side comparison study of LP and inkjet printers, Lee et al. (2001) found that LP significantly release greater levels of toluene, ethylbenzene, styrene, and PM_{10} compared with inkjet and desktop multifunction devices. Because LP and PC employ nearly identical physical process and toner formulations to create an image, Lee's findings were in good agreement with those from Stefaniak.

In a controlled chamber study of 17 PC from three different manufacturers, Hsu et al. (2005) reported PC emit measurable amounts of VOC during the idling and printing stage. The authors found benzene concentrations ranged from 90 to 121 $\mu g/m^3$. Similarly, another study investigating VOC from PC found significant concentrations of benzene, toluene, styrene, ethylbenzene, alkanes, acetophenone, and several aldehydes (Lee et al. 2006). A later assessment of LP emissions using chambers verified the presence of styrene and xylene in the emissions in addition to O_3 at levels of up to 30 ppb. Further, the same evaluation was done in an office environment and found that VOC were also emitted by LP at elevated levels (200 $\mu g/m^3$) (Kagi et al. 2007). Soon after, Barrero-Moreno et al. (2008) measured the gaseous pollutants emitted in a chamber and found the released compounds included styrene, xylenes, ethylbenzene, toluene, and particularly, 2,3-dimethyl-2,3-diisobutyl succinonitrile encompassed up to 90% of the VOC identified in the study. Later, Barrese et al. (2014) documented O_3 at levels of 45 ppb in an office-sized room and a notable increase in VOC.

Wang et al. (2012) performed qualitative analysis showing TPE emission of alkanes ranging from C21 to C45, as well as carboxylic acids, esters, and other organics. The authors found O_3 emission rates ranging from 9.7 to 21.1 $\mu g/min$ and total VOC (tVOC) concentrations of up to 400 ppb. Further, nine unsaturated organic compounds were found to originate from the toner and paper. Higher levels (up to 2889 ppb) of VOC were measured by Pirela et al. (2014) in a chamber study assessing 11 laser printers from various manufacturers. Moreover, Mullins et al. (2013) documented the release of a number of polyaromatic hydrocarbons (PAH) from toners using a simulated printing process. Benzene and trichloroethene were found to be emitted at concentrations greater than 1.15 $\mu g/m^3$ (0.36 and 0.21 ppb, respectively). Further, the authors documented 14 PAH including phenanthrene, fluoranthene, pyrene, and benzo(b)fluoranthene with maximum total emissions of 2.3 μg/page.

While a number of studies have performed chemical speciation on various gaseous co-pollutants emitted, and not just VOC, by TPE during a print job, there are still some knowledge gaps on the topic. For example, chemical interaction between the organic components in these emissions, their absorption on the surface of PM constituents, partitioning between gas/vapor and the PM phase, the impact of aging of the TPE-released pollutants, and their potential toxicological implications remain unknown.

PM. The first report on PM exposures in photocopy centers was done by Lee and Hsu (2007), who investigated indoor air quality in several photocopy centers in Taiwan for 9 months. The authors found the occupational environment was often poorly ventilated and cramped with high-volume PC with high levels of $PM_{2.5}$ ranging from 10 to 83 $\mu g/m^3$, with average measurements around 40 $\mu g/m^3$. The $PM_{2.5}$ values regularly exceeded the acceptable 24-h threshold limits of 100 $\mu g/m^3$ established by the Taiwan Environmental Protection Agency. Values as high as 1.0×10^8 particles/cm^3 were reported during the printing process, with NP accounting for the majority of the total number concentration (TNC).

Since 2007, heightened sensitivity and sophistication of real-time particle measuring devices, as well as an increased awareness and research interest on this topic resulted in a groundswell of publications from 2007 to the present, especially in controlled environmental chambers.

In the first large-scale investigation of LP emissions conducted in an open plan office environment, He et al. (2007) observed increases in TNC up to 3.8×10^4 particles/cm^3. Based on this result, the authors followed with a comprehensive chamber study where significant variation in emissions was observed over the LP studied. Furthermore, emissions varied significantly from one LP model to another, which led to a simple classification structure wherein 40% of the printers studied were classified as "high emitters".

Table 1. Summary of exposure studies from toner-based printing equipment (TPE) from 2000 to 2015. These studies focused primarily on particle emissions and gaseous pollutants.

Year	Author	Study information	Main findings
2000	Stefaniak et al.	• Photocopy centers • VOC/chemical characterization • Integrated area and personal samples	• Area concentrations include alkanes (C5–C11), aromatic solvents (ethylbenzene, trimethylbenzene(s), xylene, toluene) in ppb range • Personal samples generally mirror area concentrations • Chemical composition and concentrations vary from center to center
2001	Lee et al.	• Chamber • Integrated samples for VOC; real-time measurements (TSI Dust Trak 8553); real-time PID VOC screening; O_3	• LP emit aromatic compounds (toluene, ethylbenzene, *m–p*-xylene, and styrene) linked to toners • tVOC emissions were measured to be much higher in LP compared to inkjet • Average O_3 emission rates for LP were 100 times greater than inkjet are the MFP • LP significantly increase particulate emissions
2005	Hsu et al.	• Chamber • Integrated sampling for aromatic compounds	• PCs emit volatile aromatic compounds even at idle • Benzene (carcinogenic to humans) was found at 90–121 $\mu g/m^3$ across three manufacturers. • The hazard index exceeded 1.0 for ethylbenzene • The ELCR calculated exceeds EPA acceptable limits for hazardous waste sites for benzene
2006	Lee et al.	• Photocopy centers • Investigation of health impacts of VOC in photocopy centers • Integrated area and personal samples	• All photocopiers emitted significant VOC at idle • Benzene emitted in significant quantities. ELCR greater than the USEPA lifetime risk threshold at waste cleanup sites (1×10^{-6}) • Calculated hazard indices ranged from 1.8 to 26.2
2007	He et al.	• Chamber and Office • UFP measurements in the office from LP • Real-time measurements (TSI SMPS; TSI CPC 3022; TSI CPC 3025 A; TSI P-Trak; TSI Dust Trak) • Area monitoring	• TNC increased to 38,000 particles/cm^3 • CMD 40–76 nm • Particle emissions vary significantly from one model to another
2007	Lee et al.	• Photocopy center • Particle, VOC and O_3 assessment in the workplace • Real-time measurements (TSI SMPS 3934; TSI APS 3320; TSI Dusttrak 8553) • Integrated samples for aromatics and VOC • Area samples	• UFP fraction accounted for the majority of total number concentration • O_3 concentrations 10–70 ppb • VOC include benzene, toluene, styrene, ethylbenzene, alkanes, acetophenone, and aldehydes. Related to toner and paper
2007	Kagi et al.	• Office and Chamber • Particle, VOC and O_3 emissions assessment • Real-time measurements (TSI FMPS 3090; TSI CPC 3022A) • Integrated sampling for VOC • Area samples	• Office O_3 increased from 1.5 to 6 ppb during printing. • Office xylene & styrene up to 200 $\mu g/m^3$ during printing. • Chamber O_3 increased to 30 ppb • Xylene in the chamber 2300 $\mu g/m^3$
2008	Schripp et al.	• Chamber • Particle measurements from color and monochrome printers • Real-time measurements (TSI SMPS 3090; TSI FMPS 3091) • Area samples within the chamber	• Max TNC 1.97×10^6 particles/cm^3 • Color printers emit greater TNC than monochrome • Printers are generally "initial burst" type. • Number of printed pages influence TNC
2008	Wensing et al.	• Chamber (1 m^3) • Office (72 m^3) • Particle formation mechanisms and measurements; filter efficiency; and ventilation impact • Real-time measurements (TSI FMPS 3090; TSI SMPS 3080; TSI CPC 3022 A; TSI P-Trak) • Area samples	• Modified printer without toner or paper still emits UFP • Chamber measurements 1×10^6 particles/cm^3 • Office TNC high with and without fan • Retrofitted filters may reduce emissions by 21–84%
2008	Gehin et al.	• Chamber • Size distribution and emission rate investigation • Real-time measurements (DMS500 particle spectrometer; Grimm 1.108) • Area samples	• Nanoparticle mode diameter(s) were reported to be 21 nm • Emissions rates were calculated to be 2.3×10^{10} particles per second
2009	Morawska et al.	• Chamber • Characteristics and formation mechanisms • Real-time measurements (TSI SMPS 3080; CPC 3022A and CPC 3781) • Real-time O_3 and VOC monitoring • Integrated VOC and SVOC sampling • Area samples	• TNC and VOC emissions dependent on operating temperature • O_3 levels low, 7–10 ppb • Particles both volatile and water insoluble • Paper contributes to TNC • Positive correlation between UFP and O_3; negative correlation between UFP and tVOC concentrations • Identified VOC included xylenes, ethylbenzene, styrene, cyclohexane and toluene

(*continued*)

Table 1. Continued

Year	Author	Study information	Main findings
			• CMD 28–63 nm
2010	He et al.	• Chamber • Particle formation related to printer temperature • Real-time measurements (TSI SMPS 3934; TSI CPC 3022A) • Area samples	• Color LP higher emitters than monochrome LP • Emissions range over three orders of magnitude • Printers begin emitting when turned on • Fuser temperature significant in emission rates
2010	Koivisto et al.	• Chamber • Particle emissions from new LP • Real-time particle measurements (Grimm SMPS 5400; TSI CPC 3776) • Area samples	• Average TNC increased 11 times background over 6-h period • Maximum particle concentration up to 1.1×10^6 particles/cm^3 • Modeled peak emission rates $>7.0 \times 10^8$ particles/second • 26,000 particles/cm^3 maximum modeled concentration • Modeled ventilation does not reduce maximum particle concentration, but increases particle reduction after printing
2011	Wang et al.	• Print center and chamber • Nano particle and VOC emissions characterization, formation mechanisms and controls • Real-time measurements (TSI CPC 3010 and 3775; TSI SMPS 3080). • Integrated samples (VOC via tenax tube; TSI ESP 3100) • Area samples	• Particle emissions reached 1.6×10^6 particles/cm^3 • VOC and $PM_{0.1}$ are dependent on temperature gradient between the interior and exterior environment of the printer • Distance from LP reduces TNC • Cartridge age affects particle emissions • Cold start mode diameter ~30 nm
2011	Barthel et al.	• Chamber • Elemental analysis of UFP from LP • Real-time measurements (Grimm CPC 5414; TSI CPC 3775) • XRF analysis • Integrated area samples	• TNC ranged from 2.7×10^6–7.6×10^9 particles/page • Transition metals in $PM_{0.1}$ attributed to the toner (Si, Cr, Fe, Mn, Ni, Ti, Zn) • Toner and paper contribute to aerosol composition • P, Br, and Sb from printer components • TD analysis suggests 98–99% of the measured particles are organic in nature (SVOC)
2011	Tang et al.	• Office space • LP contaminants in office(s) • Real-time measurements (Grimm 1.108; TSI CPC 3007) • Area samples	• TNC increased by ~3 times in offices during printing • Maximum particle concentration: 1.9×10^5 particles/cm^3 • TNC ranged from 1×10^3 to 8×10^4particles/cm^3 • Detectable particles emitted in standby mode
2011	McGarry et al.	• Office space • Exposure to LP-emitted particles and offices • Real-time measurements (TSI CPC 3025 A; TSI CPC 3781; TSI P-Trak; TSI Dusttrak 8553) • Area samples	• TNC peaks exceeded 1.0×10^5 particles/cm^3 in six of 15 events recorded • TNC during operating hours is significantly greater than non-operating hours • Distance from LP reduces TNC
2011	Betha et al.	• Photocopy center • UFP and VOC in commercial print centers • Integrated samples for VOC • Real-time measurements (TSI FMPS 3091) • Carbon black measured by Aethalometer (AE-31) • Area samples	• VOC associated with printer toner and solvents increased during printing compared to non-printing • Particle size distribution dependent on ventilation rate. ACH <1.0 resulted in smaller particles and bimodal distribution • Number of printed pages and machine age affect size distribution • UFP peak diameter ~50–100 nm • Reduced ACH resulted in bimodal distribution with peak diameters of ~20 nm and 80 nm
2012	Byeon et al.	• Chamber • Particle emission rates and print speed. • Real-time measurements (TSI SMPS; TSI CPC 3025; TSI Differential Mobility Sizer 3081; Kanomax 3521 particle mass monitor). • Area samples	• Particle number concentration and print speed are inversely proportional • Average size mobility diameter is proportional to print speed • Decrease in TNC related to particle coagulation • Average mobility equivalent particle diameter 50–244 nm
2012	Wang et al.	• Chamber • O_3 initiated particle formation and precursors in a laser. • Real-time measurements (O_3 UV-106 O_3 analyzer; real-time VOC by handheld PID; particle size distributions by TSI SMPS and TSI CPC 3022) • Area samples	• Even without toner, the TPE emit particles • [tVOC] up to 400 ppb • O_3 emissions 9.7–29.1 μg/min • VOC originate from toner and paper • Toner coverage increases TNC • Average diameter ~45 nm
2012	Salthammer et al.	• Chamber • Aerosols generated by TPE • Real-time measurements (TSI FMPS 3091); TSI 3065 thermo-denuder analysis; integrated samples for GC/MS analysis; integrated samples for SEM analysis with EDX • Area samples	• TNC 3.0×10^{10}–4.0×10^{12} particles/print • CMD 30–35 nm • EDX detected Al, Cl, Ca, Co, Cr, Cu, Fe, Mg, Mn, Na, Ni, P, S, Si, Ti, and Zn • Particles have high organic content

(*continued*)

Table 1. Continued

Year	Author	Study information	Main findings
2012	Castellano et al.	• Chamber • Comprehensive particle assessment of LP emissions • Real-time measurements (TSI FMPS 3091; size segregated samples by Nanomoudi cascade impactor; integrated samples for VOC by sorbent tubes; elemental analysis by SEM EDX • Area samples	• Peak TNC 3.5×10^5 particles/cm^3 • Mass concentration ~20–100 μg/m^3 • O_3 ~100 ppb • VOC include alkanes, esters, aromatic solvents, siloxane(s) related to toner • Na, B, K, Zn, and Sr identified by EDX
2012	Jayaratne et al.	• Chamber • Emissions rates of ions from LP • Real-time measurements (TSI CPC 3022 A and P-Trak; size distribution by TSI SMPS 3934 and 3936; particle charge concentration determined by TSI aerosol electrometer 3068) • Area samples	• LP released significant quantities of ions and charged particles into the environment • Significant variation in TNC, particle charge, and CMD between print jobs • No relationship found between charge-TNC and charge-CMD
2012	Sarkhosh et al.	• Photocopy center • VOC characterization in photocopy centers • Integrated air sampling for VOC; real-time VOC monitoring by handheld PID • Area samples	• 19 VOC were detected in the area samples, which increased during business hours • Toluene was significantly higher than outdoor levels • Indoor to outdoor concentration ratio for benzene, toluene, ethylbenzene, and xylene is measured at 42 • Mean VOC concentrations were seasonally correlated with greater concentrations measured in winter compared to spring when windows are open enabling greater natural ventilation
2013	Bello et al.	• Photocopy center • Comprehensive physicochemical characterization of emissions • Real-time measurements (TSI FMPS 3091; TSI APS 3321; TSI CPC 3007; O_3 monitoring) • Size selective integrated sampling for VOC; SVOC; elemental analysis • SEM/TEM analysis on toners and particles • Area samples	• Peak TNC 2×10^5 particles/cm^3 • CMD 34.5 nm • $PM_{0.1}$ mass 4.3–4.9 μg/m^3 • First report of ENM in emitted aerosol related to toner (SEM/TEM) • Fe, Si, S, Ti, Mn, Al, Zn, Sn, P, Mg, and Ca in aerosol and toner • Alkanes C20-C40, 60–2670 ppm in aerosol, minor contributor of total mass; no PAHs in toners, traces in workplace aerosol • Mass balance: OC 49.8%, EC <1%, inorganic ~50%
2013	Mullins et al.	• Chamber • PAH assessment from LP • Simulated printing by heating toners to operating temperatures of LP • Mass loss during feeling measured by Thermogravimetric analyzer • PAH analysis conducted on toners before and after heating	• 14 PAHs detected (including Phenanthrene, fluoranthene, pyrene, and benzo(b)fluoranthene >8.5 mg/kg • Demonstrates PAH emissions from toner under printer conditions. • VOC estimated emissions 2.7 μg/page • Maximum PAH emissions estimated at 82.1 μg/minute (2.3 μg/page)
2014	Pirela et al. (a, b)	• Chamber • Development of emissions exposure platform; physicochemical characterization of LP emissions • Real-time measurements (TSI FMPS 3091, TSI APS 3321, TSI CPC 3007; O_3, VOCs) • Size selective integrated samples for VOC analysis; elemental analysis • TEM/SEM analysis with EDX on toners and printer emitted particles	• Max TNC up to 1.3×10^6 particles/cm^3 • Particle size 39–138 nm • Greater page coverage increases TNC • VOC up to 2.9 ppm • Concludes that ENM are widely used in toners, and are released in air, substantiating Bello et al. study • Si, Al, Ti, Fe, Zn, Cu Ce, and carbon black found in toners as their corresponding metal oxides EMN • Complex chemical composition: EC, 0.001–0.5%; OC, 50–90%; inorganics, 1–3%; soluble anions and cations 0.1–1.4% • Large variability in chemistry of emissions between printer manufacturers
2014	Kowalska et al.	• Chamber • Measurement of chlorinated VOC from toner-based printing equipment. • Integrated air sampling for VOC. GC MS analysis • Area samples	• All tested devices emit some carcinogens (e.g. benzene and trichloroethylene) relative to the background of the test chamber • Tested office printers and copiers emit harmful substances • Organizational structure of the work environment should consider placement of office printers and copiers
2014	Barrase et al.	• Print center • IAQ: VOC, formaldehyde, and O_3 by LP • Integrated air samples for GC/MS and HPLC analysis • O_3 analysis by 42 M O_3 Analyzer • Area samples	• VOC increase to ~4.5 mg/m^3 with printing • Printing does not add formaldehyde to indoor air • O_3 measured at ~45 ppb while printing • Filtering may reduce contaminates by up to 50%
2015, 2017	Martin et al.	• Photocopy center • IAQ at photocopy centers, physicochemical characterization of toners and PM • Real-time particle measurements (TSI FMPS 3091; TSI APS 3321; TSI CPC 3007; TSI Q-Trak 8565)	• CMD range from 23 to 35 nm; σ_g 1.7 to 2.1. • ENM found in 100% of toners studied and in workplace aerosols • Elemental composition from toner and paper: S, Ca, Na, Fe, K, Zn, Ti, Ce, Mn, Ni, Si. • $PM_{0.1}$: OC, 5.1–63.0%; EC, <1%; Inorganic fraction ~36.4–94.0% • Color printers emit less OC

(*continued*)

Table 1. Continued

Year	Author	Study information	Main findings
		• Size selective integrated samples for elemental analysis • TEM/SEM analysis of toners and PM. Area samples	• TNC at workstation(s) average 1800–23,400 particles/cm^3 • TNC max at workstation 265,000 particles/cm^3 • Number of copies most significant predictor of TNC • Most centers lacked any engineering controls, but met existing IAQ guidelines • Calls for installation of engineering controls on PCs, improved design characteristics of, and revisions/updates on indoor air quality guidelines for PC centers

ACH: air changes per hour; cm^3; APS: aerodynamic particle sizer; cubic centimeter; CMD: count median diameter; CPC: condensation particle counter; Cpd: compound; EC: elemental carbon; ELCR: excess lifetime cancer risk; ENM: engineered nanomaterial(s); TPE: toner-based printing equipment; IAQ: indoor air quality; LP: laser printer; MFP: multifunction printer; NP: nanoparticle; O_3: ozone; OC: organic carbon; PC: photocopier; $PM_{0.1}$: particulate matter $<0.1\,\mu m$ in diameter; SVOC: semi-volatile organic compounds; TD: thermo-denuder; TNC: total number concentration; tVOC: total volatile organic compound; UFP: ultrafine particle ($<0.1\,\mu m$ in diameter); USEPA: United States Environmental Protection Agency; VOC: volatile organic compound.

Significant emissions were observed with a count median diameter (CMD) that ranged from 40 to 76 nm, which suggests the majority of measured particles were $PM_{0.1}$ and this has been verified in multiple subsequent studies (Géhin et al. 2008; Salthammer et al. 2012).

Temporal characterization of emissions is an important consideration and several studies have investigated the TPE-emitted PM before, during, and after their use. Schripp et al. (2008) reported $PM_{0.1}$ emitted from LP at concentrations of 1.1×10^5 to 2.2×10^6 particles/cm^3 with CMD ranging from 22 to 40 nm and concluded LP generally fall into two categories based on their temporal emissions patterns. One type of emission profile is the "initial burst", which is characterized by a sharp increase in TNC immediately following the beginning of a print job, and a subsequent sharp decrease in particle concentration when printing has ceased. In contrast, the "constant emitter" pattern is characterized by a less pronounced increase in TNC at the onset of a print job followed by a much more gradual decrease with time. Tang et al. (2012b) assessed 59 LP and 4 PC in office environments and measured $PM_{0.1}$ at levels of up to 1.9×10^5 particles/cm^3 with average TNC from 1.0×10^3 to 80×10^3 particles/cm^3. This study found $PM_{0.1}$ concentrations tripled in offices when printing was taking place compared with non-printing even in standby mode. Further, other reports have shown indoor TNC is much greater during office hours (i.e. frequent printing events) than non-business hours.

An important development in the early 2010s was a shift in focus on the morphological and chemical composition of the size fractionated TPE-emitted PM fraction, starting with initial analysis for metal oxides and inorganics widely reported in toner SDS. For example, Barthel et al. (2011) and Pirela et al. (2015) conducted experimental work that focused on the elemental and chemical composition of TPE-emitted $PM_{0.1}$. In these chamber studies, 10 and 11 LP were assigned scripted print tasks and elevated TNC were observed and attributed to the toner used. Further, several transition metals, silicon (Si), chromium (Cr), iron (Fe), manganese (Mn), nickel (Ni), titanium (Ti), and zinc (Zn) were found and attributed directly to the toner formulations used. For the first time in the study of TPE emissions, Barthel et al. (2011) used a thermodenuder as a part of the analytical assessment in order to expose the aerosol to very high temperatures and effectively strip the particles of any organic content. The analysis showed that 98–99% of the aerosol emissions in their case were found to be organic in nature; however, no additional analysis to resolve organic content was reported. Salthammer et al. (2012) conducted similar experiments and found emissions contained high organic content, particularly long-chain aliphatic and carboxylic acid esters were detected.

A majority of assessments investigating LP emissions have occurred within controlled environmental chambers, which allow researchers to gain an understanding of the magnitude of PM emission, composition, and general mechanisms of formation. Chambers allow investigators to precisely control experimental variables (e.g. relative humidity, temperature, air exchange rate, ventilation rate, operational parameters). Further, in this experimental setting, there are no other sources of emissions but those from the TPE, thus isolating both

the PM and the gaseous co-pollutants for PCM analysis. In addition to the chamber studies, which are critical in terms of generating real world exposures and assessing their PCM and toxicological properties, exposure assessment studies in various occupational and residential settings are needed to assess human population exposure levels (Wensing et al. 2008).

More recent studies by the authors provide a comprehensive PCM characterization of size fractionated airborne PM for both LP and PC. These studies were built on the hypothesis that ENM had been incorporated into toner formulations and that wide-ranging testing approaches and platforms were needed to properly understand the problem. In the first report to affirm ENM in dry toner formulations, Bello et al. (2013) conducted extensive PCM analysis of PM sampled in a high-volume commercial photocopy center in the US. The photocopy center housed 2 PC from the same manufacturer and used toner with similar ingredients. The results of this multiphasic study included TNC in excess of 2×10^5 particles/cm^3, the majority of which was $PM_{0.1}$ with primary particle sizes ranging from 30 to 40 nm (CMD: 34.5 nm), secondary particle sizes of approximately 8–10 nm, and $PM_{0.1}$ mass concentrations of 4.3–4.9 μg/m^3.

First, the study by Bello et al. (2013) documented for the first time that toners used in PC constitute a NEP with several ENM in toners become airborne during printing, which contain transition metals and metal oxides (i.e. Fe, Si, Ti, Mn, aluminum (Al), Zn, antimony (Sb), phosphorus (P), magnesium (Mg), calcium (Ca)). Moreover, scanning and transmission electron microscopy (STEM) with energy dispersive X-ray spectroscopy (EDX) analysis found visual and analytical evidence of several types of ENM in both toner and airborne aerosols. Second, semi-volatile organic compounds (sVOC) such as long-chain alkanes in toners were found in the PM at concentrations ranging from 10 to 2670 ppm but their contribution to the total airborne mass was negligible (<2%). Third, the study provided for the first time a mass balance picture of TPE emissions. The PAH composition (phenanthrene, fluoranthene, pyrene, chrysene, and benzo(b)fluoranthene) in the airborne fraction was generally low (10–50 ppb), but no PAH were found in the toners. There are similarities in organic content of photocopy center $PM_{0.1}$ at 50–70% organic carbon (OC) by mass, suggesting a significant amount of organic material of indeterminate composition present in PC emissions. Lastly, little PM elemental carbon was found in this study (approximately 0.1–0.6%), although toners contained between 5.6 and 5.9% EC. This overall approach was subsequently used in a field study in eight copy centers Martin et al. (2015).

Pirela et al. (2014) developed a laboratory-based exposure platform designed to generate real-world exposures suitable for the PCM and *in vitro* and *in vivo* characterization studies. This exposure generation platform was utilized to characterize PM emissions from 11 most commercially used monochrome LP. This platform enabled the authors to collect size-fractionated PM samples for off-line PCM and *in vitro* characterization and was also used in animal inhalation studies. The authors confirmed for the first time that toner formulations of LP constitute a nano-enabled product in agreement with Bello et al. (2013) study for PC. They also confirmed that ENM from toners are released in the air during printing missions and found emission PM peaks of approximately 1.3×10^6 particles/cm^3, with most of the emitted PM with diameters of less than 100 nm, also consistent with the earlier observations of Bello et al. (2013). More importantly, Pirela documented at least eight different ENM in dry toner formulations and, in the $PM_{0.1}$, including oxides of Si, Ti, Fe, Zn, Al, copper (Cu), and cerium (Ce). This finding was the first to conclusively verify widespread ENM utilization in toner formulations irrespective of the manufacturer or brand for LP. Interestingly, in one particular toner formulation, there were TiO_2 nanofibers found following detailed STEM and EDX analysis. All evaluated toners contained large amounts of OC (42–89%), metals/metal oxides (1–33%), and some EC (0.33–12%). No OC speciation analysis was performed by the authors. The so-called laser printer-emitted particles (PEPs) possess a composition similar to that of toner and contained 50–90% OC, 0.001–0.5% EC, and 1–3% metals. This complex chemical makeup raises additional questions and concerns with respect to ENM that may become airborne and introduced into the general breathing zone of the user (Pirela et al. 2015).

In an expanded follow-up study in eight photocopy centers across Massachusetts, Martin et al. (2017) conducted extensive PCM characterization of 17 PC toner formulations (five black and 12 color) from five major manufacturers as well as of size fractionated PM samples collected in those PC centers. Consistent with the earlier studies of Bello et al. (2013) and Pirela et al. (2014), Martin et al. (2015) documented multiple ENM in all the toners examined. Moreover, the study documented ENM separation from the parent toner in multiple formulations, lending support to the earlier concern that ENM may become airborne during the printing/photocopying process. Authors documented high exposures in several centers with weekly average TNC ranging from 3.6×10^3 to 3.8×10^4 particles/cm^3 and maximum transient bursts of more than 1.4 million particles/cm^3, which translated to TNC over 700 times greater than background. These findings could have been anticipated as lack of engineering controls, poor ventilation, crowded rooms, and little awareness of exposures from TPE were documented in most of the 15 photocopy centers surveyed by Martin et al. (2017). Furthermore, the authors found the chemical composition of the $PM_{0.1}$ aerosol collected at eight photocopy centers was complex and qualitatively consistent with Bello and Pirela's studies. Elemental composition of $PM_{0.1}$ from PC was found to include S (0.71–10%), calcium (Ca, 0.70–2.67%), sodium (Na, 0.16–1.86%), Fe (0.10–0.99%), potassium (K, 0.13–1.56%), and zinc (Zn, 0.14–0.22%). Further, inorganic makeup varied greatly from 36 to 94% among the photocopy centers evaluated. The emitted PM elemental composition reflected qualitatively toner composition and agreed with previous findings. The data show that multiple ENM (e.g. metal oxides) are routinely used in toner formulations, which now seems to be an industry norm. Therefore, a complex chemical composition of the airborne fractions, containing organics, inorganic metal oxides, and small amounts of CB is expected.

Lastly, PM emissions from LP (and PC) also carry charged particles, as well as anions and cations in the form of salts,

which should not be surprising given the necessary charge transfer complex created within the device to facility toner deposition onto the paper as it passes the toner drum and into the fuser rollers. Jayaratne et al. (2012) found a single LP released significant quantities of charged particles into the environment. However, the authors did not find a relationship in this study between charged particles, CMD, or TNC. Pirela et al. (2015) found several cations and anions in LP $PM_{0.1}$ emissions, with individual species (i.e. chloride) all less than 1 μg/g, and the combined total of anions and cations accounting for less than 2% of the total mass of PM emissions. Similarly, Martin et al. (2015) found cation and anion mass concentration in PC emissions for one PC center to be low and in agreement with Pirela et al. (2015). With regard to sVOC, targeted structural elucidation of organics is still needed to identify its major constituents. Despite extensive screening for over 200 analytes, the majority of this organic sVOC fraction, by mass, is not characterized and no exposure markers uniquely specific to TPE emissions are yet available. The catalytic role of metal oxide ENM in formation of new organic species and as carriers of condensed organic matter on their surface is also worthy of further investigations.

Particle formation mechanisms

Equally important to PM concentration, from a mitigation and control perspective, are the mechanisms of formation and specific emissions point(s) around the LP or PC. Recurring conceptual themes with respect to particle formation from laser printers are related to the fuser or internal temperature of the housing, homogenous nucleation of sVOC and reaction of VOC with small amounts of O_3 present that create secondary organic aerosols (SOA). As research has shown clear evidence that $PM_{0.1}$ dominates the particle emissions by number, investigations now steer toward additional characterization of the elemental and chemical composition of aerosols in the work environment, as well as the formation mechanisms of emissions.

In chamber experiments, Morawska et al. (2009) showed particle and VOC emissions (including ethylbenzene, cyclohexane, *m-p*-xylene, dimethyl phthalate, and toluene) to appear dependent on temperature and correlate positively with $PM_{0.1}$ TNC and O_3 concentration. Negative correlation was found between $PM_{0.1}$ and tVOC concentration. The authors hypothesized particles may be formed in at least two separate and distinct pathways: (1) homogenous nucleation of evaporated sVOC to form particles and (2) particle formation through the reaction of VOC with small amounts of O_3 to produce SOA. Following Morawska's results, He et al. (2010) reported that fuser temperature has a significant role in particle emissions rates, which support Morawska's temperature observations. The authors went on to show that LP begin to emit particles as soon as they are turned on (in the warm-up phase), which is consistent with other reports (Wensing et al. 2008; Tang et al. 2012b).

It is worth mentioning that in earlier microscopy observation studies, the authors found small numbers of toner particles, submicron toner fragments, and agglomerates of NP. The presence of these particles cannot be explained by the first two formation mechanisms. Thus, a third mechanism (mechanical ejection of engineered NP and occasional toner fragments) should also be considered. Specifically, it has been shown that there is release of nanoscale particles from a NEP (i.e. toner powder) during the use of TPE. This is facilitated by the airflow created by the cooling exhaust fan and rollers moving paper through. This mechanism has been confirmed in two studies assessing emissions from both PC and LP (Bello et al. 2013; Pirela et al. 2015). The released PM from TPE was chemically complex and reflected the toner powder chemical profile (e.g. metal oxides). Further, the authors provide evidence of VOC condensing on the surface of the TPE-emitted solid inorganic particles, with particles acting as carriers of the VOC emitted by TPE. Understandably, the health implications for exposures to both PM and VOC are of great interest since the gaseous co-pollutants may now be able to reach the alveolar region of the lung and potentially cause adverse physiological responses.

Of additional importance is identifying where within the LP individual compositional components (i.e. paper, toner, printer housing, and solid parts) arise. Particularly, following elemental analysis of the emissions from TPE, only Ca has been linked to the paper used during the print job (Barthel et al. 2011; Pirela et al. 2015). Moreover, Pirela et al. (2015) and Martin et al. (2015) suggested that paper may contribute other elements besides Ca ($CaCO_3$), present in the paper as fillers and whitening agents, including Ti (as titania), Al (as alumina), and Mg (magnesium salts). Understanding the source material, formation mechanisms, and source apportionment are important first steps in developing effective exposure control measures and product reformulation.

Parameters that affect PM emission profiles

Few studies have evaluated the emission profile of both monochrome and color printing by LP. One study by He et al. (2010) found that color printing leads to two to three times more particle emissions than monochrome printing when comparing LP of the same manufacturer, which is consistent with previous reports (Schripp et al. 2008). The authors followed up on the premise that color printing results in greater TNC by testing both color and monochrome printers in a chamber and found that TNC ranged by three orders of magnitude and color printers were generally found to be greater $PM_{0.1}$ emitters than their monochrome counterparts.

Print speed (pages per minute) is an operational characteristic that may affect LP emission profiles. It was determined that average particle number concentration was inversely proportional to print speed, while average particle mobility diameter was directly proportional to print speed (Byeon & Kim 2012). The authors hypothesized that lower concentrations (or larger diameters) with increasing printing speed were due to higher coagulation rates with higher primary particle concentrations.

In addition to color/monochrome toner powder and printer speed, several studies have assessed the effect toner page coverage may have on the emission profile of LP. The results indicate that particle emission characteristics are affected by toner coverage, of up to 50%, of the page used

by the LP (He et al. 2007; Wang et al. 2012; Pirela et al. 2014). In contrast, some studies have reported inconclusive results regarding toner coverage and $PM_{0.1}$ emissions (Schripp et al. 2008; Morawska et al. 2009; McGarry et al. 2011). In addition to toner coverage, it has been observed that toner cartridge condition may impact TNC with used cartridges emitting fewer particles compared with newer cartridges (He et al. 2007; Wang et al. 2011).

Other operational conditions that have been shown to increase $PM_{0.1}$ emissions are startup and standby modes. Wang et al. (2011) observed cold starts and idle time longer than 30 min resulted in significantly greater $PM_{0.1}$ emission compared with emissions from the same LP when given sufficient time to warm up, or was still warm from recent use.

Deposition modeling

Understanding deposition patterns of TPE-emitted particles is critical in assessing the overall lung burden and estimating deposited doses in the various regions of the respiratory tract. By using the appropriate integrated *in vitro* dosimetric methodologies, researchers can then compare and select the proper exposure and dose range used in either animal or cellular experimental models (Cohen et al. 2013; DeLoid et al. 2014; Cohen et al. 2015; DeLoid et al. 2015; Pal et al. 2015b; DeLoid et al. 2017).

The Multiple Particle Path Dosimetry Model (MPPD) v0.2 (Anjilvel & Asgharian 1995) has been utilized to calculate the lung deposition fraction and deposition mass flux of the particles emitted from TPE on the human respiratory system. Default model parameters used in that modeling assume nasal breathing, a functional residual capacity of 3300 ml, a tidal volume of 625 ml, a head volume of 50 ml, a breathing frequency of 12 breaths/min, and an inspiratory fraction of 0.5 (Martin et al. 2015). PC center or LP specific aerosol size distribution parameters were used in the model and they included CMD of 23–40 nm (σ_g of 1.7–2.1; MMD of 89–180 nm). The estimated total particle deposition in the lungs for this set of parameters varied from 28% to approximately 40%, with increasing gradient of deposition from the head airways to the alveolar region, as follows: head airways (~6%), trachea-bronchial region (~10%), and distal alveolar region (15–20%) (Pirela et al. 2014; Martin et al. 2015). The total deposition mass flux based on the modeling of actual exposure data from the aforementioned studies was 1.732 μg/min m^2, meaning significant doses per unit surface area of lungs per unit time can be delivered to individuals running LP or PC. Of interest is to compare doses and dose rates that the upper airways may receive relative to the lower airways. Specifically, upper airways with a surface area of 150 cm^2 typically would receive 6% of the total number of TPE emitted nanoparticles, whereas the deep airways with 120 m^2 surface area receive about 15% of the total number of particles. Crude estimates $((0.06/150)/(0.15/12 \times 10^5) = 3200)$ suggest that much higher doses (number per unit surface area of the epithelium) are received by the head airways region, which may be over 3000 times higher than those received by the lower parts of the lungs (Khatri et al. 2013a, 2013b, 2013c; Pirela et al. 2014). This is important in the context of upper airway disease and possible nanoparticle translocation towards the brain (Oberdorster et al. 2009).

Toxicological assessment of PM exposure

The toxicology of PM emitted from TPE has been studied systematically only in the past 5 years (Tables 2 and 3). Most of these early studies have been conducted by the authors and include mono- and co-culture cellular models, animal intratracheal instillation, and whole-body inhalation studies, as well as small-scale exploratory studies in human volunteers and chronically exposed PC operators. Notable for these studies is the emphasis on employing realistic exposure scenarios supported by extensive PCM exposure characterization studies. The first set of such studies also aligned the toxicity endpoints around airway inflammatory pathways, enabling construction of a broader mechanistic picture on the action of these particles on cells.

Many studies have been conducted to evaluate toxicity of toner powders in both cellular and animal experimental models (Muhle et al. 1989; Bellmann et al. 1991; Muhle et al. 1991; Lin & Mermelstein 1994; Furukawa et al. 2002; Bai et al. 2010; Gminski et al. 2011; Tang et al. 2012a; Konczol et al. 2013; Morimoto et al. 2013). Such studies are directly relevant only in cases of accidental toner exposures, which can happen during toner manufacturing, toner handling, and service/repair of copy machines by copier technicians. However, studies with toner particles are not relevant to, nor are they a substitute for, actual exposures to emissions from TPE because of profound differences in PCM properties between toners and emitted PM and gaseous co-pollutants, differences in particle biokinetics (clearance rate, retention, uptake and translocation inside cells, and beyond the lungs), and dose rates. For this reason, such studies have been excluded from this analysis. It is important to note, however, that a few *in vitro* and *in vivo* studies have used toner as a surrogate of TPE-emitted nanoparticles. Such practices have little justification and toxicological relevance and we discourage future use of toners in lieu of TPE-emitted nanoparticles as they are inappropriate substitutes and such analysis may have potentially misleading outcomes. There is no replacement for the use of TPE-emitted nanoparticles, at realistic doses and exposure rates, for assessing their toxicological properties, both *in vitro* and *in vivo*.

Particles emitted from TPE

In vitro experimental model: Studies using PM emissions from TPE are more relevant to exposures during printing and consumer use than those from toner powder during manufacturing and handling of toners. Such studies have been conducted only in the past few years. Pirela et al. (2014) developed an integrated exposure generation platform that can be utilized for both PCM and *in vitro* and *in vivo* toxicological characterization of emissions from LP. This integrated platform was utilized to assess bioactivity of PM emitted from LPs including cell viability and function and identification of molecular markers and endpoints that reflect mechanistic

pathways and mode of action. More specifically, Pirela et al. (2016) exposed three physiologically relevant cell lines (small airway epithelial cells, macrophages, and lymphoblasts) to LP-emitted $PM_{0.1}$ at a wide range of administered doses (0.5–100 μg/mL) and observed significant cytotoxicity, membrane integrity damage, increase in reactive oxygen species (ROS) production as well as a rise in pro-inflammatory cytokine release, and epigenetic changes specific to the DNA methylation machinery in different cells lines at mass concentrations in the range doses at the low end of the exposure range correspond to 7.8 h, whereas at the high concentration range, they would equate to 1500 h of exposure (or six straight months) (Lu et al. 2016; Pirela et al. 2016). These concentration ranges are much smaller than what is typically used in *in vitro* nanotoxicology, and more realistic at the low end of concentrations; however, lower and more realistic doses must be used in future studies.

A co-culture system consisting of small airway epithelial cells and human microvascular endothelial cells was also used to further evaluate bioactivity of both $PM_{0.1}$ and $PM_{2.5}$ released by LP (Sisler et al. 2015). The authors found that direct exposure of epithelial cells to LP-emitted PM caused morphological changes of actin remodeling, gap formations, increased production of reactive oxygen species, and angiogenesis within the endothelial monolayer. Additionally, a significant upregulation of interleukin (IL)-1β, IL-1Rα, IL-6, IL-8, monocyte chemoattractant protein (MCP)-1, fibroblast growth factor (FGF) basic, interferon γ-induced protein (IP)-10, regulated on activation, normal T cell expressed and secreted (RANTES), and macrophage inflammatory protein (MIP)-1β levels in the cellular lysates or the condition media was found following treatment. These chemokines and cytokines play a major role in the cellular communication observed between small airway epithelial cells (SAEC) and human microvascular endothelial cells (HMVEC) and the resultant responses in HMVEC, in addition to general initiation of the immune response. In another study, PM emitted from some LP caused significant DNA damage on A549 cells following exposure via air–liquid interphase, based on the presence of micronucleus. However, the same study concluded the NP did not cause significant cytotoxic effects on the exposed cells (Tang et al. 2012a). As is common with an air–liquid interphase system, nanoparticle concentration and dose delivered to cells is often unknown. This study highlights another potentially overlooked observation related to possible significant differences in the chemical and toxicological properties of TPE emissions.

Besides LP-emitted PM, there have been studies evaluating the toxicological potential of PM sampled from various PC centers. For instance, Khatri et al. tested the effect of the $PM_{0.1}$ and $PM_{0.25–2}$ size fractions of photocopy center particles on three types of cells (primary human nasal epithelial cells, small airway epithelial cells, and induced THP-1 macrophages) at administered concentrations of 30, 100, and 300 μg/mL, which correspond to a cell surface dose of 18, 62.5, and 180 $\mu g/cm^2$, respectively. The authors back-calculated the delivered doses to cells using *in vitro* cellular dosimetric approaches (that had become available at the end of the study) and found that TPE emitted nanoparticle agglomerates would settle much slower than larger particles and control nanoparticles, such as copper oxide NP. As a result, only 1/10th of the administered concentration would have deposited to the cells at 6 h, resulting in a 10-times lower effective dose to cells. At 24 h, only ~20% of the administered concentration of particles would deposit, resulting in an effective cell dose of 1/5th of the administered one, or ~5 times lower ~6, 20, and 60 μg/mL). After considering dosimetry, effective doses to cells would in fact be 10 times and five times lower at 6 and 24 h time points, or 1.8, 6.3, and 18 and 6, 22, and 60 $\mu g/cm^2$, respectively. The message from this assessment is clear: gross errors, as big as 5–10-fold in magnitude would result in the slope of dose–response relationships *in vitro*, if *in vitro* dosimetry is not considered. A second important implication of this assessment, highly relevant to this discussion relates to relative comparison of potency of TPE-emitted nanoparticles to other types of nanoparticles. For example, in this same study, CuO nanoparticles of comparable size to TPE nanoparticles were used. Because CuO nanoparticle settles faster, 50% and 100% of the administered CuO concentration were delivered to the cells at 6 and 12 h, respectively. As we have shown elsewhere (Pal et al. 2015a), such considerations change the overall picture of the relative potency of these nanoparticles *in vitro*. In this case, when adjusting for delivered doses, the slope of tumor necrosis factor (TNF)-α production for example for TPE-emitted nanoparticles was ~7 times steeper than that of CuO ENM.

Modest cytotoxicity was observed in the aforementioned cell lines, especially for NP. The cells exhibited high levels of apoptosis and oxidative stress, in addition to an inflammatory response reflected in significant upregulation/expression of the following cytokines: granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-1α, IL-1β, IL-6, IL-8, interferon (IFN)-γ, MCP-1, TNF-α, epidermal growth factor (EGF), and vascular endothelial growth factor (VEGF) (Khatri et al. 2013b). Furthermore, gene expression confirmed upregulation of Clustered Regularly Interspaced Short Palindromic Repeat-associated protein 8 (CAS8) and tumor protein p53 (apoptosis), TNF-α (inflammation), and heme oxygenase 1 (HO1) (oxidative stress), as well as down-regulation of Superoxide Dismutase 1 (SOD1) and Glutathione Peroxidase 1 (GPX1) (oxidative stress). No DNA damage was observed in any of the tested cell lines by comet assay, and neither of the two genes (*ku70* and *RAD51*) tested was affected. Additionally, the authors performed more studies taking into account dosimetry for the nanoscale fraction (no such corrections are needed for larger fractions because they settle faster) and reported that $PM_{0.1}$ were far more potent in inducing cytotoxicity than the $PM_{0.25–2}$ fraction (Khatri et al. 2013b, 2013c).

A major issue with using *in vitro* experimental models relates to the delivery method of NP to cells. In the case of air-to-liquid delivery systems, particles are delivered to cells in a way that has physiological relevance but the exact dose to cells is difficult to measure or estimate. Delivering nanoparticles in the form of suspensions are susceptible to inaccuracies related to dispersion techniques, dispersant media, re-agglomeration, and settling behavior. Recently, methodologies that deal with protocols for ENM dispersion, agglomerate characterization, which includes measurement of effective

Table 2. Summary of findings from toxicological studies evaluating adverse health effects following exposure to toner powder and TPE-emitted PM.

Year	First author	Study information	Main findings	Comment
2010	Bai et al.	• Experimental model: male ICR mice (20–22 g bw) • Exposure treatment by intratracheal instillation ○ Toner (40 mg/kg bw) ○ PBS (vehicle control) ○ Exposure for 4 d every 2 d • Post-exposure analysis (9, 28, 56, and 84 dafter the first instillation): ○ Bronchoalveolar lavage fluid collection ○ Lung tissue homogenization ○ Lactate dehydrogenase (LDH) levels ○ Total protein levels ○ Malondialdehyde (MDA) levels ○ Histopathological examination of lavaged lungs ○ Transmission electron microscopy inspection of lavaged lungs	• Growth rate of exposed mice declined at 56 and 84 d post-exposure • Liver, kidneys, and spleen weights were lower in exposed mice when compared with controls • Number of leukocytes in toner group is significantly higher than in controls even after 84 d post-instillation • Total protein concentration in toner exposed group was significantly higher than in control • LDH levels in the toner group increased significantly after 9 and 84 d post-exposure compared with controls • Levels of NOS, IL-6, and IL-1β were higher in exposed mice • Histopathology analysis showed presence of black particle spots in lungs of exposed mice (9 and 28 d post-exposure) ○ In toner exposed mice, there were bullae observed in the lungs, pulmonary inflammation, and basement membrane damage	• Toner not an appropriate surrogate for TPE nanoparticle emissions • Doses, unrealistically high • Dose rate for IT, unrealistically high
2010	Gminski et al.	• Experimental model: human lung adenocarcinoma Type-II alveolar epithelial cells (A549) • Exposure treatment for 24 h: ○ Two types of toner powder (80, 100, 133, 200, and 400 μg/cm^2) • Post-exposure analysis ○ Erythrosin B vital exclusion assay ○ Single-cell gel electrophoresis genotoxicity assay ○ Cytokinesis block micronucleus (CB-MNvit) test	• Exposure to toner particles did not affect cell viability • Cells exposed to toner powder exhibited significant increases in DNA migration in a concentration-dependent manner • Toner exposure induced significant micronuclei formation compared to control exposures	• Toner not an appropriate surrogate for TPE nanoparticle emissions • High, unrealistic *in vitro* doses • A549, widely used but not a highly representative human airway cell model
2012	Tang et al.	• Experimental model: human lung adenocarcinoma Type-II alveolar epithelial cells (A549) • Exposure treatment for 1 h by Vitrocell air liquid interface ○ Emissions of 5 LP (5%-page coverage printing rate of 5, 10, 22, 10, and 16 pages/min) • Post-exposure analysis ○ WST-1 assay ○ Cytokinesis block micronucleus (CB-MNvit) test	• The five tested LP emitted small amounts of O_3 (13–34 μg/m^3) that were significantly different from those during stand-by • tVOC concentration ranged from 94 to 280 μg/m^3. 13 VOCs were measured and the most frequent were 2-butanone, *m,p*-xylene, and *o*-xylene • Mass concentrations of emitted PM were 2.4 μg/m^3 and a maximum of 294,460 particles/cm^3 were observed • No cellular viability changes were observed in emitted PM-treated cells • Emissions from two LP induced formation of micronuclei in the cells	• Dose – unknown • PCM characterization of TPE emissions lacking • Exposure time – possibly too short, affecting outcome?
2013	Khatri et al.	• Experimental model: human monocytic immortalized cells (THP-1), primary human nasal-, and small airway epithelial cells • Exposure treatment for 6 and 24 h ○ $PM_{0.1}$ collected at a high-volume photocopy center (30, 100, and 300 μg/mL administered concentrations) • Post-exposure analysis ○ Cell viability ○ Cytokine/chemokine analysis (13-plex) ○ Apoptosis ○ DNA damage using comet assay ○ Transcriptional analysis of biomarkers for inflammation, apoptosis, DNA and oxidative damage	• A significant dose and time dependent effect on cell viability observed in the tested cell lines • Exposure to photocopy center PM produces increase in secretion of various inflammatory cyto- and chemokines • Moderate apoptosis was exhibited by treated THP-1 cells at different concentrations of the photocopy center PM • Regulation of inflammatory, apoptotic and oxidative stress genes was observed in treated THP-1 cells • No significant DNA damage exhibited by treated; however, a slight upregulation of key DNA damage and repair genes was observed in the treated THP-1 cells • Most inflammatory markers produced by THP-1 cells • Good concordance for inflammatory markers between *in vitro* and nasal lavage following acute human exposure	• Used TPE nanoparticles from PC center • Used advanced dispersion and dosimetry modeling to cor rect for delivered concentration/cell dose • Calculation of equivalent *in vitro* dose from MPPD models • Documented major dosimetry effects *in vitro*: delivered concentration, 1/10th of administered at 6 h, 1/5th at 24 h • Background aerosol nanoparticles could not be excluded • Possible false negative by comet assay
2013	Khatri et al.	• Experimental model: human monocytic immortalized cells (THP-1), small airway epithelial cells, primary nasal epithelial cells	• Treatment with photocopy center particles ($PM_{0.25–2.0}$) lead to a modest dose- and time-dependent decrease in cell viability	• Nanoscale fraction more potent that the $PM_{0.2–2.5}$ fraction in all endpoints

(*continued*)

Table 2. Continued

Year	First author	Study information	Main findings	Comment
		• Exposure treatment for 6 and 24 h ○ $PM_{0.25-2.0}$ collected at a high-volume photocopy center (30, 100, and 300 μg/mL) • Post-exposure analysis ○ Cell viability ○ Cytokine analysis ○ Apoptosis ○ DNA damage using comet assay • Transcriptional analysis of biomarkers for inflammation, apoptosis, DNA and oxidative damage	• Four cytokines (IL-6, IL-8, TNFα, and IL-1β) were significantly higher in exposed THP-1 cells and only one cytokine (IL-8) was significantly elevated in the primary nasal and small airway epithelial treated cells • Dose-dependent apoptosis was exhibited by treated cells; however, no DNA damage was detected	• Differences with $PM_{0.1}$ possibly due to surface are and size (affecting cellular uptake & mechanisms) • The 300 concentration unrealistically high • No need for dosimetry corrections
2013	Pirela et al.	• Experimental model: male Balb/c mice • Exposure treatment by intratracheal instillation ○ $PM_{0.1}$ or $PM_{0.1-2.5}$ size fractions collected at a high-volume photocopy center (0.2, 0.6, and 2.0 mg/kg bw) • 24-h post-exposure analysis ○ Cell viability	• Mice instilled with $PM_{0.1}$ had significant increases in neutrophil number, lactate dehydrogenase, and albumin • A number of pro-inflammatory cytokines were elevated in mice exposed to $PM_{0.1}$	• Same size matched PM fractions from PC center in the Khatri studies • Overall good agreement in endpoints with Khatri studies • Dose and dose rate for the high intratracheal instillation dose may have been too high
2014	Sisler et al.	• Experimental model: small airway epithelial cells (SAEC) and human microvascular endothelial cells (HMVEC) • Exposure treatment for 24 h ○ LP-emitted $PM_{0.1}$ and $PM_{2.5}$ (0.5 and 1.0 μg/mL) • Post-exposure analysis ○ Cellular viability ○ Cellular morphology ○ Cytokine and chemokine analysis ○ Reactive oxygen species production ○ Angiogenesis assay	• Increase in ROS production in HMVEC when SAEC were treated with either LP-emitted $PM_{0.1}$ and $PM_{2.5}$ • Increase in the remodeling of actin filaments in HMVEC after the treatment of SAEC with LP-emitted $PM_{0.1}$ and $PM_{2.5}$ • Increased gap junctions in HMVEC after treatment with LP-emitted ($PM_{0.1}$ and $PM_{2.5}$) • Increase in tube formation in HMVEC after SAEC were treated with LP-emitted $PM_{0.1}$ • A number of pro-inflammatory cytokines were upregulated by both SAEC and HMVEC following exposure to LP-emitted PM	• First report documenting cross-talk between cell types in the absence of TPE nanoparticles • Much lower doses than used conventionally in nanotoxicology • Use of dosimetry models
2015	Pirela et al.	• Experimental model: small airway epithelial cells (SAEC), human monocytic immortalized cells (THP-1) and lymphoblasts (TK6) • Exposure treatment for 24 h ○ LP-emitted $PM_{0.1}$ (5, 20, 40, and 100 μg/mL) • Post-exposure analysis ○ Cellular viability ○ Reactive oxygen production ○ DNA damage ○ Cytokine and chemokine analysis ○ Methylation and expression analysis of transposable element LINE-1	• Cellular membrane integrity of all three human cell lines studied was decreased following exposure to LP-emitted PM • A clear dose–response relationship was observed in SAEC treated with LP-emitted PM • THP-1 cells displayed elevated superoxide levels following exposure to LP-emitted PM • Treatment with LP-emitted PM caused significant mem brane integrity damage, an increase in reactive oxygen species production as well as a rise in pro-inflammatory cytokine release in different cell lines • No significant DNA damage on the lymphoblasts was observed following exposure • LP-emitted Pm led to ∼70% decrease in *Alu* methylation compared to control cells • L1 ORF2 expression was 1.5 and 1.7 times higher after treatment with 0.5 and 30 μg/mL of LP-emitted PM • Significant and dose-dependent reduction in the expression of all three DNA methyltransferases (*DNMT1, DNMT3A, DNMT3B*) was detected after LP-emitted PM exposure	• Use of dosimetry models to understand dose delivered to cells • Used MPPD model to compare exposure doses in cellular studies and translate to exposures in real world scenarios

(*continued*)

Table 2. Continued

Year	First author	Study information	Main findings	Comment
2016	Pirela et al.	• Experimental model: male Balb/c mice • Exposure treatment by intratracheal instillation ○ LP-emitted $PM_{0.1}$ (0.5, 2.5, and 5.0 mg/kg bw) • 24-h post-exposure analysis ○ Bronchoalveolar lavage ○ Cytokine and chemokine analysis ○ Gene expression analysis ○ Methylation and expression analysis of transposable element LINE-1	• Exposure to LP-emitted PM did no lead no immediate changes in the lung membrane integrity • Instillation of LP-emitted PM led to a pulmonary immune response, indicated by an elevation in neutrophil and macrophage percentage • Lungs of exposed mice exhibited an upregulated expression of the *Ccl5 (Rantes), Nos1* and *Ucp2* genes • There was a significant modification of the components of the DNA methylation machinery (*Dnmt3a*) and expression of transposable element (TE) LINE-1 following exposure to LP-emitted PM	• First report on epigenetic modifications • Employed advanced dispersion and dosimetry models • Calculation of equivalent *in vitro* dose from MPPD models

density of formed agglomerates in cell culture media, and numerical fate and transport approaches that estimate delivered dose to cells as a function of time have been developed and become widely available (DeLoid et al. 2014; Cohen et al. 2015; DeLoid et al. 2015, 2017). Employing proper dispersion, characterization, and dosimetry methods in *in vitro* studies, as well as matching the dose delivered to cells to realistic exposure levels are extremely important, critical in fact, in the study outcomes (Pal et al. 2015a, 2015b).

As illustrated in earlier sections, dose ranges and lack of dosimetry consideration in *in vitro* nanotoxicology of TPE-emitted nanoparticles continued to be major problem. Nonetheless, the impact of such conceptual and experimental errors is potentially profound. With the methodology now standardized and freely available, there are no excuses to ignore them. Furthermore, lower dose ranges should be used, one to two orders of magnitude lower, and dose equivalency between airborne exposures and *in vitro* concentrations documented.

Animal experimental models: There are only a few studies focusing on studying the biological effects of exposure to the emitted PM from LP and PC. These studies are recent and have been conducted primarily by the authors. For example, an assessment was done by Pirela et al. (2013) using different size fractions of PM collected from a photocopy center intratracheally instilled in mice at various doses (0.2, 0.6, and 2.0 mg/kg bw). Particle size- and dose-dependent effects were observed in the pulmonary system of the instilled mice. The $PM_{0.1}$ size fraction led to increased neutrophil number, lactate dehydrogenase, albumin and pro-inflammatory cytokines, all markers of lung injury and inflammation. As for LP-emitted PM, Pirela et al. (2016) evaluated the toxicological potential of these particles in mice exposed by intratracheal instillation (0.5, 2.5, and 5.0 mg/kg bw). While the authors found no changes in lung membrane integrity, an immune response – indicated by elevated neutrophil and macrophage recruitment in bronchoalveolar lavage fluid (BALF) – was seen after instillation of LP emitted PM at 0.5 mg/kg bw. Gene expression and epigenetics modifications were documented on mice exposed to 2.5 mg/kg bw, mainly as upregulated expression of the *Ccl5* (*Rantes*), nitric oxide synthase 1 (*Nos1*), and uncoupling protein 2 (*Ucp2*) genes in the murine lung tissue and modified components of the DNA methylation machinery (*Dnmt3a*) as well as the expression of transposable element (TE) long interspersed nuclear element (*LINE)-1*.

Regardless of the experimental model used for the various toxicology studies reviewed here, three common themes – inflammation, oxidative stress, and gene regulation – seem to emerge throughout. In particular, upregulation of only number of important chemokines and cytokines that play various important roles, including but not limited to leukocyte migration and infiltration, cellular antioxidant status and redox balance, modulation of pro-and anti-inflammatory responses, and initiation of an immune response to exogenous particles, among other equally essential biological functions (such as apoptosis).

Understandably, the main focus of investigations to-date has been the respiratory system. Since NP from TPE represent a complex mixture, containing a large organic component, a

Table 3. Summary of human health studies exposed to emissions from toner-based printing equipment (TPE).

Year	First author	Study information	Main findings	Comments
2000	Zina et al.	• Medical case report • Subject: 30-year old male, occasionally employed as a photocopy operator • GIRDCA Standard Patch testing for formaldehyde, quaternium-15 and toner powder	• Symptoms of exposure (urticaria) on arms, neck, head and upper trunk • Symptoms regress spontaneously within a week without additional exposure • Symptoms return upon exposure and progressed to being in the same room as a working PC • Patch testing positive for formaldehyde and quaternium-15 (documented components of toner) • Patch testing of toner was unclear • Testing for components of toner released during operation was positive for formaldehyde and quaternium-15, which confirmed as a toner component • Concluded the symptoms were caused by exposure to photocopier emissions	• Typical with most case reports of this kind, occupational exposure histories, and quantitative exposure information are lacking • Exposure characteristics in the photocopy center (particle number concentration, chemical/elemental composition) are not mentioned

2004	Goud et al.	• Study to analyze genotoxic effects of PC workers
		• Subjects: 98 male PC workers with >1 year of job experience
		• Randomly selected controls chosen from the local population
		• General health information, diet, medication, social habits (e.g. tobacco and alcohol) collected during the previous three months.
		• Samples collected
		  ○ Buccal epithelial cells
		  ○ Peripheral blood lymphocytes
		• Analysis done
		  ○ Micronucleus test (MN) in buccal epithelial cells
		  ○ Cytokinesis block micronucleus assay (CBMN) in peripheral blood lymphocytes
		  ○ Chromosomal aberration test (CA) in peripheral blood lymphocytes
			• Significant increase (65.79%) in mean percentage of buccal epithelial cell MN in exposed subjects compared with control
			• The mean percentage of micronuclei in blood lymphocytes of exposed subjects increased 76.92% when compared with controls
			• The mean aberrant cell and mean percentage of aberrations in peripheral blood lymphocytes of exposed workers were notably elevated (43.52% and 38.55%, respectively) compared to controls
				• Good size cohort
				• Lack of quantitative exposure information, especially for TPE-emitted nanoparticles
				• Possible confounding by air pollution and other sources of indoor pollution
				• Healthy worker effect cannot be ruled out

2005	Gadhia et al.	• Study to analyze cytotoxic and genotoxic potential of PC emissions
		• Subjects: 12 PC operators and 12 control subjects matched according to age, gender, SES and personal habits
		  ○ Full time workers with >5 years job experience
		• Samples collected
		  ○ Blood sample for
		• Analysis done
		  ○ Chromosomal aberrations (CA)
		  ○ Sister chromatid exchange frequencies (SCE)
			• The mean percentage of aberrant cells found to be significantly higher among operators (2.5%) when compared to controls (0.33%)
			• Total aberrations of operators (3.33%) compared with control (0.33%)
			• Excluding chromatid gaps, operators exhibited 2% aberration compared with 0% in controls
			• Authors conclude photocopy workers considered a slight risk group for chromosomal aberrations after accounting for VOC, PAH, O_3, and PM
				• Small sample size
				• Blood samples were collected, which is a desirable medium to analyze
				• No quantitative exposure information on TPE-emitted nanoparticles
				• No description of chemical composition of emissions from PC
				• Possible confounding from other sources
				• Healthy worker effect cannot be ruled out

2009	Manikantan et al.	• Study to analyze DNA damage in PC operators with respect to increased exposure period and personal alcohol and smoking habits
		• Subjects: 148 participants. 74 PC workers, 74 controls; 3–13 years of experience
		• Sample collected
		  ○ Peripheral blood
		• Analysis done
		  ○ Single cell gel electrophoresis (SCGE)
			• A significant difference in DNA damage, measured by DNA strand break, was found between experimental and control subjects. Specifically, %DNA in tail in exposed males (4.63%) and females (4.08%) was notably higher than levels in control male (1.63%) and female (1.61%) subjects
			• An increase in DNA damage observed with workers who smoke compared with workers who do not smoke
			• A synergistic effect with smoking and PC emission exposure
			• Duration of exposure is associated with increased DNA damage
				• Decent sample size
				• Synergism between smoking and TP emitted nanoparticles is noteworthy
				• Association of DNA damage with length of exposure is an important finding
				• Lack of exposure information and quantitative exposure assessment
				• No other biomarker endpoints to substantiate these findings

(*continued*)

Table 3. Continued

Year	First author	Study information	Main findings	Comments
2010	Theegarten et al.	• Medical case report • Subject: 33-year-old female office worker exposed to toner dust and emissions • Analysis done ○ Biopsies taken during two colonoscopies	• Worker occupationally exposed >3 years to laser printer emissions (70 prints/daily) • Presented with intermittent abdominal pain, diarrhea, and weight loss • Carbon NP consistent in size with engineered nanomaterial and TP-emitted nanoparticles (31–67 nm) found in peritoneum confirmed by SEM/EDX • Carbon NP directly linked to the laser printer toner • No lung function abnormalities reported or tested • Conclusion that transport of carbon NP by lymphatic system and blood vessels after inhalation	• An extremely important early microscopic observation of nanoparticle translocation to the submesothelium in humans. Important in the context of nanoparticle biokinetics • Exposure characteristics (particle number concentration, chemical and elemental composition) are unclear • Inadequate chemical characterization of NP agglomerates in tissue biopsies • No attempt to reconstruct exposures

2010	Balakrishnan et al.	• Study aimed to investigate chromosomal aberrations and PC workers compared with non-workers
		• Subjects: 106 healthy subjects. 34 PC workers with smoking habits, 32 PC workers without smoking, 40 controls (non-PC worker, non-smoker)
		  ○ Selection based on age, occupational exposure, smoking/tobacco use, drug and alcohol use, viral illnesses, recent vaccinations and radiologic exams
		• Sample collected
		  ○ Blood
		• Analysis done
		  ○ Chromosomal aberrations in collected/harvested leukocytes to study DNA damage
			• Significant chromosomal aberrations observed in workers compared with non-workers
			• Results suggest a synergistic relationship between smoking and exposure to PC emissions
			• Chromosomal aberrations are related to duration of exposure/years of service in the job
				• Exposure characteristics (particle number concentration, chemical and elemental composition) are missing

2011	Kleinsorge et al.	• Study to analyze genotoxicity and oxidative stress in PC operators
		• Subjects: 26 PC operators and 50 control subjects
		  ○ Matched by age, sex, SES, personal habits (alcohol and tobacco use)
		  ○ Approximately 2 years work experience in the photocopy center
		• Sample collected
		  ○ Blood
		• Analysis done
		  ○ Reduced versus oxidized glutathione ratio (GSH/GSSG)
		  ○ Catalase activity of erythrocytes
		  ○ Lipid peroxidation in erythrocytes
		  ○ Alkaline comet assay
			• Thiobarbutric acid reactive substances (TBARS) were significantly higher (27%) in PC operators
			• PC operators show a 134% increase in damage index by comet assay (DICA) compared to controls
			• GSH/GSSG decreased 25% in PC operators compared with controls, which was statistically insignificant
			• The length of time working in PC centers is associated with DNA damage in lymphocytes and occurrence of anomalies in buccal cell nuclear
				• Association with length of time
				• No history of exposures was included
				• Exposure characteristics remain unclear as no physical and chemical characterization done on the PC emissions
				• Use of LC-MS/MS would have been preferred over TBARS assay

2012	Khatri et al.	• 9 healthy volunteers exposed to photocopier emissions for 6 h in a working high-volume photocopy center • Repeated measures design where subjects are their own controls • Compared pre-and post-exposure samples and studied clearance kinetics • Exposure occurred on 3 randomly selected days several weeks apart • Real-time particle monitoring (5.6 nm–20 μm in diameter) • Concentration of ozone and VOCs measured via real-time instruments	• Concentrations of 10 of 20 cytokines measured were significantly higher in post-exposure measurements compared to pre-exposure measurements • The concentration of four cytokines remained significantly higher 30 h post-exposure then pre-exposure concentrations • Concentration of the remaining six cytokines returned to baseline levels within 30 h of exposure • Total protein concentration was significantly higher at all post-exposure time points compared with pre-exposure concentration • The concentration of 8-OH-dG increased significantly after acute exposure, and like values remained 2× greater than baseline levels for 24 h post-exposure	• Detailed quantitative exposure assessment and chemical composition of NP • Realistic exposure scenarios and single exposure episode • Although small size, positive findings due to the study design strengths (subjects serving as their own control and kinetics approach) • Only study to focus on upper airway inflammation • High levels of 8-oxodG in urine measured with ELISA. Confirmation with LC-MS/MS would be desirable

(*continued*)

Table 3. Continued

Year	First author	Study information	Main findings	Comments
			• Mean exposure particle concentration 9500 particles/cm^3. Peak exposure 189,500 particles/cm^3 • Count median diameter $<$38 nm • Ozone and VOCs not significantly different from background	
2012	Dutta et al.	• Cross-sectional survey in India of photocopier workers divided into two categories: (1) copy production and machine operation; (2) machine maintenance and toner workers • 150 participants divided into four age groups from 20 to 45 years of age	• 88 Cases recorded as affected by PC environment • Machines operated 4–8 h/d • Self-reported symptoms include rhinorrhea, conjunctivitis, allergic rhinitis, upper and lower respiratory tract irritation, cough, and sinusitis • Chronic exposure symptoms include headache, dizziness, gastric disorders, and respiratory ailments • Age and duration of exposure correlated with severity and number of symptoms	• Reports respiratory airway symptoms (upper and lower) • Lack of quantitative exposure information on TPE emissions
2013	Elango et al.	• Cross-sectional design • 81 photocopier operators (64 M and 17 F); 43 healthy controls (31 M, 12 F) • Participants were 5 year workers in PC centers. Chronic exposure • Pulmonary function testing performed on subjects • Aimed to assess differences in lung function, hematological changes, and oxidative and inflammatory markers • PM_{10} and $PM_{2.5}$ measured gravimetrically • Integrated samples for lead, arsenic, nickel, benzene, and benzo (a) pyrene • Detailed copy center information collected	• Elevated PM_{10} and $PM_{2.5}$ • Indistinguishable elemental results between background and indoor • Pulmonary function testing did not reveal differences between PC operators and controls • Hematocrit, mean corpuscular volume, and red blood cell distribution were significantly higher among PC operators • Eosinophils, basophils, and monocytes were significantly lower in PC operators compared to controls • Plasma UCAM-1, leukotriene B_4 levels, ECP, and IL-8 were significantly higher in PC operators compared with controls	• Most comprehensive study to date with multiple endpoints • Important findings on inflammation, oxidative stress, and high prevalence of symptoms • Detailed quantitative information about numerous air pollutants, but missed the most important one: TPE nanoparticles • Health worker effect possible • ELISA assays susceptible to interferences. Reconfirmation by LC-MS/MS desirable • TBARS and FRAC, non- specific measures
2014	D'Alessandro et al.	• MCR: 40-year-old female office worker occupationally exposed to emissions • No history of smoking or allergies • Controlled exposure after 4 weeks of non-exposure and no symptoms • Provocation test after 4 weeks in a 63 m^3 room with normal ventilation	• Pulmonary function tests were normal • Allergy skin test (patch testing) were normal • Chest radiography did not reveal abnormalities • Provocation test after 4 weeks was associated with immediate onset of coughing • Bronchoscopy and BAL/mucosal biopsy found BAL cellularity showed an increase of neutrophils and lymphocytes • Biopsy of the bronchial mucosa revealed moderate signs of inflammation with infiltration of lymphocytes and plasma cells	• Important study in that effects were documented via challenge testing with printer exposures • Exposure characteristics (particle number concentration, chemical and elemental composition) in office environment and during the provocation test are missing
2015	Awodele et al.	• Exposure assessment among PC operators in Nigeria • 50 workers (61% male, 60.30% 15–25 years of age) partici pating in the study at high-volume copy centers at multiple university sites • The selected subjects were divided into five categories based on the length of time at the job (2–3 years, 3–4 years, 4–5 years and $>$5 years). Four groups of 10 photocopy workers each, and one group of 10 controls • Aimed to determine the level of oxidative stress markers, PAH and hematological parameters in the blood of PC operators • Airborne emissions/concentrations determined by real-time emission analyzer (Testo 350) but not reported	• No levels of CO, NO_3, NO, NO_2, CO_2, SO_2 were detected at the PC operation site • High levels of hydrocarbons (152 ppm) were detected at the site of PC operation using petrol generator • Statistically significant differences among the levels of various oxidative stress parameters (SOD, GSH, CAT, MDA) in the blood of PC operators that seemed to have an association with amount of years working at the PC center • Common health complaints among study population are eye irritation, headache, dizziness, and catarrh • Blood analysis did not detect VOC or PAH in the blood plasma of PC operators • Hematologic parameters showed no noteworthy difference in the levels of hemoglobin, white blood cells, lymphocytes, monocytes, or eosinophils between PC operators and controls	• Description of the study area and subjects • Sample size is small ($n = 10$) • Airborne concentration data was collected but not mentioned in the study • Insufficient information regarding subject selection process and lack of detail on confounding factors • Concise study evaluating hematotoxicity in PC operators

(*continued*)

Table 3. Continued

Year	First author	Study information	Main findings	Comments
2016	Karimi et al.	• Retrospective cohort study • Aimed to assess the respiratory health of PC workers in Shiraz, Iran • A total of 150 exposed individuals were surveyed, while 114 individuals served as the unexposed group • A questionnaire was used to assess occurrence of respiratory symptoms; pulmonary function tests assessed VC < FVC, FEV1, FEV1/FVC	• FVC and FEV1 significantly decreased by 3.97% and 4.49%, respectively, in exposed individuals compared with control subjects • Frequency of cough and wheezing in exposed workers was higher than in unexposed individuals (OR =2.6 and 2.92, respectively) • Respiratory protection used by 5% of the exposed workers	• Performed assessment on the characteristics of the workplace but the information was not reported • Three questionnaires were used simultaneously to evaluate, along with respiratory function tests, the demographic variables, occupational hygiene and respiratory disorders. However, this information was incompletely incorporated into the manuscript • Exposure information is lacking

sizeable amount of metal oxides, and other more volatile organics absorbed onto surface of PM, the toxicological testing framework for these particles should be expanded to evaluate other organ systems and endpoints – including but not limited to those related to cardiovascular, neurological, and blood systems. For example, Lee et al. (2015) demonstrated a concentration-dependent inhibition of synaptic signaling by photocopy center NP in mouse cultured cortical neurons over 0.1–50 μg/mL of administered concentrations (corresponding to delivered concentrations of 0.2–10 μg/mL at 24 h after taking into account dispersion and dosimetry modeling), and a synergistic effect between amyloid beta and NP. Equally important is utilization of screening assays to understand the impact of variability in chemical composition on toxicity, synergistic effects between organics and metals, and biokinetics of such particles from real-world exposures, studies yet to be conducted. Recently, Setyawati et al. (2015) published a review detailing the differences in properties and behavior of the various types of endothelial cells found in the human body, particularly as they relate to interaction with NP with diverse physico-chemical properties. The authors highlighted the importance in considering the extensive variation in endothelial cell characteristics when evaluating the biological potential of NP in the field of nanomedicine, especially as it refers to pharmacokinetics and drug targeting. Particularly, it was found that PM from classrooms led to significantly more cytotoxicity and leakiness in human microvascular endothelial cells than that of the corridor due to the markedly high content of endotoxin found in the indoor PM sample (Chua et al. 2017).

Evaluation of a relevant dose range that reflects realistic human exposures and data-driven tissue dosimetry for neurons in the brain of humans (or animals for that matter) is extremely difficult at present, because reliable data are scarce and the data on biokinetics of nanoparticles to the brain are nanomaterial and system dependent. In all likelihood, unrealistically high doses are being used in such systems, including in this study.

Human studies. *Medical case reports:* Relevant, high- quality studies in humans are limited. Here, we review evidence from human medical case reports, controlled human studies with well-documented exposure information, and studies that incorporate biomolecular markers along well-established disease mechanisms induced by airborne particles. Some landmark studies have been summarized in Figure 1, bottom panel. In an early medical case report (MCR) to link work-related TPE exposure to clinical observations, Zina et al. (2000) reported an otherwise healthy 30-year old male presented with rash on the arms, neck, and upper trunk. Symptoms were reported to resolve when the subject was removed from the PC environment for several days and would recur upon return to work, even for a short period of time. While patch tests of toner material were unclear, formaldehyde and quaterinium-15 were found to be in the used toner, and becoming airborne during operation. The examiner concluded the observed symptoms were caused by exposure to PC emissions.

D'Alessandro et al. (2013) reported a case of a 46-year-old worker with no history of smoking or allergies who exhibited chronic coughing caused by exposure to PC emissions. Bronchoscopy and BAL biopsy found a significant increase in neutrophils and lymphocytes, and biopsy of the bronchial mucosa suggested moderate signs of inflammation. The authors conducted a controlled challenge test where the subject was exposed to TPE emissions for 1 hour after 4 weeks of no workplace exposure and no symptoms. The test was conducted in a 63-m^3 room with normal ventilation and the exposure resulted in immediate and severe coughing, which provided additional evidence that TPE emissions may have a negative impact on workers, patrons, and occupants of the indoor space where TPE are operated without any type of exposure control.

Another published case is that of a worker occupationally exposed to more than 3 years of emissions from a LP that averaged approximately 70 prints per day. The worker presented to medical personnel with intermittent abdominal pain, gastrointestinal distress, and weight loss. Large agglomerates of NP, with primary sizes in the 30–70 nm range and consistent in size with PEPs, was found in the peritoneum of the subject, which was confirmed by SEM with EDX analysis. Upon auscultation, there were no abnormalities observed in lung function; therefore, no further investigation was performed. The authors from the study believed that the deposition of the carbon NP and translocation via the lymph nodes was a contributory agent in this work-related illness (Theegarten et al. 2010).

Epidemiological studies. Beyond MCR, at least one cross-sectional survey of PC workers shows an increase of self-reported symptoms that include rhinorrhea, allergic rhinitis, cough, and sinusitis (Dutta & Deka 2012). The results are consistent with our observations through casual communication with PC workers and managers that the most common complaints from this group are headache, upper respiratory irritation, and epiphora (overflow of tears onto the face) (Khatri et al. 2017; Lucas & Maes 2013). Workplace studies, such as that by Goud et al. (2001), reported a significant increase in DNA damage measured by the comet assay in photocopy center workers compared to unexposed controls. In a follow-up study, Goud et al. studied the genotoxic effects of PC emissions on 98 healthy fulltime PC center workers. The authors found a significant increase of micronuclei in buccal epithelial cell and blood lymphocytes in addition to increased frequency in chromosomal aberrations among exposed workers compared to study controls (Goud et al. 2004). Studies published soon after Goud's arrived to similar conclusions (Gadhia et al. 2005). Further, other studies found a potential synergistic relationship between PC emissions and smoking, and that chromosomal aberrations are related to the duration of exposure and years of service (Balakrishnan & Das 2010). An investigation targeted to oxidative and genotoxic damage in workers at photocopy centers processing 32,000 sheets of paper each 8-h working day was done by Kleinsorge et al. (2011). The authors measured catalase activity (CAT), reduced versus oxidized glutathione ratio (GSH/GSSG), level of lipid peroxidation (TBARS), damage index by Comet assay (DICA), and buccal cells with micronuclei (BCMN). Results showed that exposed PC operators exhibited increased TBARS, DICA, and BCMN numbers, consistent with earlier studies on oxidative stress.

It is important to emphasize that all these studies provided no quantitative exposure information, other than a categorical classification "yes/no" to working in a photocopy center. Therefore, it is difficult to ascertain if effects were caused by NP from PC or other exposures, even though such as assumption is not unreasonable.

Khatri et al. (2013a) conducted a controlled study where nine healthy volunteers, who served as their own controls, were exposed to emissions from PC for a day (6 h). Several biomolecular markers of inflammation were measured in the nasal lavage and urine, before exposure, at the end of exposure, as well as 24- and 36 h pre-exposure. Authors measured PM, VOC, and O_3 concentrations during the exposure and observed a significant increase for several pro-inflammatory markers in nasal lavage, total protein (2.25-fold), infiltrated neutrophils (2.66-fold), as well as 10 pro-inflammatory cytokines (IL-6, IL-8, TNF, IL-1β, GC-CSF, EGF, IL-10, MCP-1, fractalkine, and VEGF) in post-exposure measurements when compared with pre-exposure measurements. While most markers would return to baseline pre-exposure levels within 24 h, 4 cytokines (IL-6, IL-8, EGF, and fractalkine) remained significantly higher 36 h post-exposure than pre-exposure measurements. Similarly, a significant increase in the level of 8-oxodG concentrations in urine was observed post-exposure relative to controls and pre-exposure, which returned to baseline levels after 24 h. These markers did not change with time in the control group and were not significantly different from the pre-exposure levels. The study overall established that emissions from photocopiers induced upper airway inflammation and systemic oxidative stress.

The marker 8-oxodG is commonly regarded in the published literature as a biomarker of oxidatively damaged DNA and assumed to originate from excise repair of damaged DNA. Its precise origin is not fully documented, and diet and cell turnover have been ruled out (Evans et al. 2016). The authors could not establish whether 8-oxodG derives from the 2′-deoxyribonucleotide pool, and conclude that "8-oxodGuo is most accurately described as a non-invasive biomarker of oxidative stress derived from oxidatively generated damage to 2′-deoxyguanosine". It should be further noted that for obvious reasons (ease of use and low cost), 8-oxodG in urine is often measured with commercially available ELISA kits and this includes almost all studies mentioned above. Such kits are susceptible to interferences and tend to generate much more variable and higher results relative to the more robust liquid chromatography/tandem mass spectrometry techniques, which should be the preferred analytical method for this 8-oxodG (Barregard et al. 2013; Ellegaard & Poulsen 2016) and other biomarkers.

Elango et al. (2013) conducted a more detailed survey in copier operators in India. The authors collected information on the respiratory symptoms (via symptoms questionnaires), measured lung function by spirometry, and conducted comprehensive blood biochemistry. Among various markers in blood, they measured hematocrit, total protein, oxidative

stress (ferric reducing antioxidant capacity of serum (FRAC), serum TBARS, glutathione peroxidase, and myeloperoxidase), as well as several inflammatory and cardiovascular markers, including leukotriene B_4 (LTB_4), 8-isoprostane, C-reactive protein (CRP), IL-8, intercellular adhesion molecule 1 (ICAM-1), Clara cell protein (CC-16), and Eosinophilic Cationic Protein (ECP). The authors reported significantly higher prevalence of nasal blockage, cough, excessive sputum production, and breathing difficulties in copier operators relative to controls. Several serum markers confirmed elevated oxidative stress in copier operators. Additionally, serum TBARS were significantly higher in copier operators than controls. Similarly, the FRAC was lower in copier operators than controls. Plasma ICAM, IL-8, and LTB_4 were also higher in copier operators relative to controls. Among several blood parameters, authors also found higher hematocrit values for copier operators, and a lower albumin to globin ratio. However, lung function tests and other biomarkers in plasma/serum were not statistically different from controls. Although authors measured numerous air pollutants, including $PM_{2.5}$ and PM_{10}, they did not measure the most important exposure component – PC-emitted nanoparticles and their chemistry. Awodele et al. (2015) performed a study in Nigeria evaluating the level of oxidative stress markers, PAH, and other hematological parameters in PC operators. The authors concluded that there was a significant increase in the levels of various oxidative stress markers in the blood of PC operators when compared with unexposed controls. However, white blood cell differentials remained unchanged across the two subject groups. Additionally, Karimi et al. (2016) recently published a cross-sectional study of respiratory symptoms and lung function in 150 copier operators and 114 controls in Iran. The respiratory symptoms were measured via a questionnaire, whereas spirometry was used to assess lung function using forced expiratory volume in 1 s (FEV1), forced vital capacity (FVC), and FEV1/FVC ratio. The authors found significantly higher cough (odds ratio, OR, of 2.6) and wheezing (OR 3) in copier operators relative to controls. All other respiratory symptoms, except for shortness of breath, were also more prevalent in copier operators. Lung function capacity (FEV1 and FVC) was also reduced in copier operators relative to controls. Of note, this study lacked any quantitative exposure information or exposure histories.

A relevant cross-sectional epidemiological study is one performed by Yang and Haung (2008) in Taiwan, which investigated the prevalence of chronic respiratory symptoms and acute irritative symptoms among 74 photocopy workers (exposure group) and 69 controls (employees of optical stores). The authors reported a higher, albeit statistically insignificant, rates of chronic cough (adjusted OR 2.9), wheezing (adjusted OR 1.9), chronic bronchitis (adjusted OR 2.2), and dyspnea (3.1) in the copy center workers. It must be noted, however, that the study had no exposure information available since the objective of the study was solely on identifying whether VOC was the trigger agent, rather than the emitted NP.

There is a need for a large-scale human epidemiological or mechanistic study assessing exposures from engineered nanomaterials released from TPE. Such comprehensive studies can enhance our understanding on nano-bio interactions and the potential environmental implications of exposure to emissions from this unique NEP. Further, it is worth highlighting the observed agreement among the toxicological findings described in this review despite the experimental model used, which points to a common outcome: upper airway inflammation, that could potentially lead to exacerbation of existing asthma in addition to potential cardiovascular effects.

Discussion

Potential health risks

There are dozens of reports in the literature documenting high exposure to nano-sized particles emitted from LP and PC in both consumer and occupational settings. Toxicological studies conducted across different test platforms, cell monoculture, cell co-culture, animal intratracheal instillation studies, human case reports, and limited epidemiological studies to date, provide compelling evidence in our opinion that emissions from LP and PC are indeed biologically active in that at the molecular/cellular level, they trigger oxidative stress, inflammatory responses, likely DNA damage, and possible epigenetic effects. While such effects have been documented in cellular and animal studies, at somewhat high doses and possible dose rates (Baisch et al. 2014), similar effect has been documented using transcriptomics and cell co-cultures in a few studies even at low doses, comparable with doses currently experienced by workers and users of these technologies over a day or week. More importantly, studies from our group have shown good agreement in the inflammatory, oxidative stress, and cellular damage endpoints between *in vitro*, *in vivo* (rats) and in humans. Good concordance also exists in the biomolecular endpoints of systemic oxidative stress, inflammation, and respiratory symptoms (nasal blockage, cough, excessive sputum production, breathing difficulties, irritation of eyes and upper airways, headaches, dizziness, irritant, and allergic rhinitis), and, in some cases, these symptoms were correlated in a quantitative fashion with duration of employment (Table 3). Elevated cardiovascular markers in one study (Elango et al. 2013; Table 3) also raise the possibility of cardiovascular disease, but this has not been looked at closely yet.

Familiarity with this technology seems to feed a sense of safety, one that is not supported by the existing exposure and toxicological findings: that exposures can be at times quite high; that exposure controls are practically nonexistent, and indoor ventilation insufficient; and that the exposures are not benign, as we have discussed earlier. As air pollution research reminds us, one does not need continuous high exposures to become ill. Instead, chronic exposures, at low to moderate levels (averages of 10–30 particles/cm^3), may be sufficient. Martin et al. (2017) documented 13 out of the 15 commercial photocopy centers in the greater Boston area have workstations that were positioned less than approximately 3 m from the nearest TPE. Justified concern for exposure to PM and gaseous pollutants released during a print/photocopy job warrants both the

design and the implementation of both engineered and administrative controls, as well as the development of regulatory limits governing policy to mitigate exposures to building occupants and patrons.

To-date, risk assessments of inhaled NP from LP and PC are lacking, in part because the necessary data were not available. Hänninen et al. (2009) conducted a risk assessment of exposure to $PM_{0.1}$ released by LP based on particle number and mass concentration, in addition to size distributions reported in a previous publication. The authors did not conduct their own physico-chemical analysis of TPE emissions, and such characterization became available years later. This was done at a time when the chemistry of these emissions was not understood and the black color of emissions and toners feed the assumption (we hear this often) that such emissions are carbon black. The authors made assumptions that LP emissions are expected to exhibit similar toxicological responses to CB or (urban) soot particles, which is not accurate. In fact, such risk assessment exercises could be quite problematic, because they make several inaccurate assumptions (chemical composition is inaccurate and disease endpoints may not be the more sensitive ones), which may lead to wrong conclusions – with bias in either direction (safe or not safe). The recently published (Sisler et al. 2015; Lu et al. 2016; Pirela et al. 2016) toxicological profiles of LP-emitted PM point to biological activity that is higher to that of mild steel welding fumes, as well as other metal oxide NP. Nevertheless, the authors found that using the relative added mortality risks based on particle number concentration and mass concentration as separate metrics corresponded to 34 and 14 excess annual deaths/million users in an office environment, respectively. Similarly, for home offices and casual consumer uses, the mortality risks were found to be 12 and 4 excess deaths/million users, respectively. The calculated excess mortality surpasses the acceptable risk level (1 death/million users) presently held by USEPA for hazardous waste sites. The excess mortality found in the Hänninen et al. (2009) study may be an underestimate given the limited available data at the time as well as the erroneous assumptions about the chemical composition of and toxicological properties of nanoscale emissions from LP.

Policy or regulations on laser-based printing technology

The authors are unaware of any existing policy, regulation or standard at the local, state, or federal level that address PM emissions, emissions control, or emissions exposure to workers or the general public for such printing technology. Based on the increasing evidence in the medical case reports, toxicological, and epidemiological literature, there is an emerging and apparent need to develop awareness programs informing workers, managers, and patrons of the hazards; emission controls for existing TPE; new printing technology; and possibly new indoor air quality guidelines that are protective to worker and public health in indoor micro-environments that house TPE (i.e. photocopy centers).

The exposure characterization data that have aggregated in the literature thus far paint a reasonably clear picture of the complex chemistry of TPE $PM_{0.1}$ emissions. However, several reports indicate a large, yet unresolved, fraction of organic compounds that remain to be fully characterized. Based on these missing data, additional analytical assessment with a particular focus on the sVOC fraction of TPE $PM_{0.1}$ is warranted. Filling in the organic data gap would enable researchers to construct a complete risk assessment using data collected within actual working offices and commercial PC centers.

There is a notable absence of data with respect to available and emerging control technology to mitigate PM emissions. To date, the authors are aware of only one study to evaluate controls that may be available to managers/owners, hygienists, and health and safety practitioners. Wensing et al. (2008) showed commercially available filters retrofitted to the LP can reduce emissions to the indoor environment of up to 84%. Moreover, other controls such as local exhaust ventilation and isolation have not been evaluated in the LP or PC micro-environment as means to minimize exposures. Similarly, reformulation of toners to reduce toxicological properties of PM emissions might offer the toner industry a safer by design approach to address this issue.

The regulatory environment and indoor occupational exposure guidelines present in the United States, and presumably elsewhere, with respect to $PM_{0.1}$ emissions from TPE are lacking. Generally speaking, both OSHA and USEPA have adopted provisions and recommendations developed by the American Society of Heating, Refrigerating, and Air-Conditioning Engineers (ASHRAE) with respect to indoor air quality, ventilation, and comfort. Similarly, state and local agencies generally adopt ASHRAE standards on ventilation (ASHRAE 62.2–2010) (ASHRAE 2010). The ASHRAE standard merely mentions PM as PM_{10} as a selected contaminant with a concentration of interest threshold of $50\,\mu g/m^3$. Failing to bring attention to nano-sized PM fractions, particularly $PM_{0.1}$, in the ASHRAE standards is a clear indication that ultrafine particles do not seem to be a priority pollutant in the indoor environment.

Conclusions

Presently, the available information clearly indicates substantial exposures to NP due to LP and PC use. Although toxicology and molecular epidemiology of these emissions warrants further research as outlined below, current state of evidence also warrants efforts towards exposure mitigation strategies. Martin et al. (2017) outlines a number of strategies and options to achieve this. The knowledge base for developing engineering solutions and the necessary tools are available. In the case of TPE emissions, besides developing effective control technologies on existing devices as outlined above, promotion of a new generation of clean printing and photocopying technologies, which are now commercially available, must be encouraged. Moreover, an awareness campaign to inform workers and the general public of the potential hazards associated with TPE use is also important. The indoor air quality guidelines for indoor spaces, which even when met do not provide sufficient ventilation, should be revised to address this pollution source. Likewise, further research is needed to untangle contribution of the various organic and inorganic

components of the TPE-emitted PM, as well as to identify possible chemical signature patterns and exposure markers for organics in the released pollutants. The catalytic role of metal oxide ENM in the formation of other pollutants is also suspected, given that such effects are known in air pollution research. Furthermore, these metal oxides may play an important role in the formation of ROS and oxidative stress, as well as in serving as condensation nuclei for organics. Transition metals corresponding to metal oxide ENM in toners may account from 2 to 10% of the airborne $PM_{0.1}$ fraction.

Despite there being a growing database on *in vitro* and *in vivo* adverse effects (oxidative stress, inflammation, DNA damage, and respiratory disease such as cough, wheeze, increased sputum production, etc.) following exposure to emissions from TPE, there are still several questions regarding the toxicological mechanism/s of action of these particles, metal oxide ENM, and gaseous co-pollutants. Lastly, large-scale toxicological and epidemiological studies are warranted to understand potential disease development in chronically exposed populations of workers and consumers. Such studies should assess the impact of emissions from TPE on the respiratory (e.g. lung function, rhinitis), immune (allergies), cardiovascular, nervous system, and possibly liver.

It is of critical important that future studies do not repeat the same mistakes of past studies. With regard to cellular and animal testing, these include the use of TPE-emitted nanoparticles and not toners, dispersion, and dosimetry considerations, *in vitro* and *in vivo* dose equivalency calculations, realistic dose ranges and dose rates, true sub-chronic and chronic inhalation studies in animals, and a combination of biomolecular markers and histopathology of the target organs. The studies should also attempt to address directly quantitative links between biomolecular markers and disease development. As mentioned earlier, the use of ELISA kits for urinary 8-oxodG and other analytes, as well as global/non-specific assays such as TBAR and FRAC should be substituted with more specific markers, and measured whenever possible with liquid chromatography-mass spectrometry techniques.

Large-scale human epidemiological studies should first and foremost ensure good quality quantitative exposure assessment, lack of which has been one of the most common limitations of most studies to date. Personal nanoparticle monitors are now encouraged because they are now available. Furthermore, they should collect and report contextual information on exposures and activities; and quantify and verify health effects in a more objective manner rather than rely on self-reported symptoms. Most importantly, these studies should quantify prevalence of disease in such populations and strive to provide quantitate exposure-response analysis.

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