Engineered Nanoparticles Emitted From Laser Printers: Environmental Health Implications

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ABSTRACT

We present a multi-tiered methodology designed to thoroughly characterize the engineered nanomaterials (ENMs) released from nano-enabled toners during printing. A printer exposure generation system (PEGS) suitable for the physico-chemical and toxicological assessment of printer-emitted particles (PEPs) was developed to screen commercially available laser printers. Results from our extensive analysis show laser printers emitted nanoparticles at concentrations of up to 1.3 million particles/cm^3. Moreover, toner powders and PEPs share a similar chemical composition (organic, metal, metal oxides). Toxicological assessment using *in vitro* and *in vivo* models showed PEPs have the potential to be biologically reactive (*e.g.*, inflammation, cytotoxicity, epigenetic modifications). Overall, our results suggest that laser printer emissions are chemically complex and bioactive and thus, may be deleterious to the physiology of individuals exposed to these particles both in a residential and occupational setting.

Keywords: laser printer emissions, nanoparticles, life cycle, particulate matter, toner.

1 INTRODUCTION

The use of printing equipment, such as laser printers and photocopiers, has grown exponentially over the last decade, driven primarily by the substantial increase in the number of home-based businesses in the USA and the use of personal computing[1]. Aside from exposures at printing centers, there is also the risk of occasional exposures in many other settings, such as schools, hospitals, offices and homes. Thus, it is of growing importance to both evaluate laser printer emissions and perform a proper science-based risk assessment.

There are numerous studies associating the process of printing with emission of particulate matter (PM) and gaseous pollutants, such as semi-volatile organic compounds (sVOCs) and ozone, among others[2-5]. However, while these studies looked at particle concentration and size distribution of the emissions from laser printers, there is limited literature on the physico-chemical properties of PEPs, and more significantly there is no evidence on the incorporation of engineered nanomaterials (ENMs) in the toner formulation and their possible emission into the air.

Further, the toxicological potential of printer-emitted particles (PEPs) is currently poorly understood, but circumstantial evidence continues to grow. A major limitation of some studies is the use of toner powder particles in both *in vitro* and *in vivo* test platforms[6-10]. Several *in vivo* studies revealed that long-term inhalation exposures using toner powders can cause chronic inflammation and fibrosis in rats, while intratracheal instillation leads to development of lung tumors in rats[9, 10]. Furthermore, *in vitro* cellular bioassays using toner powder reported increased levels of reactive oxygen species, cyto- and genotoxicity markers, fibrosis, reduced pulmonary clearance and cell proliferation[6, 7]. The use of toner particles rather than actually emitted PM does not accurately reflect the actual exposures and properties of PEPs and prohibits interpretation of the findings.

In the study described here, an integrated platform suitable for the physico-chemical, morphological and toxicological characterization of realistic PEPs was developed and tested. The exposure system was utilized to thoroughly assess the emissions and toner powder from eleven comercially available laser printers.

2 RESULTS AND DISCUSSION

2.1 Exposure platform development

A laser printer exposure generation system (PEGS, Figure 1)[11] was developed to generate real world PEPs exposures associated with commonly used laser printers. The PEGS was found to be versatile and allowed for the generation of realistic PEP exposures that were suitable for physico-chemical, morphological and toxicological characterization.

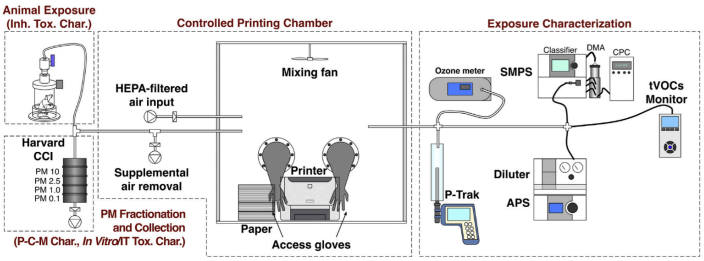

Figure 1. Printer Exposure Generation System used to collect freshly generated PEPs for subsequent assessment.

2.2 PEPs profiles for commercial printers

The real-time monitoring of the emissions from the tested laser printers provided evidence that particles are emitted at substantially high levels (*i.e.*, 1.3 million particles/cm^3) in addition to other pollutants (*e.g.*, ozone, tVOCs)[11]. The data also showed that the majority of PEPs are in the nanoscale with modal diameters ranging from 49 to 208 nm, with the majority of PEPs in the nanoscale (<100 nm) size. Moreover, most of the printers, regardless of the manufacturer/model, had an ''initial burst'' emission pattern characterized by a transient peak in particle number concentration within the first 10–20 min of printing, followed by a steady decay until completion of printing. This observation is in agreement with other studies and it has been attributed to the rise in temperature of the fuser unit[2, 5, 12]. Furthermore, PEPs can be affected by certain operational parameters.

The elevated particle concentrations during the use of a laser printer was concerning as these were higher than those observed in highly polluted highways[13]. Similarly, the $PM_{2.5}$ mass concentration levels for one printer was 99.81 mg/m^3, which surpasses the Environmental Protection Agency retained 24-hour ambient PM standard of 35 mg/m^3.

2.3 Chemical and morphological assessment of PEPs and toner powder

Following the development and testing of the PEGS, a thorough investigation was done to assess the presence of nanoscale materials in the toner formulations and study the release of such ENMs in the air during printing (consumer use) using state of the art analytical methods. The data[14] confirmed that a number of ENMs incorporated into toner formulations (*e.g.*, silica, alumina, titania, iron oxide, zinc oxide, copper oxide, cerium oxide, carbon black among others) and released into the air during printing. All evaluated toners contained large amounts of organic carbon (OC, 42–89%), metals/metal oxides (1–33%), and some elemental carbon (EC, 0.33–12%). The PEPs possess a composition similar to that of toner and contained 50–90% OC, 0.001–0.5% EC and 1–3% metals. While the chemistry of the PEPs generally reflected that of their toners, considerable differences are documented indicative of potential transformations taking place during consumer use (printing). The results confirm there is routine incorporation of ENMs in toners -classifying them as a nano-enabled product (NEPs)- and these ENMs become airborne during printing.

2.4 *In vitro* toxicological assessment of PEPs

Once a comprehensive evaluation on the physico-chemical and morphological properties of PEPs was completed, we sought to evaluate the potential toxicity of varying doses of PEPs on physiologically relevant cell lines using both mono- and co-culture systems at doses that approximate those associated with current inhalation exposures[15-17].

Firstly, our data from using the alveolar-capillary co-culture model with Human Small Airway Epithelial Cells (SAEC) and Human Microvascular Endothelial Cells (HMVEC) demonstrated that direct exposure of SAEC to low concentrations of PEPs (0.5,1.0 mg/mL) caused morphological changes of actin remodeling and gap formations within the endothelial monolayer. Increased production of reactive oxygen species (ROS) and angiogenesis were observed in the HMVEC. Further, certain cytokines and chemokines may play a major role in the cellular communication observed between SAEC and HMVEC and the resultant responses in HMVEC.

Secondly, using a mono-culture system, three physiologically relevant cell lines (SAEC, macrophages and lymphoblasts) were exposed to PEPs (0.5-100 µg/mL) for varying exposure times and the biological response was assessed. Results showed that both the epithelial and the macrophage cell lines were negatively affected by treatment with PEPs and experienced >50% cell death. It seems that macrophages were particularly sensitive to exposure to PEPs. SAEC also secreted significant levels of mediators that are critical in the innate immune responses to foreign particles, leading to recruitment of various leukocytes to the site of injury/inflammation[18]. Aside from inflammatory responses, an increase in superoxide levels was also evident in epithelial cells post-treatment with PEPs. Lastly, we observed a dysfunction of DNA methylation and demethylation machinery that was associated with the loss of methylation and reactivation of transposable elements, whose reactivation may lead to genomic instability. Overall, the results from such a comprehensive battery of *in vitro* toxicological assessments on PEPs are indicative of the cyto- and genotoxic potential of laser printer emissions at relevant doses comparable to current consumer and occupational settings. Most importantly, laser printer-emitted engineered nanoparticles can be deleterious to lung cells and may cause persistent genetic modifications that could translate to pulmonary disorders.

2.5 *In vivo* toxicological assessment of PEPs

The next aim was to continue to use "real world" PEPs, rather than raw toner powder, and assess the pulmonary responses following exposure by intratracheal instillation. *Balb/c* mice were exposed to various doses of PEPs (0.5, 2.5 and 5 mg/kg body weight), which are comparable to

real world human inhalation exposures ranging from 14 to 142 hours of printing. Toxicological parameters reflecting distinct mechanisms of action were evaluated, including lung membrane integrity, inflammation and regulation of DNA methylation patterns[19].

Results from this *in vivo* toxicological analysis showed that while intratracheal instillation of PEPs caused no changes in the lung membrane integrity, there was a pulmonary immune response, indicated by an elevation in neutrophil and macrophage percentage over the vehicle control and low dose PEPs groups. Additionally, exposure to PEPs upregulated expression of the *Ccl5* (Rantes), *Nos1* and *Ucp2* genes in the murine lung tissue and modified components of the DNA methylation machinery (Dnmt3a) and expression of transposable element LINE-1 compared to the control group. These genes are involved in both the repair process from oxidative damage and the initiation of immune responses to foreign pathogens. The results are in agreement with findings from previous *in vitro* studies and suggest that PEPs may cause immune responses in addition to modifications in gene expression in the murine lung at doses that can be comparable to real world exposure scenarios, thereby raising concerns of deleterious health effects.

3 CONCLUSIONS

In this study, we have developed and tested an integrated realistic exposure generation platform that is suitable for the physico-chemical, morphological and toxicological characterization of PEPs.

The results from this extensive assessment show that laser printers emit substantially high levels of PM (*i.e.*, 1.3 million particles/cm^3) in addition to other pollutants (*e.g.*, ozone, tVOCs). Further, it was confirmed that chemically-complex ENMs are being incorporated into current toner formulations, and most importantly, these ENMs are becoming airborne during the use of a laser printer. The toxicological evaluation of PEPs showed these particles are biologically reactive in cellular and animal experimental models there is lung injury, inflammation and changes in gene expression. This raises concerns as to possible adverse cardiopulmonary effects of laser printer emissions. It is clear, however, that these acute studies should be followed by more detailed sub-acute and chronic studies in order to have more conclusive evidence on deleterious physiological effects of such a widely used NEP.

Lastly, this integrated approach provides a testing platform for nano-risk assessors to understand the properties of released PM from NEPs and their link to toxicological outcomes and can be used for other nanomaterials. Such a methodological approach will improve our understanding of the potential impact of ENM exposures on human health in both occupational and non-occupational settings and generate suitable data for science-based risk assessment. Predominantly, the established platform described here to link exposures to particulate matter released across life cycle (called LCPM) could be used to study other NEPs for a more realistic risk assessment throughout the life cycle of nanomaterials.

4 METHODS

4.1 Development of exposure platform

The PEGS was developed to generate real world PEPs exposures associated with eleven commonly used laser printers. It consists of (a) a glovebox type environmental chamber to house the printers for uninterrupted operation; (b) real-time and time-integrated PM particle sampling and monitoring instrumentation to quantify particle size distribution and collect size-fractionated PEPs for analysis and (c) an animal inhalation exposure system for toxicological evaluation.

4.2 Sampling of PEPs

Laser printer emission profiles were assessed using real-time monitoring instrumentation such the water-based condensation particle counter (Model 3785, TSI Inc.) and the scanning mobility particle sizer (Model 3080, TSI Inc.). The Harvard compact cascade impactor (CCI)[20] was used to size fractionate and collect PM samples in sampling substrates (teflon filter and polyurethane foam).

4.3 Characterization of PEPs and toner powder

Post-sampling, the PEPs were extracted in deionized water (DI H_2O) using a sonication protocol to allow for maximum extraction efficiency, without chemical alteration of the extracted particles and thus; we have a final particle suspension that is representative of the aerosol composition[21]. Particle suspensions were prepared and characterized using a published protocol[22, 23].

Physico-chemical characterization of the size fractionated PEPs and toner powders included testing for total and water-soluble fraction of multiple metals (50 elements), and for organic and elemental carbon per previously published methods[24]. Further chemical and morphological assessment of both the PEPs and the toner powder were done using scanning and transmission electron microscopy (STEM) coupled with energy dispersive spectroscopy (EDX).

4.4 Dosimetry considerations

To express *in vivo* and *in vitro* doses on the same scale, the dosimetric approach recently developed by the authors was followed[21, 25]. In summary, the Multiple-Path Particle Dosimetry (MPPD2)[26] model was used to calculate the dose deposited in the lung for a range of inhalation exposure times. Successively, that lung dose deposited is converted into cell deposited dose. The hybrid Volumetric Centrifugation Method-*In Vivo* Sedimentation, Diffusion and Dosimetry (VCM-ISDD) dosimetry methodology was used to convert the administered-to-cell dose to delivered-to-cell dose[23, 27].

4.5 *In vitro* and *in vivo* toxicological methods

For the cellular toxicology assessment, human monocytic immortalized cells (THP-1), SAEC, TK-6 human lymphoblastoid cells and HMVEC were cultured and maintained following manufacturer protocol. The cells were treated with PEPs ($PM_{0.1}$ and $PM_{2.5}$) at doses ranging from 0.5 to 100 μg/mL. Post-treatment evaluation of cell death, inflammation, oxidative and DNA damage, and epigenetic changes was performed.

For the animal toxicology assessment, mice were exposed to various exposure doses of PEPs ($PM_{0.1}$) by intratracheal instillation. Following the exposure, animals were sacrificed and bronchoalveolar lavage (BAL) was performed. The BAL fluid, blood and lung tissue were subsequently used to measure biochemical markers of inflammation, albumin and hemoglobin levels, white blood cell differentials and expression of a number of genes in addition to epigenetic analyses.

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