Occupational inhalation exposures to nanoparticles at six Singapore printing centers

Setyawati, Magdiel Inggrid; Singh, Dilpreet; Krishnan, Sriram P. R.; Huang, Xian; Wang, Mengjing; Jia, Shenglan; Goh, Bernice Huan Rong; Ho, Chin Guan; Ridhwan Yusoff; Kathawala, Mustafa H.; Poh, Tuang Yeow; Nur A'tikah Binte Mohamed Ali; Chotirmall, Sanjay Haresh; Aitken, Robert J.; Riediker, Michael; Christiani, David C.; Fang, Mingliang; Bello, Dhimiter; Demokritou, Philip; ...Ng, Kee Woei

2020

Setyawati, M. I., Singh, D., Krishnan, S. P. R., Huang, X., Wang, M., Jia, S., ... Ng, K. W. (2020). Occupational inhalation exposures to nanoparticles at six Singapore printing centers. *Environmental Science and Technology*, 54(4), 2389-2400. doi:10.1021/acs.est.9b06984

<https://hdl.handle.net/10356/146776>

<https://doi.org/10.1021/acs.est.9b06984>

This document is the Accepted Manuscript version of a Published Work that appeared in final form in *Environmental Science and Technology*, copyright © American Chemical Society after peer review and technical editing by the publisher. To access the final edited and published work see <https://doi.org/10.1021/acs.est.9b06984>

Downloaded on 26 Apr 2023 23:18:19 SGT

Occupational Inhalation Exposures to Nanoparticles at Six Singapore Printing Centers

*Magdiel I. Setyawati[†], Dilpreet Singh[‡], Sriram P. R. Krishnan^{†,§}, Xian Huang[§], Mengjing Wang[†],
Shenglan Jia[†], Bernice Huan Rong Goh[†], Chin Guan Ho[†], Ridhwan Yusoff[†], Mustafa H.
Kathawala[†], Tuang Yeow Poh^Δ, Nur A'tikah Binte Mohamed Ali^Δ, Sanjay H. Chotirmall^Δ,
Robert J. Aitken[§], Michael Riediker^{†,§}, David C. Christiani[‡], Mingliang Fang[†], Dhimiter Bello[‡],
Philip Demokritou^{‡,†}, Kee Woei Ng^{†,‡,||,¶,*}*

[†]School of Materials Science and Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798, Singapore.

[‡]Center for Nanotechnology and Nanotoxicology, Harvard T.H. Chan School of Public Health, Harvard University, 665 Huntington Ave., Boston, MA 02115, USA.

[§]IOM Singapore, 237 Alexandra Rd., Singapore 159929, Singapore.

^ΔSchool of Civil and Environmental Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798, Singapore.

^ΔLee Kong Chian School of Medicine, Nanyang Technological University, 11 Mandalay Road, Singapore 308232, Singapore.

^{||}Skin Research Institute of Singapore, Biomedical Science Institutes, Immunos, 8A Biomedical Grove, Singapore 138648, Singapore.

[¶]Environmental Chemistry & Materials Centre, Nanyang Environment and Water Research Institute (NEWRI), Nanyang Technological University, 1 Cleantech Loop, CleanTech One, Singapore 637141, Singapore.

KEYWORDS photocopy centers, occupational exposure to nanoparticles, engineered nanomaterials, indoor air quality, polycyclic aromatic hydrocarbons, toners

ABSTRACT

Laser printers emit high levels of nanoparticles ($PM_{0.1}$) during operation. Although it is well established that toners contain multiple engineered nanomaterials (ENMs), little is known about inhalation exposures to these nanoparticles and work practices in printing centers. In this report we present a comprehensive inhalation exposure assessment of indoor microenvironments at six commercial printing centers in Singapore, the first such assessment outside of the United States, using real-time personal and stationary monitors, time-integrated instrumentation and multiple analytical methods. Extensive presence of ENMs, including titanium dioxide, iron oxide and silica, were detected in toners and in airborne particles collected from all six centers studied. We document high transient exposures to emitted nanoparticles (peaks of $\sim 500,000$ particles/cm³, lung deposited surface area of up to $220 \mu m^2/cm^3$, and $PM_{0.1}$ up to $16 \mu g/m^3$) with complex $PM_{0.1}$ chemistry that included 40-60 wt% organic carbon, 10-15 wt% elemental carbon, and 14 wt% trace elements. We also record 271.6 – 474.9 pmol/mg of Environmental Protection Agency-priority polycyclic aromatic hydrocarbons. These findings highlight the potentially high occupational inhalation exposures to nanoparticles with complex compositions resulting from widespread usage of nano-enabled toners in the printing industry, as well as inadequate ENM specific exposure control measures in these settings.

INTRODUCTION

Toner-based printing equipment (TPE) like laser printers (LP) and photocopiers (PC) have become permanent fixtures in many homes and work establishments. TPE, originally developed to accelerate the process of repetitive printing and document reproduction, employs a digital printing

process that continuously heats the dry toner powder and fuses the “melted” powder on the paper to affix an images.¹⁻³ Studies have shown that TPE releases gaseous pollutants and high levels of aerosolized particulate matter (PM) containing engineered nanomaterials (ENMs) used in the toner formulations such as metal oxides and silica, with some studies showing temporal PM emission bursts up to 1 million particles/cm³.^{1, 4-7} Though much work has been dedicated to measure and understand PM_{0.1} emissions from TPE (summarized here¹), the majority of these studies were performed in controlled printer chambers. Only a handful of high quality field studies, primarily conducted in the US and Europe, measured real-world PM_{0.1} exposures in an occupational setting,¹ and even a lesser number of studies have been done to understand the personal exposure levels of TPE operators in printing and copier centers, where multiple printing equipment are concurrently operated to fulfil high-volume printing jobs.

More specifically, an indoor exposure study conducted at 8 copier centers in the greater Boston area (USA) reported weekly mean nanoparticle number concentrations reaching approximately 12 times higher than the background levels (before start of printing activity), with maximum temporal emission recorded to be 700-fold higher than the background average.² Physicochemical characterization of PM_{0.1} sampled at these copier centers revealed the presence of ENMs,² which were shed from the nano-enabled toners utilized in the printing or copying processes.^{2, 5-6, 8} In a recent comprehensive toxicological assessment of LP emissions, the transition metal/metal oxide ENMs present in the printer-emitted particles (PEPs) were reported to contribute in the production of reactive oxygen species (ROS) and short-lived radicals that may lead to oxidative stress induction in the workers and patrons of the printing centers.⁹ Khatri *et al.* documented acute oxidative stress and upper airway inflammation in healthy volunteers exposed to copier center PM_{0.1} fractions at realistic levels.¹⁰ More recently, elevated levels of oxidative

stress and upper airway inflammation biomarkers were detected in 6 chronically exposed copier operators relative to their control peers.¹¹

The bioactivity and toxicity of nanoparticles emitted from LP and PC have also been assessed extensively in a series of cellular *in-vitro* and animal *in-vivo* studies. A subacute whole-body inhalation study conducted on rats exposed to PEPs showed significant changes in anti-inflammatory cytokine and chemokine levels in the respiratory tract and lung in addition to elevated serum biomarkers of oxidative stress and inflammation.¹² Intratracheal instillation of PEPs in a mouse model showed evidence of lung injury and inflammation as well as epigenetic alterations in the lung and peripheral blood such as changes in global DNA methylation and upregulation of genes involved in oxidative damage repair and initiation of immune responses to foreign pathogens.¹³⁻¹⁵ Numerous *in-vitro* studies in physiologically relevant human cell lines have also documented effects of acute PEPs exposure resulting in cell membrane integrity damage, increase in ROS production and upregulation of antioxidant and inflammation genes, pro-inflammatory cytokines, cell apoptosis, structural changes in the alveolar-capillary endothelium, and epigenetic modifications.^{13, 15-18} These initial exploratory studies have demonstrated the toxic potential of nanoparticles emitted during toner-based printing processes.

While emission profiles (i.e., particle number concentration, PNC) from printers with similar setup and activity are not expected to differ between the US and Singapore, it is hypothesized that environmental differences and air composition could lead to composition and behavioral differences in the particles emitted during toner-based printing processes. Moreover, to maintain indoor comfort in a hot and humid climate, Singapore buildings rely heavily on air conditioning and mechanical ventilation systems which were reported to have low fresh air intake, leading to the buildup of indoor pollutants.¹⁹ Considering these geographically relevant

differences, it is meaningful and novel to conduct a comprehensive assessment of real-world exposures, exposure controls and indoor air quality inside Singapore printing centers to understand the associated potential occupational health and safety implications of such exposures.

Here, we provide a comprehensive exposure assessment of indoor airborne printing center nano/particle (PCP) exposures using both area and personal monitors, indoor air quality (IAQ) parameters, exposure controls, and operational setups at 6 commercial printing centers in Singapore using real-time and time-integrated approaches, followed by a detailed physicochemical and morphological characterization of the airborne $PM_{0.1}$ fractions of the sampled PCP and toners to generate several exposure metrics for molecular epidemiological studies of the health effects of these nanoparticle exposures in printer and copier operators. In subsequent studies we will present findings on systemic and upper airway inflammation, oxidative stress, allergic potency, gene expression and respiratory disease and pulmonary function in the operators at these centers.

MATERIALS AND METHODS

Printing center walkthroughs

Six different printing centers (designated as center #1-6) distributed across Singapore were recruited for this study (Institutional Review Board of Nanyang Technological University approval number IRB-2017-07-023). Participating centers utilized TPE in their 9- to 10-hr daily operations, and were visited on randomly selected monitoring weeks, during which the physical characteristics of each center (size, layout, HVAC (heating, ventilation, and air conditioning) system, engineering and other exposure controls) and their printing production information (TPE model and brand, toner brand and composition, number of active printers and production volume) were systematically collected. In addition, toner samples were collected from each printer and photocopier in these centers.

Exposure assessment strategy

The overall exposure assessment strategy employed at these printing centers, as depicted in **Figure 1** and described in greater detail in the **Supplementary Information (SI)**, was based on earlier work by the authors.^{2, 8} Briefly, exposure measurements of indoor airborne PCP and various IAQ parameters were accomplished through stationary (fixed) monitors (real-time and time-integrated), as well as personal exposure monitoring (real-time) of the printer operators, as described below. In addition to the exposure measurement campaign, detailed contextual information related to the occupancy of the printing room, workers' real-time activities and deployment of the monitoring instrument (location and timing) was also collected.

Stationary exposure measurements

Stationary monitoring locations for PCP and IAQ measurements were chosen as close as possible to the printing stations without causing any interference to the workers' operational activities. Stationary measurements in the printing room comprised of: (i) real-time measurements of PCP number concentration and its mobility size distribution in the nano-regime (10-420 nm) using a Scanning Mobility Particle Sizer (NanoScan SMPS Nanoparticle Sizer 3910; TSI Inc., USA); (ii) time-integrated and size-fractionated ($PM_{>2.5}$, $PM_{0.1-2.5}$, and $PM_{0.1}$) particle sampling using the Harvard Compact Cascade Impactor (CCI)²⁰ to quantify the average airborne PCP mass concentration and for detailed physicochemical and morphological analysis of collected $PM_{0.1}$; and (iii) real-time measurements of IAQ parameters: temperature, relative humidity, CO_2 , volatile organic compounds (VOC) were measured using a Qtrak IAQ meter (model 7575; TSI Inc., USA), while gaseous co-pollutants NO_2 and ozone (O_3) were recorded using a portable Series 500 air quality monitor (Aeroqual, New Zealand). Exposures were measured over 2 weeks of monitoring at each center and typically lasted for 4 days/week and followed the centers' operational hours.

For comparison purposes, real-time PCP and IAQ measurements were also done in the office areas (physically separated from the printing rooms) of centers #2-6 and outdoor areas of all centers. Indoor background PM samples in the printing rooms were also collected using the CCI over a weekend (when no printing operations occur) to compare with PM collected during regular operating hours. A representative outdoor PM sample was collected at center #6.

Personal exposure measurements

TPE operators' personal exposures to PCP were monitored in real time with portable personal nanoparticle dosimeters (Partector, Naneos Particle Solutions GmbH, Switzerland), that measure the estimated alveolar lung deposited surface area (LDSA) concentration of particles within the size range of 10 nm to 10 μ m. The instruments were randomly assigned to participating centers' employees, who were monitored continuously for at least 4 hours/day while conducting their work activities. Personal exposures of these workers were measured for at least three monitoring days over the course of the 2-week monitoring period.

Physicochemical and morphological analysis of airborne PCP and toner powders

Detailed physiochemical and morphological analyses of PCP samples and toner powders (collected from printer cartridges at the centers) were done as previously described by Bello *et al.*,⁸ which comprised of: (i) morphological analysis of electrostatically precipitated (using Partector TEM sampler, Naneos Particle Solutions GmbH, Switzerland) airborne PCP with Transmission Electron Microscopy (TEM) and that of toner powders using Scanning Electron Microscopy (SEM); (ii) organic and elemental carbon (EC-OC) characterization of the PCP PM_{0.1} size fraction and toner powders; (iii) total elemental analysis of PCP PM_{0.1} and toner powders using Inductively Coupled Plasma Mass Spectrometry (ICP-MS), and (iv) polycyclic aromatic hydrocarbon (PAH)

analysis (16 EPA-priority PAHs)²¹ of PCP PM_{0.1} and toner powders²²⁻²³. Detailed experimental methods are described in the **SI**.

Data analysis

Real-time measurement data from the SMPS and Partector were transferred and analyzed with Origin Pro 2018 (Originlab, USA). Upon examination of the histograms (Shapiro-Wilks test), these data were found to fit the log-normal distribution better, and thus were summarized as geometric mean (GM) $\pm$ geometric standard deviation (GSD). IAQ parameters were reported as daily or weekly averages $\pm$ standard deviation (SD). Details on gravimetric data analysis to determine average PM mass concentration and the mass-weighted proportions of EC, OC and inorganic content in PM_{0.1} are presented in the **SI**.

RESULTS

Physical characteristics of printing centers

Figures S1-S6 outline the interior layouts and physical characteristics of the printing centers recruited into the study. Across these 6 centers, which were distributed across different parts of Singapore, eight different types of commercially available printers originating from four major brands (designated as A, B, C, and D) were identified. Most printing centers use 2-3 high-speed TPE (**Figure 2A**). Typically, mechanical ventilation and air conditioning systems (cassette and/or split-AC) were used in the printing room, and the windows and doors in the premises remained closed year-round. Printing volumes varied between centers with daily average prints ranging from 652 pages/day (center #6) to 59,686 pages/day (center #1). Maximum daily prints in a single production day could reach 147,000 pages (center #1), while the minimum daily prints registered for a single day were found to be 8 pages (center #6). No dedicated engineering nor other exposure controls were utilized in any of the participating printing centers, though in one center (center #1),

local exhaust ventilation systems were installed on 2 of 4 photocopiers but not operational. The stationary (fixed) monitoring locations in each of the printing rooms are denoted by an orange-colored star (*) in the layouts. Detailed information on the physical characteristics of each printing center is provided in the **SI**. Manufacturer-provided technical specifications of the printers utilized in these centers and the chemical composition of their respective toners (designated as TON A1 – D1 to correspond with the printer designation) are summarized in **Table S1**. Based on the information provided by the toner manufacturers' Safety Data Sheets (SDS), the toners in general contain polyester resin, iron oxide, carbon black, polymer wax, titanium dioxide, and silica (in TON A1 and D1) in addition to styrene acrylate copolymer, iron oxide, wax, carbon black, amorphous silica, and titanium dioxide formulations found in TON A2, B1 and B2.

PCP characterization from stationary real-time measurements

Real-time SMPS measurements revealed that printing activity contributed to the airborne particle generation, as evident from the significant increase of measured PNC at the onset of the printing activity (**Figure S7**). Pre-printing activity background PNC was measured to be in the range of 1,400 – 10,000 particles/cm³ (**Table S2**), and the resultant printing activity to background ratio of PNC ($\text{GM PNC}_{\text{Print}}/\text{GM PNC}_{\text{bckgd}}$) was determined to be 1.2 – 6.4 fold (**Figure 2 B, C**). No background PNC measurement was collected in center #1 due to limited access granted by the center. Nonetheless, work activity observation coupled with the real-time measurement of PNC reiterated the notion that printing activity was the primary contributor to the PCP detected in the printing room of center #1. The particle counts steadily decreased in the absence of printing activity over an extended period of time (*e.g.*, during lunch break period), and then rose significantly (approximately 3.2-fold increase, **Table S2**) once printing activity resumed (**Figure S7A**). Continuous real time SMPS measurement over the course of 36 hours at selected centers (*i.e.*,

centers #2 and #3) showed the PNC to decay once printing activity in the room was completed at the end of the work shift, reaching background levels within 4 hours afterwards (green dotted line, **Figure S8**). We observed no “accumulative effect” due to residual particles contributing to the PNC measurement (**Figure S8**), even when there was overtime printing activity in the printing room (center #2).

Full-shift assessments in these printing centers revealed that the particle count and particle size fluctuated throughout the operational day following the printing activities in the room, as depicted in **Figure S7**. Daily GM of PNC of a given center had day-to-day variations due to inter-day workload variabilities (**Figure 2B**). Daily GM PNC in center #2, for example, ranged between 20,000 to 52,600 particles/cm³ (**Figure 2B**) with close to 426,000 particles/cm³ of transient burst of PCP number concentration detected during the peak production period (**Figure 2C**). It is well established that production volumes, TPE setups utilized, printing room designs and HVAC performance could affect PM_{0.1} emissions. For example, color printers will generally emit more PM_{0.1} compared to their monochrome counterparts,²⁴ while utilizing used cartridges resulted in lower PM_{0.1} emission compared to new cartridges.^{1, 7} PM_{0.1} emissions were reported to increase proportionally with toner print coverage.^{1, 7} These inter-center variabilities further magnified the exposure level differences observed between the participating centers. Weekly GM PNC was determined to be 1,600 - 50,000 particles/cm³ across all centers, registering more than 30-fold difference between the low- and high- PCP emitting centers, centers #6 and #1, respectively.

The detected PCP were predominately in nanoscale range with a broad and approximately unimodal particle mobility size distribution that ranged between 10 – 200 nm (**Figure S9**). Slight differences were observed in the weekly count median diameter (CMD) across the different centers, ranging from 30 – 45 nm, with corresponding GSD ranging from 1.19 - 1.52 (**Figure 2C**).

Particle counts at control sites (*i.e.*, office room and mail room) situated away from the printing area, were generally found to be significantly lower as compared to the printing rooms (**Table S3**). However, office PNC was slightly higher than in the printing room at centers #4 ($\text{PNC}_{\text{Print}} / \text{GM PNC}_{\text{Office}} = 0.91$) and #6 ($\text{GM PNC}_{\text{Print}} / \text{GM PNC}_{\text{Office}} = 0.95$), which could be due to the low production volume at these centers. We documented respective average daily volumes of 4000 prints (corresponds to ~10% daily printing capacity) and 652 prints (corresponds to ~2% daily printing capacity) at centers 4 and 6 (**Figure 2A**), respectively. In addition, in both of these centers our monitoring revealed that production processes were dominated by intermittent printing which has been reported to promote a higher particle count decay rate, due to agglomeration, and effectively reduced the airborne PCP concentration, resulting in lower PNC detected in the printing rooms compared to the respective control sites.²⁵

PCP characterization from stationary time-integrated measurements

Weekly average $\text{PM}_{0.1}$ mass concentration in the 6 participating printing centers determined from size selective sampling with the CCI,²⁰ ranged from 4.4 - 15.1 $\mu\text{g}/\text{m}^3$, which accounted for approximately 21 – 37 wt% of the total mass concentration of airborne particles sampled in the printing room (**Table S4**). The weekly average $\text{PM}_{2.5}$ mass concentrations (including both $\text{PM}_{0.1}$ and $\text{PM}_{0.1-2.5}$ fractions) ranged between 10.8 – 31.4 $\mu\text{g}/\text{m}^3$ across the different centers.

Workers' personal exposures to airborne PCP

Nineteen healthy, full-time PCP-exposed workers (*i.e.*, those who are primarily working in the printing room) were recruited from the 6 participating centers. Demographic details of the recruited workers will be reported in our subsequent publications. Workers were observed to work without any personal protective equipment (PPE). Representative real-time measurements depicted a good correlation between workers' LDSA concentration and the PNC in the respective

printing rooms in which they work (**Figure 3A, Figure S10**), indicating that area measurements of PCP using stationary particle counters could serve as a reasonably good surrogate for personal exposure measurements. Personal exposures to PCP were found to vary based on the workers' job-task activities which affected the amount of time they spent in the printing room (**Figure 3B**). Daily GM LDSA level for worker #1 (center #1), who predominantly stayed in the printing room (approximately 90% of the working hours) to operate the TPE printers and copiers, was found to range between 62 – 212 $\mu\text{m}^2/\text{cm}^3$ with overall GM LDSA of 106.1 $\mu\text{m}^2/\text{cm}^3$ and corresponding GSD of 1.67 (**Figure 3B**). Whereas, worker #2 (center #1), who supervised the production process and spent lesser time in the printing room (approximately 46% of the working hours), was found to have daily GM LDSA between 28 – 220 $\mu\text{m}^2/\text{cm}^3$ with overall GM LDSA of 54.2 $\mu\text{m}^2/\text{cm}^3$ and corresponding GSD of 2.00 (**Figure 3B**). Consistent with the stationary real-time PCP measurements depicted in **Figure 2**, the workers' personal exposure levels varied between participating centers with registered GM LDSA between 20.4 – 81.9 $\mu\text{m}^2/\text{cm}^3$ (**Figure 3C**). More information about workers' exposure variability is provided in the **SI section B**.

Morphological and elemental characterization of airborne PCP and toner powders

TEM observation of airborne PCP revealed the presence of complex aggregates formed by irregularly shaped nanoparticles (NPs) with a size ranging from 30 – 300 nm (**Figure 4**). Energy-dispersive X-ray spectroscopy (EDX) analysis identified these NPs as metal/metal oxide NPs, including SiO_2 , TiO_2 , MnO , and Fe_2O_3 , as clearly depicted in the elemental scan map of airborne PCP collected from center #1 (**Figure 4A**). Further EDX analysis on the airborne PCP collected from the other participating printing centers detected several inorganic elements such as Mn, Mg, Cr, Ni, Al, Zn, Si, Sn, Ti, and Fe (**Figure 4B-F**).

SEM images show the presence of NPs on the surface of the toner particles (**Figure S11**). EDX analysis of these toners yielded Si, Ti, Fe, and Mg, validating the incorporation of metal/metal oxide NPs into toners as indicated in previous studies.^{2, 5, 8}

Chemical analysis of size-fractionated PCP (PM_{0.1}) and toner powders

The PCP PM_{0.1} collected in the printing rooms during operating hours was characterized chemically for carbonaceous (organic and inorganic carbon) and inorganic compounds (**Figure 5A**). Elemental and organic carbon (EC-OC) analysis shows the carbonaceous population of PM_{0.1} was dominated by organic carbon (OC/TC: 75 – 93 wt%; **Figure 5B**) which accounted for 40-60 wt% of the particles whereas elemental carbon made up only 10-15 wt% across the different centers, similar to the composition of ultrafine PM observed at other photocopy centers in the US.⁸ The remaining fraction was assumed to be inorganic (30-50 wt%), containing numerous metals (*e.g.*, Na, K, Al, Mg, Ca, Fe, Mn, Ti, Zn, Ni, Cu, Cr, V), metalloids (B, Si) and non-metals (P, S) in trace concentrations (0.1 – 9 wt%), as determined from ICP-MS and Si analysis (see **SI**). The overall total concentration of the trace metals in PM_{0.1} ranged from 3 wt% (center #6) to 14 wt% (center #2), as shown in **Figure 5B**. For comparison purposes, the background PM_{0.1} collected over a weekend (no printing operation) in centers #2, #3 and #4, and the PM_{0.1} collected in the office microenvironment of one center (# 5) during regular work hours were also characterized to identify changes in chemical composition of PM as a result of printing activities (**Figure S12**). Results show that the background and office PM_{0.1} have similar overall chemical composition as reflected in global markers of OC: (~40-50 wt%), EC: (~5-10 wt%), and inorganics: (~40-50 wt%) to that of PM_{0.1} collected during printing operations in the printing room. The total trace element concentration of the office PM_{0.1} was ~9 wt%, which was within the range observed for the printing room PM.

The toner powder particles sampled from the TPE of the different centers were also characterized similarly to determine their chemical composition (**Figure S13, Table S5**). Organic carbon constituted 30-85 wt% of the toner particles whereas the elemental carbon content was minimal in most samples (0.1-7 wt%), consistent with previous reports.^{5, 8} The balance inorganic content ranged from 15-70 wt% and exhibited a similar profile of trace elements as PCP PM_{0.1}. The overall concentration of trace elements characterized in toner particles varied from 2 wt% (TON B2, center #3) to 34 wt% (TON C1, center #3; TON C2, center #5).

PAH analysis of size-fractionated PCP (PM_{0.1}) and toner powders

PM_{0.1} fractions of the PCP collected in the printing rooms during normal operating hours were also analyzed for their content of 16 EPA-priority PAHs. The respective background PM_{0.1} collected over a weekend (no printing operation), and the corresponding toner powders utilized in the centers were also analyzed as comparative controls. Total PAH (Σ PAH) levels in toner and background PM_{0.1} were determined to be in the range of 18.9 – 75.2 pmol/mg (median: 53.2 pmol/mg) and 125.8 – 210.1 pmol/mg (median: 138.5 pmol/mg), respectively (**Figure 6A, Figure S14**). Total PAH levels detected in PCP PM_{0.1} amounted to 271.6 – 474.9 pmol/mg (median: 382.6 pmol/mg), corresponding to 48.5 – 86.1 ppm (median: 68.74 ppm; **Figure S14**). This registered as a significant increase compared to PAHs found in background PM_{0.1} (range: 2.1 – 3.4 fold, median: 2.7-fold) and toner powders (range: 5.1 – 11.2 fold, median: 5.4-fold), respectively (**Figure 6A, B**). The increase was noted to be consistent across all printing centers for the 16 individual PAH congeners (**Figure 6B**).

A significantly higher amount of high molecular PAHs (HMPAHs) was detected in PCP PM_{0.1} (range: 81.8 – 144.3 pmol/mg, median: 125.3 pmol/mg) compared to background PM_{0.1} (range: 34.6 – 54.7 pmol/mg, median: 35.4 ng/mg) and toner powders (range: 3.1 – 6.9 pmol/mg,

median: 4.9 pmol/mg). In addition, a notable shift was observed in the PAH speciation profile (**Figure S14**). HMPAHs detected on toner powders only accounted for 4 – 18 mol% of total PAHs (median: 10.6 mol%), while HMPAHs associated with the background PM_{0.1} and PCP PM_{0.1} ranged from 24 – 31 mol% (median: 25.9 mol%) and 27 – 37 mol% (median: 30.6 mol%), respectively (**Figure S14**). The greatest PAH speciation change was noted in center #2, where HMPAH composition shifted from 24 mol% (background PM_{0.1}) to 37 mol% (PCP PM_{0.1}).

Representative outdoor PM_{0.1} sample (at center #6) was analyzed to determine the ambient PAH levels and the contribution of outdoor pollutants to the total PAHs observed in the printing rooms. Singapore is a small city-state with uniform climate and narrow temperature and pressure changes.²⁶⁻²⁸ Therefore, it is reasonable to assume that outdoor PAH concentrations would remain relatively constant across the different participating centers and throughout the year. Total PAH concentration associated with outdoor PM_{0.1} was found to be comparable with that of background PM_{0.1} (**Table S6**), which was significantly lower than that of PCP PM_{0.1}, suggesting that the elevated PAH level in the printing rooms levels was primarily caused by the printing activity.

The level of 7 PAHs (*i.e.*, benz[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, chrysene, dibenz[a, h]anthracene, and indeno[1,2,3-c, d]pyrene), which are classified as possible human carcinogens (Σ carPAH),²⁹ were determined to understand the potential carcinogenicity that could arise from workplace exposures to PCP. The level of Σ carPAH was found to increase in PCP PM_{0.1} (range: 2.3-10.4 fold, median: 4 fold) when compared to the background PM_{0.1} (**Figure 6B**, **Figure S14**). Correspondingly, the computed benzo[a]pyrene-equivalent (BaP_{eq}) concentration associated with PCP PM_{0.1} (based on toxic equivalency factors of individual PAHs; see **SI**) was determined to range between 6.1 – 15.4 pmol/mg (median: 12.7 pmol/mg; **Figure 6C**).

Indoor air quality (IAQ) from real-time monitoring

In addition to extensive PCP characterization, the levels of various gaseous co-pollutants and environmental parameters in the printing rooms were also assessed in real time (**Table S7**). Weekly average levels of CO₂ and total VOC in the 6 participating printing centers were measured to be in the range of 494 – 837 ppm and 0.4 – 8.8 ppm, respectively. NO₂ and ozone were detected at relatively low concentrations. Weekly averages for NO₂ reached up to 70 ppb while maximal hourly peak values reached 118 ppb. Weekly average ozone levels were measured at up to 4 ppb and hourly peak values as high as 33 ppb were recorded in some centers. The ambient temperature and relative humidity levels in the various printing centers remained fairly well controlled, with weekly averages ranging from 25.2-26.6°C and 38.0-56.6%, respectively. These measured parameters were mostly agreeable to the recommended IAQ standards specified in the Singapore building code (SS 554: 2016, Code of practice for indoor air quality for air-conditioned buildings, Building and Construction Standards Committee, Singapore).³⁰ The code specifies acceptable range of thermal comfort parameters such as operative temperature (24-26°C) and relative humidity (<65%), and chemical parameters such as CO₂ concentrations (upper limit: 700 ppm above outdoor, which would be approximately 1,100 ppm in this study, **Table S8**). Total VOC levels were noted to exceed the recommended code (upper limit: 1 ppm) in 3 of 6 printing centers (centers #1, #3, and #6).

The ventilation performance in each of the printing rooms was also estimated using the CO₂ steady-state mass balance method specified in ASHRAE Standard 62,³¹ and compared to the ventilation requirements (5.5 L/s/person or 0.6 L/s/m², whichever is higher) specified in the relevant Singapore building code (SS 553: 2016, Code of practice for air-conditioning and mechanical ventilation in buildings, Building and Construction Standards Committee, Singapore).³² Calculations of the ratios of calculated and required ventilation rates (Q/Q_r) showed that all but one printing center (center #3, $Q/Q_r = 0.7$) had sufficient ventilation rates (Q/Q_r : 1.0 – 8.2) appropriate for the full-time personnel occupancy of the printing area (**Table S7**).

IAQ monitoring in the office areas (**Table S9**) revealed similar environmental conditions between the printing and office environments, except for centers #2 and #6, where weekly average CO₂ concentration in the office spaces was observed to be significantly higher than that in the printing room by at least 300 ppm. This might be due to a higher number (unknown) of occupants in the offices of those centers compared to the printing rooms and/or poorer ventilation performance in those office areas.

DISCUSSION

Commercial printing center toners confirmed as nano-enabled products

Incorporating ENMs in product formulations has been the general trend in many industries over the past decade, as evidenced by the burgeoning numbers of nano-enabled products that have penetrated the market.³³ It comes as no surprise that powder toners for LP and PC have also transitioned into a nano-enabled product through incorporation of ENMs in their formulations. The extent of ENM incorporation is not clearly known as toner formulation is proprietary information, and the toners' SDS show no indication whether the listed compounds were added in the form of ENMs. This was expected as no regulatory bodies require manufacturers to distinguish the ENMs

from their bulk counterparts in their products' SDS. Nonetheless, EDX and ICP-MS analyses identified the presence of TiO₂, SiO₂, MgO, Al₂O₃, MnO₂ and Fe₂O₃ ENMs in the toner powders utilized at the participating printing centers. Toner analysis by Morawska et al.³⁴ revealed the presence of Fe, Ti, Sr, Al and Si. Similarly, Barthel et al. identified Si, Sn, Ca, Ti, V, Cr, Mn, Ni, Zn, and Fe which was identified as the dominant element in the analyzed toner powder.³⁵ Furthermore, carbon black, a nanoscale ENM, is also often added to toners in large quantities (up to 20 wt% in toner D1). Additionally, ENMs such as CuO, ZnO, CeO₂, SiO₂, and TiO₂ are added as part of toner formulations to improve performance.^{5, 36-37} Overall, our results are in good agreement with the previous studies, in which toner powders from LP^{5, 34-35} and PC^{2, 8} have been identified to contain similar ENMs, verifying that nano-enabled toners have widespread penetration in the digital printing industry.

Printing activity generates chemically complex airborne PM

Physicochemical and morphological characterization revealed that digital printing process produces highly complex airborne PCP PM_{0.1} emissions that contain both organic and inorganic constituents. Though the exact compositions of the emitted PCP varied between centers (toner formulations, copier make/model, age, level of printing activity), similar elemental fingerprints (*i.e.*, Mn, Mg, Cr, Ni, Al, Zn, Si, Sn, Ti, and Fe) have been consistently identified in the PCP PM_{0.1} across all centers recruited. Many of these elements could be traced back to the toner formulations that have been used in the respective printing centers, and chamber studies have established nano-enabled toners as the potential source of transition metal/metal oxide ENMs in PCP. As in most formulations the ENMs are incorporated on the surface of the toner particles, these ENMs could be easily shed and become airborne during the printing process. This is in line with previous reports in which significant transition metal/metal emission from LP and PC has been noted.^{2, 5-6, 8, 35} ICP-

MS analysis has also identified several other elements such as Na, K, Ca, B, S, and P which, in addition to toners, may also originate from the paper used for printing.^{5, 8, 35}

In addition to various transition metal/metal oxide ENMs, the PCP contain considerable amounts of organic constituents (~40-60 wt%), whose exact composition continues to be largely unknown. A small fraction of this organic fraction has been previously identified as waxes, long chain hydrocarbons and hopanes, that contributed <2% of total mass of the PCP PM_{0.1}.⁸ Pirela *et al.*⁵⁻⁶ and Martin *et al.*² detected the presence of anions and cations that contributed to <2% of the total mass of the PM_{0.1} collected from the chamber studies and the copy centers in the US, respectively. Interestingly, PM_{0.1} collected in the photocopier centers in the US were found to contain insignificant amount (ppb level) of USEPA priority PAHs; while PCP PM_{0.1} collected in the participating centers in Singapore contained close to three order of magnitude of these PAHs (~50-90 ppm). The majority of the organic fraction presumably originated from the condensation of the semi-volatile organic compounds vaporized from the toners during the printing/copying process³⁸ and additional degradation of the polymeric materials within toners, under the influence of heat and catalytic activity of metal oxide ENM.³⁴

The presence of these inorganic and organic mixtures is of particular interest as synergistic interactions would likely occur between the constituents. A recent study implicated the interaction of transition metal/metal oxides and carbonyl-rich organic constituents in PEPs in the formation of short lived radicals and ROS.⁹ In another study, Chalbot *et al.* identified transition metal ENMs in toner formulations as the key players involved in toner polymer catalytic degradation as well as catalytic conversion of LMPAH to HMPAH in the PEPs.²³ In good agreement, the PAH analysis carried out in this study detected an overall increase of PAH amounts in the PCP PM_{0.1} collected from participating centers as compared to the toner (median: 5.4 fold) and background PM_{0.1}

(median: 2.7 fold). Moreover, the relative abundance of PAHs congeners was observed to shift distinctively in favor of HMPAHs. The greatest HMPAH composition shift was noted to occur in center #2, which also exhibited one of the highest peak PCP number concentrations of all centers (peak PNC = 426,000 particles /cm³). This further supports the notion that the inherently catalytic transition metal/metal oxide ENMs present in the PCP are responsible for the LMPAH-to-HMPAH conversion. Similarly, overall increase of PAH levels and conversions from LMPAHs to HMPAHs have been documented in the various lifecycle scenarios involving nano-enabled products containing ENMs such as TiO₂, Ag, NiO, Ceria, and CNT.³⁹⁻⁴¹ Overall, these results highlight the chemical complexity of PCP sampled at printing centers during the digital printing process, and underscore the need to better characterize the organic constituents in the PCP. Likewise, further investigation is warranted to fully understand the synergistic interactions between the organic and inorganic constituents of PCP.

Complex worker exposures in printing environment: potential health effects and exposure risk management

Exposure measurement revealed that these chemically complex PCP were present in high concentrations at the participating printing centers. Daily GM PNC could reach close to 93,000 particles/cm³ with peak emissions as high as 426,000 particles/cm³. High nanoparticle emission was also reported in six out of eight copy centers in the US where daily GM could reach up to 52,000 particles/cm³.² Similarly, assessment in Taiwan copy centers documented peak emission up to 10 million particles/cm³,⁴² while Bello *et al.* recorded over 1 million particles being released during printing in a copy center in Boston (USA).⁸ The daily GM alveolar LDSA for a typical TPE operator could reach up to 212 $\mu\text{m}^2/\text{cm}^3$, registering approximately 8 times higher exposure as compared to the “control worker” working in the TPE-free environment. With no dedicated

engineering or exposure controls in place, one could postulate that high exposures to airborne nano-sized PCP would result in significant lung deposition in the workers' respiratory tract (upper and lower airways) that potentially could lead to the systemic translocation of the PCP to other organs.⁴³ This raises a genuine concern about the potential adverse health effects resulting from prolonged exposures to highly complex and oxidatively damaging PCP.

In the context of risk assessment, this study provides the understanding with regards to exposure to ENMs in a printing center. However, uncertainties remain in our understanding of the hazards associated with such exposures. While toxicity of several metal/metal oxide nanoparticles in this mixture has been well documented,⁴⁴⁻⁵¹ their interplay with complex organics might affect the bioavailability, bioactivity, and toxicological profile of constituents such as PAHs. One possibility is that PAHs may be deposited with higher efficiency deeper in the airways, because of their adsorption onto metal oxide nanoparticles, than in the absence of those nanoparticles. In a comparative study against SiO₂ ENMs and welding fume nanoparticles, a low dose PEPs exposure was demonstrated to induce the highest inflammatory response that resulted in the dysfunction of the epithelial and endothelial barriers.¹⁶ Lu *et al.* documented that PEPs at relevant exposure doses could induce significantly higher oxidative stress response in smooth airway epithelial cells as compared to TiO₂ and CuO ENMs.¹³ These studies therefore point to the potential biochemical complexity of PCP exposures and warrant the need for in-depth biological studies to understand the interplay between the constituents in PCP nanoparticles and their potential synergistic toxicological consequences. Questions from the standpoint of sustainable nanotechnology remain: How do ENM additives in toners modulate the chemical composition and consequent deposition patterns in the respiratory tract? What are the health risks of PCP exposure, if any? How can toner formulations be modified so that PCP exposures are safe?

In summary, a comprehensive exposure assessment of inhalation nanoparticle exposure at 6 Singapore printing centers revealed that high-level emissions of NPs is a common occurrence in typical printing centers that utilize TPE. Due to the widespread utilization of nano-enabled toners, the PCP collected from all participating printing centers contained a substantial amount of transition metal/metal oxide ENMs, in addition to complex organic constituents such as carcinogenic PAHs. These real-world occupational exposure data are being utilized to support our ongoing molecular epidemiology and mechanistic studies aiming to further understand the nano-bio interactions, exposure-dose-effect relationships and potential adverse health consequences within the occupational cohort recruited from these participating printing centers. In addition, these findings would be useful to provide the much-needed exposure data to establish protective regulatory guidelines for TPE-associated emissions at both occupational and consumer levels, and to develop safer-by-design approaches to address the potential occupational and environmental health and safety implications of commercial printing activities.

ASSOCIATED CONTENT

Supporting Information. The following files are available free of charge on the ACS Publications Website. Detailed exposure measurements, experimental and characterization methods, printing room layouts and printers specification at each participating centers; Representative real time measurement for area and personal monitoring conducted in printing room: Weekly size distribution of airborne PCP detected in printing room; Summary statistics of PNC, IAQ parameters measured at the printing centers' control areas and outdoor environment; ICP-MS, EC-OC, TEM/EDX analysis of PCP; SEM/EDX analyses for toners; ICP-MS, EC-OC analyses for background samples; Comparative concentration and mass distribution of individual PAH

497 congeners detected in PCP, background indoor ambient air, toners, and outdoor environment
498 (PDF).

499 **AUTHOR INFORMATION**

500 **Corresponding Author**

501 *Corresponding author:

502 Kee Woei Ng (KWNG@ntu.edu.sg)

503 **Author Contributions**

504 The manuscript was written through contributions of all authors. All authors have given approval
505 to the final version of the manuscript.

506 **Funding Sources**

507 Nanyang Technological University – Harvard School of Public Health Initiative for Sustainable
508 Nanotechnology (NTU-Harvard SusNano; Ref. No. NTU-HSPH 17001).

509 **ACKNOWLEDGMENT**

510 This study was supported by Nanyang Technological University – Harvard School of Public Health
511 Initiative for Sustainable Nanotechnology (NTU-Harvard SusNano; Ref. No. NTU-HSPH 17001).

512 The authors acknowledge Dr. Martin Shafer from the Trace Elements Clean Lab at Wisconsin State
513 Laboratory of Hygiene for his help with EC-OC and ICP-MS analyses.

514 **ABBREVIATIONS**

515 CCI, Compact Cascade Impactor; CMD, count median diameter; EC-OC, elemental carbon -
516 organic carbon; EDX, Energy-dispersive X-ray spectroscopy; ENMs, engineered nanomaterials;
517 GM, geometric mean; GSD, geometric standard deviation; HVAC, heating, ventilation, and air
518 conditioning; HMPAH, high molecular weight polycyclic aromatic hydrocarbons; IAQ, Indoor air
519 quality; ICP-MS, Inductively coupled plasma mass spectrophotometry; LDSA, alveolar lung

520 deposited surface area; LMPAH, low molecular weight polycyclic aromatic; LP, laser printers;
521 NPs, nanoparticles, PAHs, polycyclic aromatic hydrocarbons; PC, photocopiers; PCP, printing
522 center particles; PEPs, printer-emitted particles; PM, particulate matter; PNC, particle number
523 concentration; ROS, reactive oxygen species; SDS, Safety Data Sheet; SEM, scanning electron
524 microscopy; TEM, transmission electron microscopy; TPE, toner-based printing equipment; VOC,
525 volatile organic compounds.

526 REFERENCES

- 527 1. Pirela, S. V.; Martin, J.; Bello, D.; Demokritou, P., Nanoparticle Exposures from Nano-
528 Enabled Toner-Based Printing Equipment and Human Health: State of Science and Future
529 Research Needs. *Crit. Rev. Toxicol.* **2017**, *47*, 683-709.
- 530 2. Martin, J.; Bello, D.; Bunker, K.; Shafer, M.; Christiani, D.; Woskie, S.; Demokritou, P.,
531 Occupational Exposure to Nanoparticles at Commercial Photocopy Centers. *J. Hazard Mater.*
532 **2015**, *298*, 351-60.
- 533 3. Martin, J.; Demokritou, P.; Woskie, S.; Bello, D., Indoor Air Quality in Photocopy
534 Centers, Nanoparticle Exposures at Photocopy Workstations, and the Need for Exposure
535 Controls. *Ann. Work Exposures Health* **2017**, *61*, 110-122.
- 536 4. Schripp, T.; Wensing, M.; Uhde, E.; Salthammer, T.; He, C.; Morawska, L., Evaluation
537 of Ultrafine Particle Emissions from Laser Printers Using Emission Test Chambers. *Environ. Sci.*
538 *Technol.* **2008**, *42*, 4338-4343.
- 539 5. Pirela, S. V.; Sotiriou, G. A.; Bello, D.; Shafer, M.; Bunker, K. L.; Castranova, V.;
540 Thomas, T.; Demokritou, P., Consumer Exposures to Laser Printer-Emitted Engineered
541 Nanoparticles: A Case Study of Life-Cycle Implications from Nano-Enabled Products.
542 *Nanotoxicology* **2015**, *9*, 760-768.
- 543 6. Pirela, S. V.; Pyrgiotakis, G.; Bello, D.; Thomas, T.; Castranova, V.; Demokritou, P.,
544 Development and Characterization of An Exposure Platform Suitable for Physico-Chemical,
545 Morphological and Toxicological Characterization of Printer-Emitted Particles (PEPs). *Inhal.*
546 *Toxicol.* **2014**, *26*, 400-8.
- 547 7. He, C.; Morawska, L.; Taplin, L., Particle Emission Characteristics of Office Printers.
548 *Environmental Science & Technology* **2007**, *41*, 6039-6045.
- 549 8. Bello, D.; Martin, J.; Santeufemio, C.; Sun, Q.; Lee Bunker, K.; Shafer, M.; Demokritou,
550 P., Physicochemical and Morphological Characterisation of Nanoparticles from Photocopiers:
551 Implications for Environmental Health. *Nanotoxicology* **2013**, *7*, 989-1003.
- 552 9. Zhang, Y.; Demokritou, P.; Ryan, D. K.; Bello, D., Comprehensive Assessment of Short-
553 Lived ROS and H₂O₂ in Laser Printer Emissions: Assessing the Relative Contribution of Metal
554 Oxides and Organic Constituents. *Environ. Sci. Technol.* **2019**, *53*, 7574-7583.
- 555 10. Khatri, M.; Bello, D.; Gaines, P.; Martin, J.; Pal, A. K.; Gore, R.; Woskie, S.,
556 Nanoparticles from Photocopiers Induce Oxidative Stress and Upper Respiratory Tract
557 Inflammation in Healthy Volunteers. *Nanotoxicology* **2013**, *7*, 1014-1027.
- 558 11. Khatri, M.; Bello, D.; Martin, J.; Bello, A.; Gore, R.; Demokritou, P.; Gaines, P., Chronic
559 Upper Airway Inflammation and Systemic Oxidative Stress from Nanoparticles in Photocopier
560 Operators: Mechanistic Insights. *NanoImpact* **2017**, *5*, 133-145.
- 561 12. Pirela, S. V.; Bhattacharya, K.; Wang, Y.; Zhang, Y.; Wang, G.; Christophi, C. A.;
562 Godleski, J.; Thomas, T.; Qian, Y.; Orandle, M. S.; Sisler, J. D.; Bello, D.; Castranova, V.;
563 Demokritou, P., A 21-Day Sub-Acute, Whole-Body Inhalation Exposure to Printer-Emitted
564 Engineered Nanoparticles in Rats: Exploring Pulmonary and Systemic Effects. *NanoImpact*
565 **2019**, *15*, 100176.
- 566 13. Lu, X.; Miousse, I. R.; Pirela, S. V.; Melnyk, S.; Koturbash, I.; Demokritou, P., Short-
567 Term Exposure to Engineered Nanomaterials Affects Cellular Epigenome. *Nanotoxicology* **2016**,
568 *10*, 140-50.

14. Pirela, S.; Molina, R.; Watson, C.; Cohen, J. M.; Bello, D.; Demokritou, P.; Brain, J., Effects of Copy Center Particles on the Lungs: A Toxicological Characterization using A Balb/c Mouse Model. *Inhal. Toxicol.* **2013**, *25*, 498-508.
15. Pirela, S. V.; Lu, X.; Miousse, I.; Sisler, J. D.; Qian, Y.; Guo, N.; Koturbash, I.; Castranova, V.; Thomas, T.; Godleski, J.; Demokritou, P., Effects of Intratracheally Instilled Laser Printer-Emitted Engineered Nanoparticles in A Mouse Model: A Case Study of Toxicological Implications from Nanomaterials Released during Consumer Use. *NanoImpact* **2016**, *1*, 1-8.
16. Sisler, J. D.; Pirela, S. V.; Friend, S.; Farcas, M.; Schwegler-Berry, D.; Shvedova, A.; Castranova, V.; Demokritou, P.; Qian, Y., Small Airway Epithelial Cells Exposure to Printer-Emitted Engineered Nanoparticles Induces Cellular Effects on Human Microvascular Endothelial Cells in An Alveolar-Capillary Co-Culture Model. *Nanotoxicology* **2015**, *9*, 769-779.
17. Khatri, M.; Bello, D.; Pal, A. K.; Cohen, J. M.; Woskie, S.; Gassert, T.; Lan, J.; Gu, A. Z.; Demokritou, P.; Gaines, P., Evaluation of Cytotoxic, Genotoxic and Inflammatory Responses of Nanoparticles from Photocopiers in Three Human Cell Lines. *Part. Fibre Toxicol.* **2013**, *10*, 42.
18. Khatri, M.; Bello, D.; Pal, A. K.; Woskie, S.; Gassert, T. H.; Demokritou, P.; Gaines, P., Toxicological Effects of PM0.25–2.0 Particles Collected from A Photocopy Center in Three Human Cell Lines. *Inhal. Toxicol.* **2013**, *25*, 621-632.
19. Zuraimi, M. S.; Roulet, C. A.; Tham, K. W.; Sekhar, S. C.; David Cheong, K. W.; Wong, N. H.; Lee, K. H., A comparative study of VOCs in Singapore and European office buildings. *Build. Sci.* **2006**, *41*, 316-329.
20. Demokritou, P.; Lee, S. J.; Ferguson, S. T.; Koutrakis, P., A Compact Multistage (Cascade) Impactor for the Characterization of Atmospheric Aerosols. *J. Aerosol Sci.* **2004**, *35*, 281-299.
21. Keith, L. H., The Source of U.S. EPA's Sixteen PAH Priority Pollutants. *Polycyclic Aromatic Compounds* **2015**, *35*, 147-160.
22. Fang, M.; Getzinger, G. J.; Cooper, E. M.; Clark, B. W.; Garner, L. V.; Di Giulio, R. T.; Ferguson, P. L.; Stapleton, H. M., Effect-Directed Analysis of Elizabeth River Porewater: Developmental Toxicity in Zebrafish (*Danio rerio*). *Environ Toxicol Chem* **2014**, *33*, 2767-2774.
23. Chalbot, M.-C. G.; Pirela, S. V.; Schiffman, L.; Kasaraneni, V.; Oyanedel-Craver, V.; Bello, D.; Castranova, V.; Qian, Y.; Thomas, T.; Kavouras, I. G.; Demokritou, P., Synergistic Effects of Engineered Nanoparticles and Organics Released from Laser Printers Using Nano-Enabled Toners: Potential Health Implications from Exposures to the Emitted Organic Aerosol. *Environ. Sci.: Nano* **2017**, *4*, 2144-2156.
24. He, C.; Morawska, L.; Wang, H.; Jayaratne, R.; McGarry, P.; Richard Johnson, G.; Bostrom, T.; Gonthier, J.; Authemayou, S.; Ayoko, G., Quantification of the Relationship between Fuser Roller Temperature and Laser Printer Emissions. *J. Aerosol Sci.* **2010**, *41*, 523-530.
25. Zhao, B., Ultrafine Aerosol Particles from Laser Printing Process: Response Relationship between Operating Parameters and Emission Characteristics. *Aerosol Air Qual. Res.* **2017**, *17*, 2139-2151.
26. Urbančok, D.; Payne, A. J. R.; Webster, R. D., Regional Transport, Source Apportionment and Health Impact of PM10 Bound Polycyclic Aromatic Hydrocarbons in Singapore's Atmosphere. *Environ. Pollut.* **2017**, *229*, 984-993.

27. He, J.; Zielinska, B.; Balasubramanian, R., Composition of Semi-Volatile Organic Compounds in the Urban Atmosphere of Singapore: Influence of Biomass Burning. *Atmos. Chem. Phys.* **2010**, *10*, 11401-11413.
28. Ang, K. P.; Gunasingham, H.; Tay, B. T., The Distribution of Polynuclear Aromatic Hydrocarbons in Ambient Air Particulates in Singapore. *Environ. Monit. Assess.* **1986**, *6*, 171-80.
29. Kim, K. H.; Jahan, S. A.; Kabir, E.; Brown, R. J., A Review of Airborne Polycyclic Aromatic Hydrocarbons (PAHs) and Their Human Health Effects. *Environ. Int.* **2013**, *60*, 71-80.
30. Singapore Standards Council, SS 554 : 2016: *Code of Practice for Indoor Air Quality for Air-Conditioned Buildings*; Singapore, 2016.
31. American Society for Heating, Refrigerating, and Air-Conditioning Engineers, Standard 62-1981: *Ventilation for Acceptable Indoor Air Quality*; Atlanta GA, 1981.
32. Singapore Standards Council, SS 553: 2009: *Code of Practice for Air-Conditioning and Mechanical Ventilation in Buildings*; Singapore, 2009.
33. Vance, M. E.; Kuiken, T.; Vejerano, E. P.; McGinnis, S. P.; Hochella, M. F., Jr.; Rejeski, D.; Hull, M. S., Nanotechnology in the Real World: Redeveloping the Nanomaterial Consumer Products Inventory. *Beilstein J. Nanotechnol.* **2015**, *6*, 1769-1780.
34. Morawska, L.; He, C.; Johnson, G.; Jayaratne, R.; Salthammer, T.; Wang, H.; Uhde, E.; Bostrom, T.; Modini, R.; Ayoko, G.; McGarry, P.; Wensing, M., An Investigation into the Characteristics and Formation Mechanisms of Particles Originating from the Operation of Laser Printers. *Environ. Sci. Technol.* **2009**, *43*, 1015-1022.
35. Barthel, M.; Pedan, V.; Hahn, O.; Rothhardt, M.; Bresch, H.; Jann, O.; Seeger, S., XRF-Analysis of Fine and Ultrafine Particles Emitted from Laser Printing Devices. *Environ. Sci. Technol.* **2011**, *45*, 7819-7825.
36. Ni, W.; Wu, S.; Ren, Q., Silanized TiO₂ Nanoparticles and Their Application in Toner as Charge Control Agents: Preparation and Characterization. *Chem. Eng. J.* **2013**, *214*, 272-277.
37. Zhang, H.; Ding, W.; Law, K.-Y.; Cetinkaya, C., Adhesion Properties of Nanoparticle-Coated Emulsion Aggregation Toner. *Powder Technol.* **2011**, *208*, 582-589.
38. Mullins, B. J.; Bertolatti, D.; Mead-Hunter, R., Assessment of Polyaromatic Hydrocarbon Emissions from Laser Printers. *Atmos. Environ.* **2013**, *79*, 428-432.
39. Singh, D.; Schiffman, L. A.; Watson-Wright, C.; Sotiriou, G. A.; Oyanedel-Craver, V.; Wohlleben, W.; Demokritou, P., Nanofiller Presence Enhances Polycyclic Aromatic Hydrocarbon (PAH) Profile on Nanoparticles Released during Thermal Decomposition of Nano-enabled Thermoplastics: Potential Environmental Health Implications. *Environ. Sci. Technol.* **2017**, *51*, 5222-5232.
40. Vejerano, E. P.; Leon, E. C.; Holder, A. L.; Marr, L. C., Characterization of Particle Emissions and Fate of Nanomaterials during Incineration. *Environ. Sci.: Nano* **2014**, *1*, 133-143.
41. Vejerano, E. P.; Holder, A. L.; Marr, L. C., Emissions of Polycyclic Aromatic Hydrocarbons, Polychlorinated Dibenzo-p-Dioxins, and Dibenzofurans from Incineration of Nanomaterials. *Environ. Sci. Technol.* **2013**, *47*, 4866-4874.
42. Lee, C.-W.; Hsu, D.-J., Measurements of Fine and Ultrafine Particles Formation in Photocopy Centers in Taiwan. *Atmos. Environ.* **2007**, *41*, 6598-6609.
43. Poh, T. Y.; Ali, N. A. t. B. M.; Mac Aogáin, M.; Kathawala, M. H.; Setyawati, M. I.; Ng, K. W.; Chotirmall, S. H., Inhaled Nanomaterials and The Respiratory Microbiome: Clinical, Immunological and Toxicological Perspectives. *Part. Fibre Toxicol.* **2018**, *15*, 46.

44. Colvin, V. L., The Potential Environmental Impact of Engineered Nanomaterials. *Nat. Biotechnol.* **2003**, *21*, 1166-1170.
45. Aillon, K. L.; Xie, Y.; El-Gendy, N.; Berkland, C. J.; Forrest, M. L., Effects of Nanomaterial Physicochemical Properties on *In Vivo* Toxicity. *Adv. Drug Delivery Rev.* **2009**, *61*, 457-466.
46. Gautam, A.; Rakshit, M.; Nguyen, K. T.; Kathawala, M. H.; Nguyen, L. T. H.; Tay, C. Y.; Wong, E.; Ng, K. W., Understanding the Implications of Engineered Nanoparticle Induced Autophagy in Human Epidermal Keratinocytes *In Vitro*. *NanoImpact* **2019**, *15*, 100177.
47. Ng, K. W.; Khoo, S. P. K.; Heng, B. C.; Setyawati, M. I.; Tan, E. C.; Zhao, X.; Xiong, S.; Fang, W.; Leong, D. T.; Loo, J. S. C., The Role of the Tumor Suppressor p53 Pathway in the Cellular DNA Damage Response to Zinc Oxide Nanoparticles. *Biomaterials* **2011**, *32*, 8218-8225.
48. Setyawati, M. I.; Khoo, P. K. S.; Eng, B. H.; Xiong, S.; Zhao, X.; Das, G. K.; Tan, T. T.-Y.; Loo, J. S. C.; Leong, D. T.; Ng, K. W., Cytotoxic and Genotoxic Characterization of Titanium Dioxide, Gadolinium Oxide, and Poly(Lactic-co-Glycolic Acid) Nanoparticles in Human Fibroblasts. *J. Biomed. Mater. Res., Part A* **2013**, *101A*, 633-640.
49. Gminski, R.; Decker, K.; Heinz, C.; Seidel, A.; Könczöl, M.; Goldenberg, E.; Grobéty, B.; Ebner, W.; Gieré, R.; Mersch-Sundermann, V., Genotoxic Effects of Three Selected Black Toner Powders and Their Dimethyl Sulfoxide Extracts in Cultured Human Epithelial A549 Lung Cells *In Vitro*. *Environ. Mol. Mutagen.* **2011**, *52*, 296-309.
50. Máté, Z.; Horváth, E.; Papp, A.; Kovács, K.; Tombácz, E.; Nesztör, D.; Szabó, T.; Szabó, A.; Paulik, E., Neurotoxic Effects of Subchronic Intratracheal Mn Nanoparticle Exposure Alone and in Combination with Other Welding Fume Metals in Rats. *Inhal. Toxicol.* **2017**, *29*, 227-238.
51. Zaitseva, N. V.; Zemlyanova, M. A.; Zvezdin, V. N.; Akafieva, T. I.; Saenko, E. V., Acute Inhalation Toxicity of Manganese Oxide Nanoparticles. *Nanotechnol. Russ.* **2015**, *10*, 468-474.

687

688 **Figure 1.** General schematic of the study design. SF-ICP-MS, Sector Field Inductively Coupled
689 Plasma Mass Spectroscopy; EC-OC, Elemental Carbon – Organic Carbon analysis; PAH,
690 Polycyclic Aromatic Hydrocarbon analysis; CCI, Compact Cascade Impactor; TEM- EDX,
691 Transmission Electron Microscopy – Energy Dispersive X-ray Spectroscopy; SEM-EDX,
692 Scanning Electron Microscopy – Energy Dispersive X-ray Spectroscopy; SMPS, Scanning
693 Mobility Particle Sizer, IAQs, Indoor Air Quality.

A Physical characteristics and printing production information of each participating printing center

Center ID	Operating hours	Full-time TPE operators	No. of active TPE	Printer ID	Average prints/ day	Daily print range	Printing room dimensions (m), L×W×H = V (m ³)	No. of doors / windows in printing room	Printing room floor level	HVAC type
1	9	3	4	A1, A2	59,686	7,087 – 147,171	8 × 6 × 2.8 = 134.4	2/0	1	1 cassette AC; 3 split AC; 2 local exhausts (not in operation)
2	9	2	2	A1	10,465	8,328 – 16,960	5 × 4 × 2.8 = 56	2/0	2	1 cassette AC
3	10	6	5	A3, B1, B2, C1	38,684	12,943 – 65,047	18 × 6 × 3.8 + 8 × 8 × 3.8 = 653.6	8/0	3	3 cassette AC
4	10	14	1	D1	4,000	3,200 – 4,850	17 × 14 × 2.8 = 666.4	3/0	2	7 cassette AC
5	10	3	2	A1, C2	2,712	1,272 – 4,730	6 × 5 × 3.8 = 114	1/0	3	2 cassette AC; 2 split AC; 1 industrial fan
6	9	1	1	D1	652	8 – 1,724	10 × 6 × 3.8 = 228	2/0	2	1 cassette AC; 1 window AC; 1 exhaust fan

B

C Summary statistics of PCP detected at six printing centers in Singapore

Center ID	Max PNC (#/cm ³)	Weekly average PNC (#/cm ³)		Weekly average diameter (nm)		PM _{0.1} mass conc. (µg/m ³)	PNC _{print} / PNC _{bkgd}
		GM	GSD	CMD	σ _g		
1	197,700	49,900	1.77	31.3	1.19	9.2	3.17
2	426,000	30,500	2.12	30.6	1.29	15.1	6.36
3	36,000	8,900	1.41	38.5	1.16	4.4	1.78
4	54,100	12,200	1.55	40.3	1.37	13.2	2.00
5	61,400	14,500	2.24	36.2	1.52	15.2	3.74
6	6,200	1,680	1.54	44.9	1.19	7.7	1.18

Figure 2. Comparative environmental exposure assessment of PCP at 6 printing centers in Singapore. (A) Physical characteristics and printing production of each participating center. (B) Comparative daily (8h time weighted average) geometric mean of particle number concentration (PNC). The minimum and maximum boundary lines of each box indicate the 25th and 75th percentile values, respectively. The line within the box marks the median. Whiskers (to the left and right of each box) indicate minimum and maximum detected daily PNC. (C) Summary statistics of total PNC, PNC ratio, particle diameter and mass concentration of the airborne PM_{0.1} PCP at these 6 printing centers in Singapore over 2 monitoring weeks. GM, geometric mean; GSD, geometric standard deviation; CMD, Count median diameter; σ_g, geometric standard deviation; PNC_{print}/PNC_{bkgd}, GM of process measurement / GM of background measurement in printing area.

Figure 3. Workers' personal exposure assessment to PCP. (A) Representative real time measurement of lung deposited surface area (LDSA) obtained from Partector personal dosimeter shows positive correlation with the measured PNC level in the printing room. Complete data set could be found in Supplementary Information. (B) Workers' time weighted average exposure to PCP as expressed in LDSA concentration. The minimum and maximum boundary lines of each box indicate the 25th and 75th percentile values, respectively. The line within the box marks the median. Whiskers (above and below each box) indicate minimum and maximum detected workers' exposure level. (C) Summary statistics of exposure to PCP for workers recruited from 6 participating printing centers in Singapore.

Figure 4. Representative transmission electron microscopy (TEM) images and the respective energy dispersive X-ray spectroscopy analysis (EDX) of airborne PCP collected during active printing periods in printing rooms at (A) center #1, (B) center #2, (C) center #3, (D) center #4, (E) center #5, and (F) center #6. Cu signal originated from the Cu grid.

Figure 5. Chemical composition of PCP PM_{0.1}. (A) Pie chart breakdown of PCP PM_{0.1} collected from representative printing centers (centers #2, #3, and #4), represented as mass-weighted fractions of elemental carbon, organic carbon, and inorganic contents, which are further subdivided into various trace metals. (B) Summary chemical composition of PCP PM_{0.1} collected from 6 printing centers in Singapore as determined by EC-OC and SF-ICP-MS analysis. Elemental analysis included 50 elements, of which the most abundant elements with toxicological relevance are presented.

Figure 6. Comparative analysis of PAHs associated PCP PM_{0.1} collected from 6 participating printing centers. **(A)** Total PAHs (left panel) and HMPAHs (right panel) concentrations. **(B)** PAH concentration ratios show increase amounts of PAHs associated with PCP PM_{0.1} when compared to the level of PAHs in toner powders (n=6), and background PM_{0.1} (n=5). Black line marks the median. **(C)** BaP equivalent concentrations. PAH_{PCP}/PAH_{Toner}, concentration ratio of PAHs associated with PCP PM_{0.1} / toner powders; PAH_{PCP}/PAH_{Bckgrd}, concentration ratio of PAHs associated with PCP PM_{0.1} / background PM_{0.1}; Nap: naphthalene; Acy: acenaphthylene; Ace: acenaphthene; Flu: fluorene; Phe: phenanthrene; Ant: anthracene; Fla: fluoranthene; Pyr: pyrene; BaA: benz[a]anthracene; Chr: chrysene, BbF: benzo[b]fluoranthene; BkF: benzo[k]fluoranthene; BaP: benzo[a]pyrene; ΣcarPAH: total concentration of 7 PAHs considered as possible human carcinogen; ΣPAH: total PAHs concentration; ΣBaP_{eq}: total BaP-equivalent concentration; ΣLMPAH = total low molecular weight PAH; ΣHMPAH: total high molecular weight PAH.

743 TOC graphic

744