Physico-chemical and toxicological characterization of engineered nanoparticles emitted from laser printers: A case study of consumer exposures across life cycle of nano-enabled products

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QEEN Workshop:
Quantifying Exposure to Engineered Nanomaterials (QEEN) from Manufactured Products
Addressing Environmental, Health, and Safety Implications
Focuses on Applications and Implications of engineered nanomaterials and nanotechnology

- Mission: Integrate material & exposure science and nanotoxicology risk assessment to facilitate science-based decision-making regarding nano-EHS.
- Current research activities: Development of *in vitro* and *in vivo* toxicological screening platforms for ENMs, assess nano-EHS issues across life cycle of NEPs, safer by design development of ENMs and NEPs, Environmental Nanotechnology applications
- Industrial Partners: BASF, Panasonic, Nanoterra, STERIS, Profector Life Sciences.
- International in nature: Current collaborations with Federal Agencies, and Universities around the world (ETH, NTU- Singapore, MIT, SUNY, UMass, Northeastern Univ., NIOSH, CPSC, etc.)
Engineered Water Nanostructures

Our recently published work was featured on the cover of Environmental Science: Nano, published by the Royal Society of Chemistry.

Read More...

About NanoCenter

Harvard NanoCenter draws on decades of experience with environmental pollutants and the health effects of particles to address the unique environmental health and safety (EHS) concerns raised by engineered nanomaterials (ENM) and nanotechnology applications.

Our mission is to integrate exposure science and nanotoxicology risk assessment to facilitate science-based decision-making regarding nano-EHS. In doing so, we are bringing together stakeholders including industry, academia, policy-makers, and the general public to maximize benefits and minimize risks.
Funding Sources

Grant Numbers

NIOSH & CPSC grant #: 212-2012-M-51174
NIEHS grant #: ES-000002
Background: Laser printers

- Widely available in office spaces and businesses everywhere

Exposure studies
- Laser printers release both particulate matter (PM) and gaseous pollutants during their use
- Particle release from board cooler, rear of printer, paper tray, fan and toner waste

Has the laser-based printing industry shifted to the use of ENMs in toners? If yes, are laser printers now releasing PM in the nanoscale?

Toxicology studies
- Using toner powder as the test material instead of printer-emitted particles (PEPs)
- Intratracheally instilling toner powder to mice at unrealistic doses (e.g., 40 mg/kg)
- No inhalation studies evaluating biological responses post PEPs exposure

Not enough data for adequate science-based risk assessment of consumer exposure scenarios

1 Barthel et al., 2011; He et al., 2010; Morawska et al., 2009; Lee et al., 2009
2 Wensing et al., 2008
3 Bai et al., 2010
Research Objectives

- Develop lab-based exposure platform to generate real-world PEPs suitable for pcm and tox characterization studies

- Utilization of developed platform to evaluate PEPs from commonly used printers
  - Assess emission profile
  - Evaluate operational parameters and their effect on emission profile
  - Physico-chemical and morphological characterization of black toner powders and PEPs

- In vitro evaluation of biological outcomes using both mono- and co-culture systems
  - Endpoints: genotoxicity, cytotoxicity, reactive oxygen production, cytokine/chemokines levels

- In vivo evaluation of biological outcomes following whole-body inhalation or intratracheal instillation of PEPs
  - Endpoints: lung injury and inflammation, epigenetics, gene expression
Study Design

Source: Laser printer

Development of exposure platform → Generation of printer-emitted particles (PEPs) → Physicochemical and morphological assessment

- in vitro
- in vivo

Toxicological assessment
Background: How do laser printers work?

1. Charge
2. Write image
3. Add toner
4. Transfer toner to paper
5. Fuse toner to paper (~225 °C)
6. Clean
Features:
- Uninterrupted operation
- Real time aerosol and gaseous emission monitoring
- Particle generation and collection
- Animal exposures
- Simulation of different exposure scenarios (ACH)
- Versatile: can be used for characterization of particle released from various NEPs
Results: Size distribution and number concentration of PEPs

- Emission profiles of 11 laser printers (4 manufacturers)
  - It varies across manufacturers and model
  - Peak concentrations levels: 2,990 - 1.27 million particles/cm³
  - Initial burst within 10-12 min
  - Mean diameters: 39 - 122 nm, majority of particles by number < 100 nm
  - Mass concentrations: up to 100 μg/m³

- Emission profiles identified for printers → rank them based on maximum particle released
## Ranking of commonly used laser printers

<table>
<thead>
<tr>
<th>Ranking</th>
<th>Printer</th>
<th>Maximum particle number concentration (#/cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A1</td>
<td>1.27 x 10⁶</td>
</tr>
<tr>
<td>2</td>
<td>B1</td>
<td>1.26 x 10⁶</td>
</tr>
<tr>
<td>3</td>
<td>B2</td>
<td>6.78 x 10⁵</td>
</tr>
<tr>
<td>4</td>
<td>C1</td>
<td>2.62 x 10⁵</td>
</tr>
<tr>
<td>5</td>
<td>C2</td>
<td>2.12 x 10⁵</td>
</tr>
<tr>
<td>6</td>
<td>C3</td>
<td>1.70 x 10⁵</td>
</tr>
<tr>
<td>7</td>
<td>C4</td>
<td>1.52 x 10⁵</td>
</tr>
<tr>
<td>8</td>
<td>C5</td>
<td>1.02 x 10⁵</td>
</tr>
<tr>
<td>9</td>
<td>C6</td>
<td>3.27 x 10⁴</td>
</tr>
<tr>
<td>10</td>
<td>D1</td>
<td>5.27 x 10³</td>
</tr>
<tr>
<td>11</td>
<td>A2</td>
<td>2.99 x 10³</td>
</tr>
</tbody>
</table>

Pirela et al., *Inhalation Toxicology* 2014
Physicochemical and morphological assessment of toner powder and PEPs

Toner powder

- Diameter 10-15 µm
- ENMs on the surface and embedded in the toner particle
- EDX: traces of carbon, oxygen, aluminum, silicon, cerium, iron, Mn, among others
- Chemistry matched that of MSDS sheet

**Confirmation:** toner formulations are nano-enabled products

PEPs

- Different aggregate shapes/sizes of ~ 20 – 200 nm
  - Consistent with RT monitoring data
- EDX: traces of carbon, oxygen, aluminum, silicon, zinc, iron, cerium, copper, tellerium, titanium, sulfur, among others

**Confirmation:** ENMs become airborne during consumer use of laser printer

Pirela et al., *Inhalation Toxicology*, 2014
Pirela et al., *Nanotoxicology*, 2014
Complex Chemical composition of PEPs and toner powder

- **Elemental carbon**: toner powder 0.14-12.10%, PEPs 0.20%
- **Organic carbon**: toner powder 43.02-88.65%, PEPs 0.42-99.8%
- **Metals**: toner powder 1-34%, PEPs 1-3%. CeO₂, ZnO, CuO, SiO₂
- **Other elements**: …

Pirela et al., *Nanotoxicology*, 2014
Toxicology Study Design

Toxicological evaluation

**In vitro**

- mono- and co-culture

  - Epithelial cells
  - Endothelial cells
  - Macrophages
  - Lymphoblasts

**In vivo**

- Inhalation and Instillation

  - *Balb/c* mice

PEPs (PM$_{0.1}$, PM$_{0.1-2.5}$, PM$_{2.5}$), comparative particles (SiO$_2$, Welding Fumes)

PEPs, PEPs+ gaseous pollutants

Cell viability, ROS, Gap junctions, Epithelial-Endothelial interactions, DNA damage, Epigenetics, Lung injury, Inflammation

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1 Pirela et al., *EHP* 2015  
2 Lu et al., *Nanotoxicology*, 2015  
3 Sisler et al., *Nanotoxicology*, 2014
Toxicological characterization of PEPs: *in vitro* experimental design

**Cells**
- Mono-¹,² and co-culture³ systems
- SAEC, HMVEC, THP-1

**Test Particles**
- PEPs (PM$_{0.1}$, PM$_{0.1-2.5}$, PM$_{2.5}$)
- Comparative particles (SiO$_2$, Welding Fumes)

**Exposure/Doses**
- Duration: 24 hours
- Doses: 0.5, 1, 5, 20, 30, 40, 100 µg/ml

**Endpoints**
- Cell viability
- Morphology
- Cell junctions
- Inflammation
- ROS generation
- Epigenetics

¹ Pirela et al., *EHP* 2015  ² Lu et al., *Nanotoxicology*, 2015  ³ Sisler et al., *Nanotoxicology*, 2014
Dosimetric considerations for toxicological assessment

Lung deposition model

Deposited mass in vitro

Administered dose using the Harvard In vitro dosimetric platform

Breathing parameters + Airborne PEPs properties

1 Angilvel, 1995 | 2 Demokritou et al., 2013 | 3 Cohen et al., 2014 | 4 DeLoid et al., 2014
**Dosimetric considerations for toxicological assessment**

<table>
<thead>
<tr>
<th>Duration of exposure to PEPs (inhalation)</th>
<th>Mass deposited in human lungs</th>
<th><strong>in vitro</strong></th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cell delivered mass</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SAEC</td>
</tr>
<tr>
<td>24 hours</td>
<td>174.6 μg</td>
<td>0.08 μg</td>
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<tr>
<td><strong>Volumetric dose (μg/ml)</strong></td>
<td></td>
<td>0.8 μg/ml</td>
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<table>
<thead>
<tr>
<th>Real world exposure at consumer level</th>
<th><strong>in vivo</strong></th>
<th><strong>in vitro</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Inhalation (hours)</td>
<td>Rodent Inhalation (hours)</td>
<td>Rodent Instillation (mg/kg)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<tr>
<td>15</td>
<td>6.5</td>
<td>0.4</td>
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<tr>
<td>3006</td>
<td>1295</td>
<td>83</td>
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</tbody>
</table>

in vitro: effect of exposure to PEPs on cell viability and ROS production

**Cytotoxicity**

- PEPs led to significant cell death in epithelial cells (at highest delivered mass) and in macrophages in a dose-dependent pattern
  - THP-1 more responsive than SAEC

**Reactive oxygen species**

- PEPs led to a dose dependent increase in ROS production in epithelial cells and in macrophages
  - SAEC more responsive than THP-1
PEPs affect cytokines associated with cell division and immune responses (recruitment of leukocytes to injury site, immune response stimulation, neutrophil production)
PEPs decreased expression levels of DNA methyltransferases (DNMTs) and TET in a dose-response pattern → possible change in methylation patterns affecting overall gene expression
**in vitro (co-culture): effect of exposure to PEPs on endothelial cells**

- Co-culture system allows for investigation of alveolar-capillary interaction

- Following epithelial cell treatment with PEPs, endothelial cells exhibited:
  1. Increased reactive oxygen species
  2. Substantial gap formation (arrows)
  3. Elevated cytokines levels: IL-1β, IL-8, IP-10, FGF-basic, IL-1RA, IL-6, MCP-1, MIP-1b, RANTES

### Reactive oxygen species

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>PEPs (PM&lt;sub&gt;0.1&lt;/sub&gt;)</th>
<th>SiO&lt;sub&gt;2&lt;/sub&gt;</th>
<th>MS-WF</th>
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<tbody>
<tr>
<td>Catalyst</td>
<td>-</td>
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<tr>
<td>Particle</td>
<td>+</td>
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### Gap formation

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<th>PEPs (PM&lt;sub&gt;0.1&lt;/sub&gt;)</th>
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<tr>
<td>Particle</td>
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</table>
Intratracheal instillation: effect of PEPs
- Male Balb/c mice
- PEPs (PM$_{0.1}$) extracted from CCI
- Dose: 0.5, 2.5 and 5.0 mg/kg bw
- Samples obtained: blood, heart, liver, spleen, lungs, bronchoalveolar lavage
- Parameters examined: lung injury and inflammation, epigenetics, reactive oxygen species

Whole-body inhalation: effect of VOCs and PEPs
- Male Balb/c mice
- Exposure: 6 hours/day, 5 days. Control: gaseous pollutants
- Particle concentration: 408,000 particles/cm$^3$
- Samples obtained: blood, heart, liver, spleen, lungs, bronchoalveolar lavage
- Parameters examined at Day1 and 5: lung injury and inflammation, reactive oxygen species
What are the effects of the PEPs + gaseous pollutants emitted by laser printers? Is there a synergistic effect?

- Exposure duration: 6 hours/day (1 and 5 consecutive days)
- Average particle concentration: 408,000 particles/cm^3
- Average aerodynamic particle diameter: 35.70 nm
- Average mass concentration: 32.4 μg/m^3
- Average ozone concentration: 13.8 ppbv
- Average VOC concentration: ~13 ppm
No change in lactate dehydrogenase (LDH) following instillation of PEPs

- Agreement with results from epithelial cell cytotoxicity experiments

Dose comparisons (IT vs. inhalation)
- 0.5 mg/kg = 8.13 hours
- 2.5 mg/kg = 40.63 hours
- 5 mg/kg = 81.25 hours
Exposure to PEPs led to:

- No effect in neutrophil degranulation after instillation
- Significant elevation in percent of lavaged neutrophils at 5.0 mg/kg
Significantly increased levels of LIF post-PEPs exposure vs. control group

- Involved in pulmonary response to inflammation (e.g., repair processes, airway responsiveness)
Instillation: evaluation of gene expression following exposure to PEPs

- Upregulated expression of genes due to exposure to PEPs (2.5 mg/kg)
  - Cell survival, inflammatory responses
- CCL5 (RANTES) also significantly elevated in vitro → consistency in results from both experimental platforms
Exposure to PEPs led to a reduction in DNMT3a and TET1
- Important components of DNA methylation machinery

Similar responses in lung and alveolar macrophages to PEPs

Results consistent with *in vitro* experiments for the case of the lung and alveolar macrophages
Inhalation: evaluating lung injury and inflammation following PEPs + VOCs exposure (1/2)

- No synergistic effects from presence of gaseous co-pollutants. Levels of lactate dehydrogenase (LDH) is same between gas pol. only and gas+ PEPs groups for both time points.

- Difference in LDH levels between 6- and 30-hour exposure durations:
  - Acclimatization of the mice to laser printer emissions (gaseous)?

Unpublished data
Summary

- Toner formulations are considered nano-enabled products.
- Laser printers emit high numbers of ENMs used in the toners during consumer use (~1.3 million particles/cm³).
- In both *in vitro* and *in vivo* experimental conditions, PEPs had an effect on cell viability, production of ROS, cytokine levels and epigenetics, among other parameters.
  - PEPs are biologically reactive at concentrations comparable to customer exposure scenarios (at as low as 8 hours of exposure).
Acknowledgements

- Dr. Sandra Pirela, HSPH
- Dr. Georgios Pyrgiotakis, HSPH
- Dr. George Sotiriou, HSPH
- Dr. Xiaoyan Lu, HSPH
- Dr. Bingtao Zhao, HSPH
- Dr. Vincent Castranova, NIOSH
- Dr. Treye Thomas, CPSC
- Dr. Jennifer Sisler, NIOSH
- Dr. Yong Qian, NIOSH
- Dr. Nancy Gao,
- Dr. Joel Cohen, HSPH
The only true wisdom is in knowing you know nothing.

Socrates
Dosimetric considerations for toxicological assessment – Dose table

Real world exposure at consumer level

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<th>Human Inhalation (hours)</th>
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MPPD2 Model 1

Deposited mass
In the lung

VCM-ISDD Model 2-4

Deposited mass
in vitro

in vitro administered dose

in vitro delivered dose

SAEC

THP-1