Physico-chemical and toxicological studies of engineered nanoparticles emitted from printing equipment

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Background (1 of 3)

- Nanotechnology has increased significantly over last decade: benefits of unique 'nano' properties.

- A new industrial "motto" for the 21st Century: "**The NANO the better"**

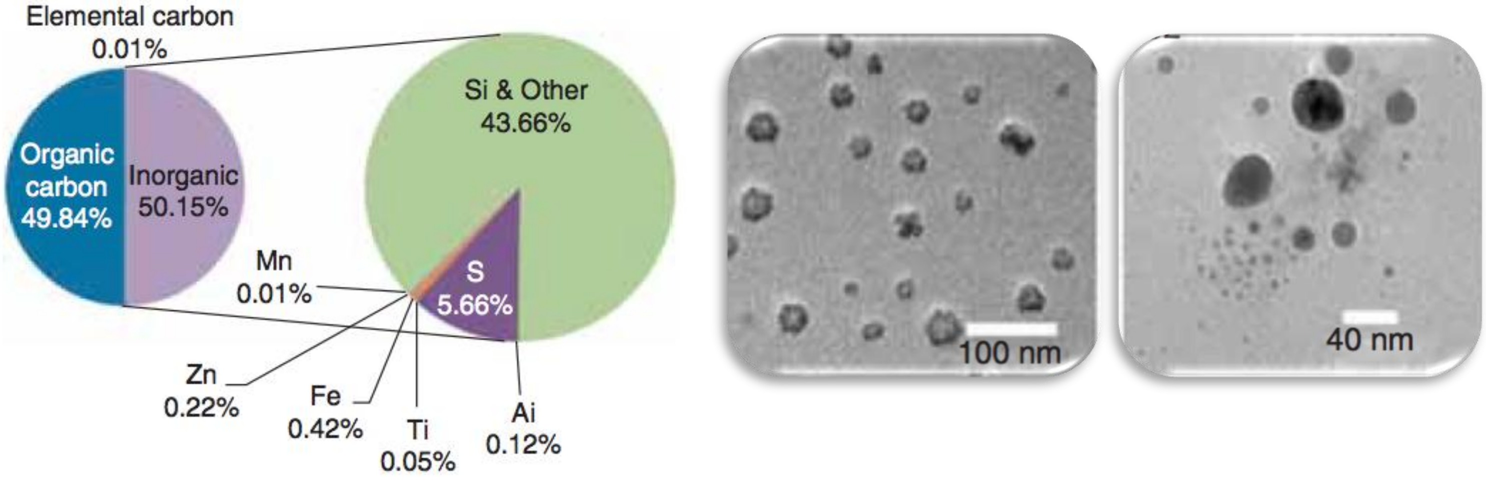
- There is a growing number of products in the market which contain nanoscale materials (e.g. sunscreens, building materials, food packaging, etc).

- There is growing evidence linking nano-enabled products (NEPs) to possible exposures and adverse health effects

Background (2 of 3)

- Our group has been investigating the possible exposure to engineered nanomaterials (ENMs) included in the toner formulations and emitted from printing equipment.
- New photocopier toner formulations have switched to ”nano” in order to improve quality.
  - Shift in toner composition → incorporation of nanoparticles [1].
  - Complex elemental mixture (organic/inorganic composition)[1].
- Our recent studies from a photocopy center linked emitted PM to toxicity potential in both *in vitro* and *in vivo* experimental models [2,3].

Chemical profile and imaging of the emitted $PM_{0.1}$ size fraction



References: 1. Bello et al, 2012. 2. Pirela et al., 2013. 3. Khatri et al., 2012

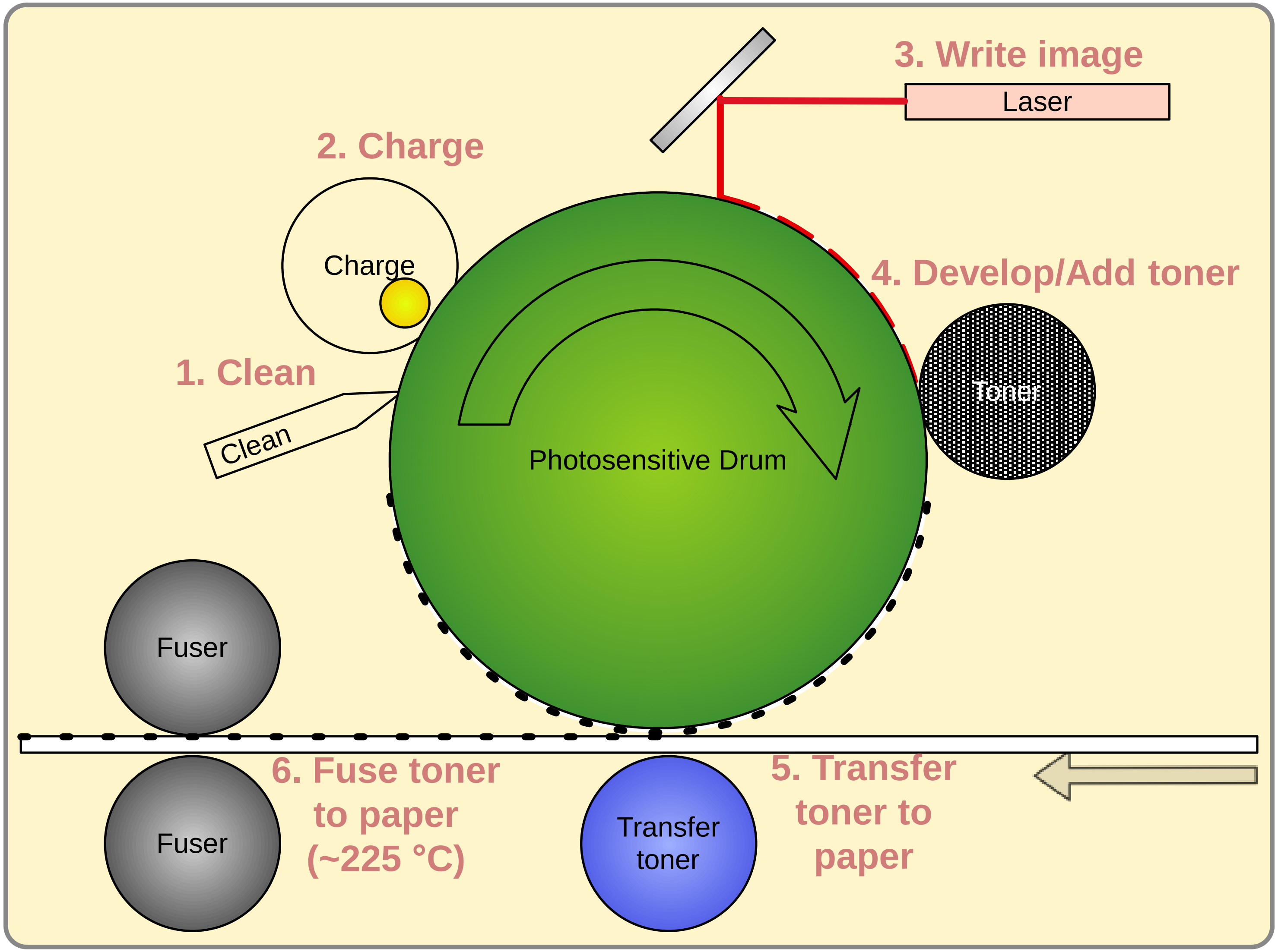
Background- Printers (3 of 3)

- Currently, our group is involved in a new study focusing on the **physico-chemical and toxicological** implications of particles emitted from consumer grade printers.
- It is well known that laser printers emit high number of nanoparticles, VOCs and ozone.
- Not much is known about engineered nanoparticles emitted from printers.
- Complex physico-chemical and morphological characteristics of both toner formulations and PEPs are unknown.

Gaps in the literature

- Physicochemical and morphological properties of PEPs remain poorly characterized.
- Unsure of the source apportionment of these emissions.
- Toxicological studies use toner particles instead of "real world" emitted particles and physiologically irrelevant doses.
- Uncertainty in the implications of shift from micron to nano-sized particles in toner formulations.
- Insufficient data for adequate science-based risk assessment of exposure in both occupational and non-occupational settings.

How do laser printers work?

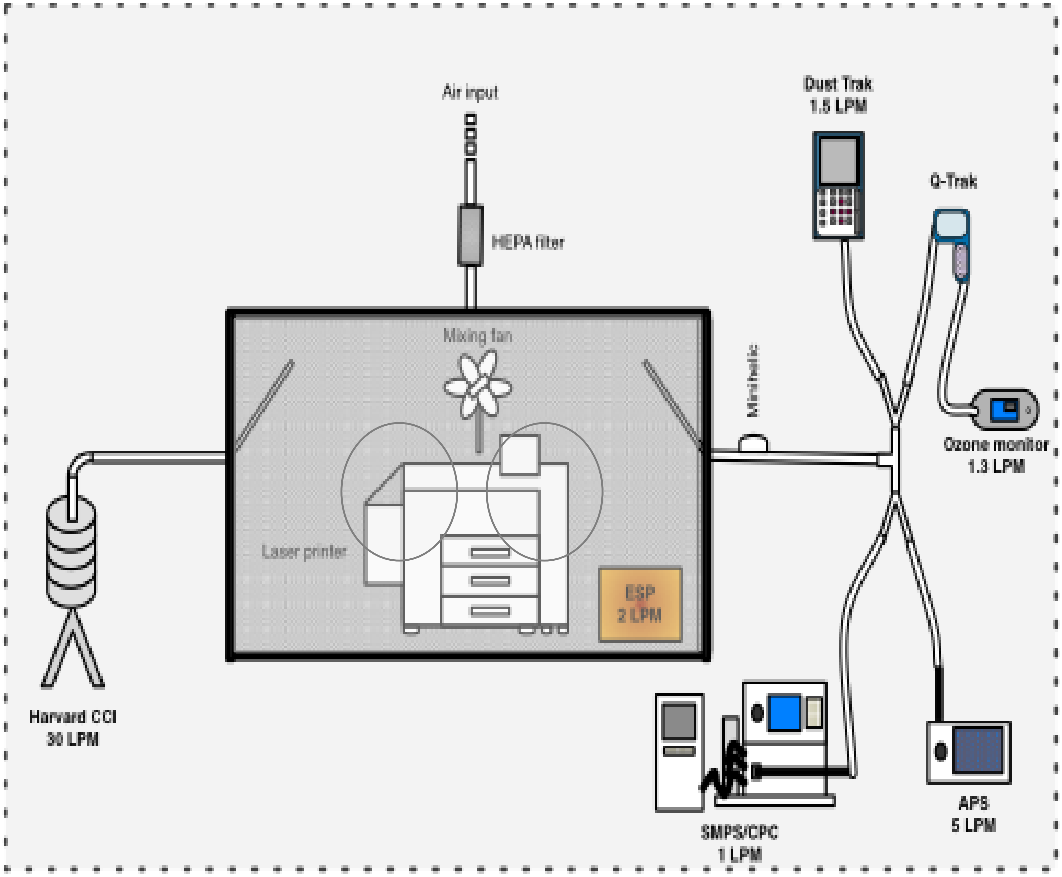


Research Objectives

- Develop a lab-based exposure system and p-c-m characterization of printer emitted particles (PEPs).
  - Generation of relevant real-world particles emitted from laser printers
  - Evaluation of effect of operational parameters on printer emission profile
  - Use of the exposure system to assess emissions of commonly used printers (11 printers, various models and manufacturers).
- Physico-chemical and morphological evaluation of toner powder and PEPs.
- *In vitro* evaluation of biological outcomes
  - Exposure: Cell suspensions (single- and co-culture systems).
  - Endpoints: genotoxicity and cytotoxicity.

Objective 1: Develop lab-based exposure system to generate controlled-exposure atmospheres from laser printers

- Glovebox chamber design allowed for uninterrupted operation.
- Evaluated 11 printers of various manufacturers/brands
  - Emission profiles
  - Operational parameters
- Real time and integrated PM sampling/monitoring systems:
  - SMPS/CPC and APS
  - Ozone monitor
  - Dust track
  - Q-trak



Results:
Size distribution and number concentration of PEPs

- 11 printers from 4 manufacturers were assessed and ranked in terms of emitted particles during a continuous print job.
- Particle number concentration ranged across manufacturers and varied from 2.99×10^3 to 1.27×10^6 particles/cm^3.
- Size distribution properties:
  - Mean: 39 - 122 nm
  - Majority < 100nm (none/few particles > 200 nm)
- "Initial burst" of particles at initiation of printing visible for almost all printers.
- Certain operational parameters affect printer emission profile: warm up period, page coverage, and printing mode (continuous vs. intermittent).
- No observable association between printer model/maker and emission profile.

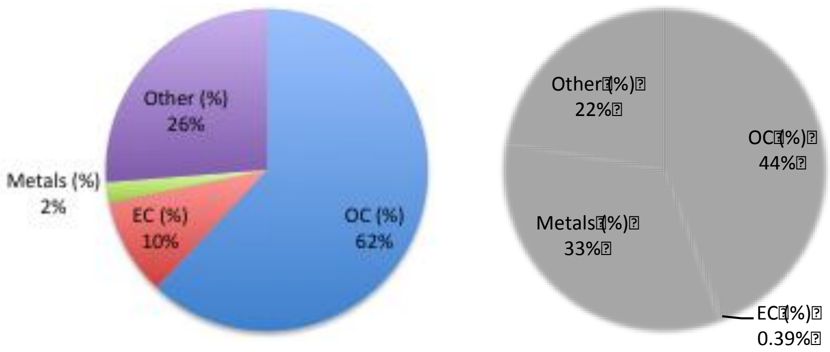
Results:
Detailed chemical and morphological characterization of toners and PEPs

- Collected size-fractionated PEPs and toner powder from the 6 highest-emitting laser printers.

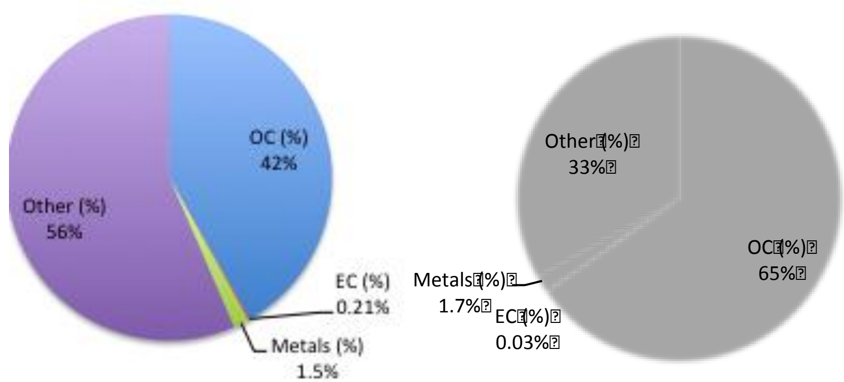
- Performed the following chemical and morphological analyses:
  - SF-ICP-MS: metals
  - GC-MS: organic and elemental carbon
  - TEM/SEM: morphology
  - EDS: surface chemistry

Chemical composition of toner powder and PEPs

Toner powder (Manufacturer A and B)



PEPs (Manufacturer A and B)

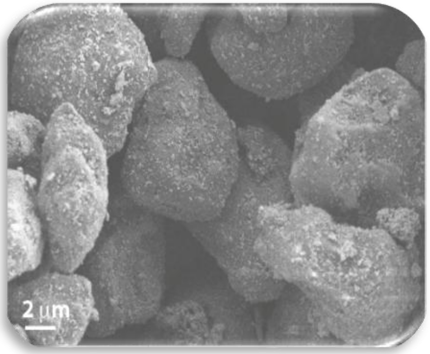


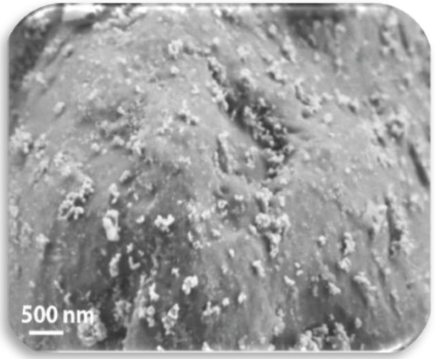
- Toner powder and PEPs **share** complex chemical composition (metals, OC, EC, organometallic salts).
- Organic carbon: toner powder 42-89%, PEPs 40-99%.
- Metals: toner powder 1-34%, PEPs 1-3%. These include: CeO_2, ZnO and CuO.
- Elements from toner were identified in PEPs at different concentrations, indicative of PM emitted from toner.
- Some metals found in PEPs and paper, but not in toner powder → paper as a PM emission source.

(Unpublished data, manuscripts in preparation)

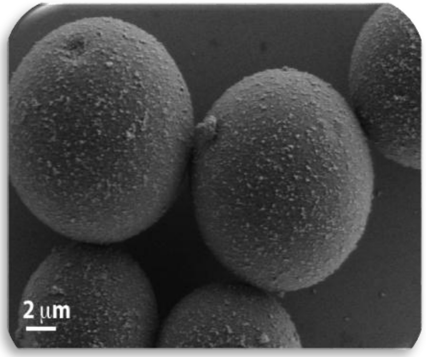
Results:
Morphological characterization of toner powders

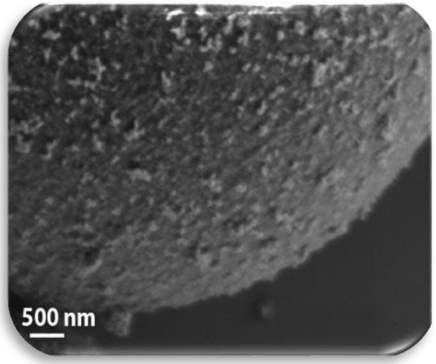
Manufacturer 1, Model A





Manufacturer 1, Model B:





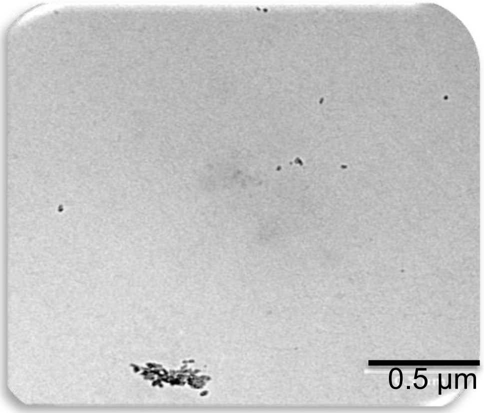
- Nanoparticles are visible on surface of toner powder of two models of same manufacturer.
- Irregular particles with diameter of 10 µm.
- Traces of carbon, oxygen, aluminum, silicon, cerium and lanthanum, among others was confirmed in EDS analysis.

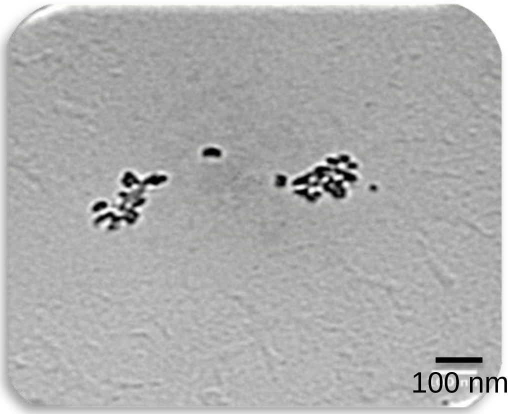
Confirmation: toner formulations switched from micron to nano.

(Unpublished data, manuscripts in preparation)

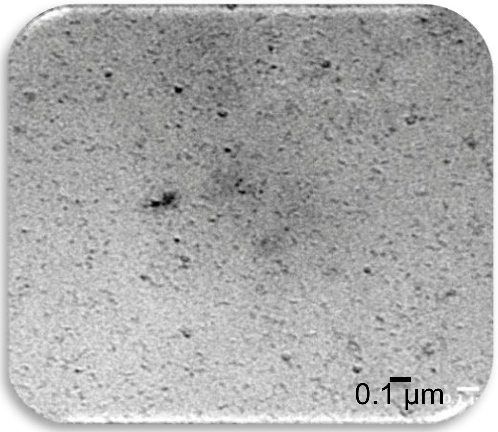
Results:
Morphological characterization of PEPs

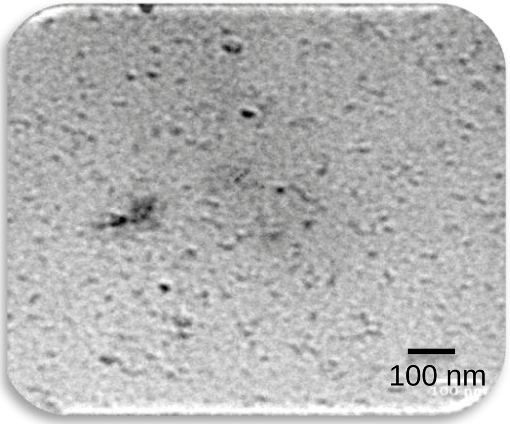
Manufacturer 1, Model A





Manufacturer 1, Model B





- Size of aggregates ranged from 20 – 200 nm.
  - Consistent with real-time monitoring instrumentation.
- Irregularly-shaped particles.

Confirmation: ENMs are making their way to room air during printing.

(Unpublished data, manuscripts in preparation)

Objective 3: Characterize biological outcomes *in vitro* due to exposures to PEPs

Cells

- Single and co-culture systems
- SAEC, HMVEC, THP-1 and TK-6

Exposure

- Duration: 24 hours
- Dose: 5, 20, 40 and 100 µg/ml

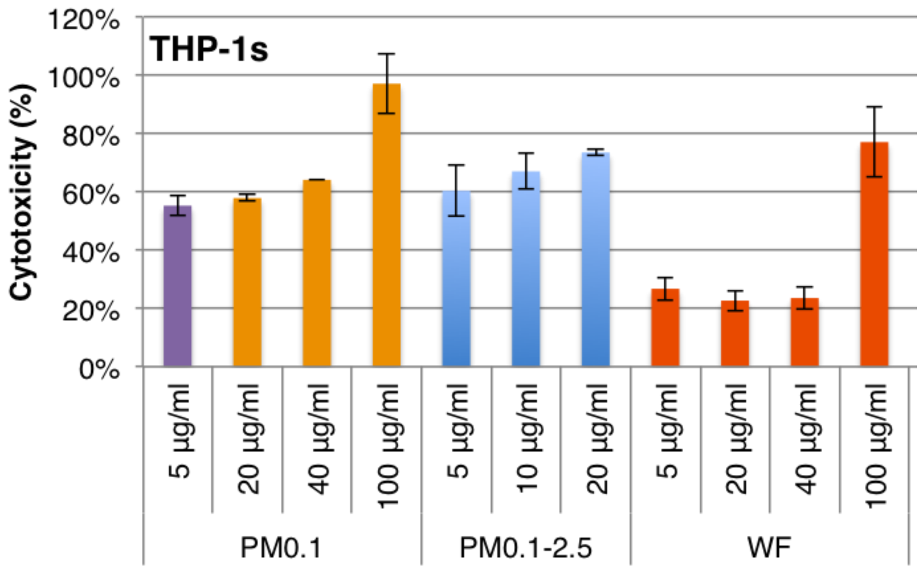
Particles

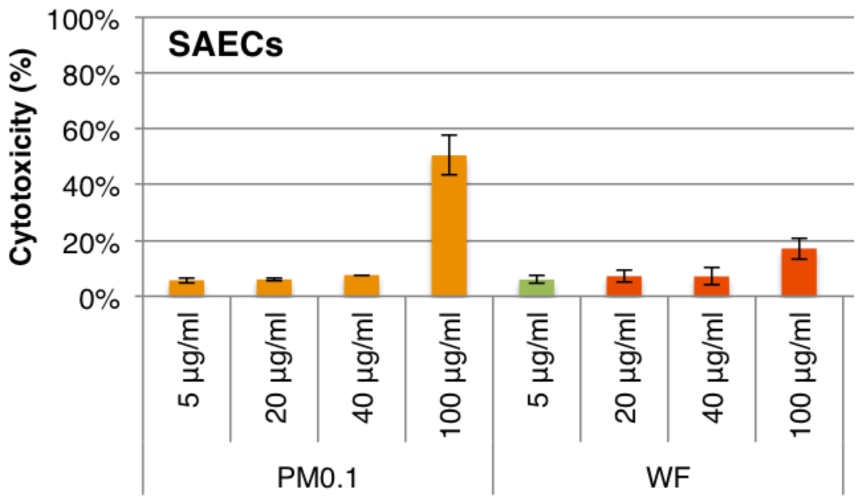
- PEPs ($PM_{0.1}$, $PM_{0.1-2.5}$ and $PM_{<2.5}$)
- Comparative particles (SiO_2, MS-welding fumes)

Endpoints

- DNA damage
- Cell viability
- Morphology
- Membrane injury
- Migration
- Inflammation
- ROS generation

Results:
preliminary *in vitro* toxicological characterization of PEPs





- LDH assay performed 24 hrs post-exposure to PEPs
- Dose response
- Different toxicity of $PM_{0.1\text{-}2.5}$ and $PM_{0.1}$
- $PM_{0.1}$ more toxic than welding fumes.

Cellular membrane integrity has been affected by the exposure to PEPs → concerns for lung toxicity

Conclusions

- A substantial number of engineered nanoparticles are emitted during printing.
- Toner formulations contain nanoscale materials, which become airborne during the printing process.
- Complexity of chemical composition of PEPs raises concern for toxicity.
- Preliminary *in vitro* data → possible adverse health implications.

Future directions: *in vivo* toxicological characterization using a mouse model to evaluate biological responses in the lung following exposure to PEPs.

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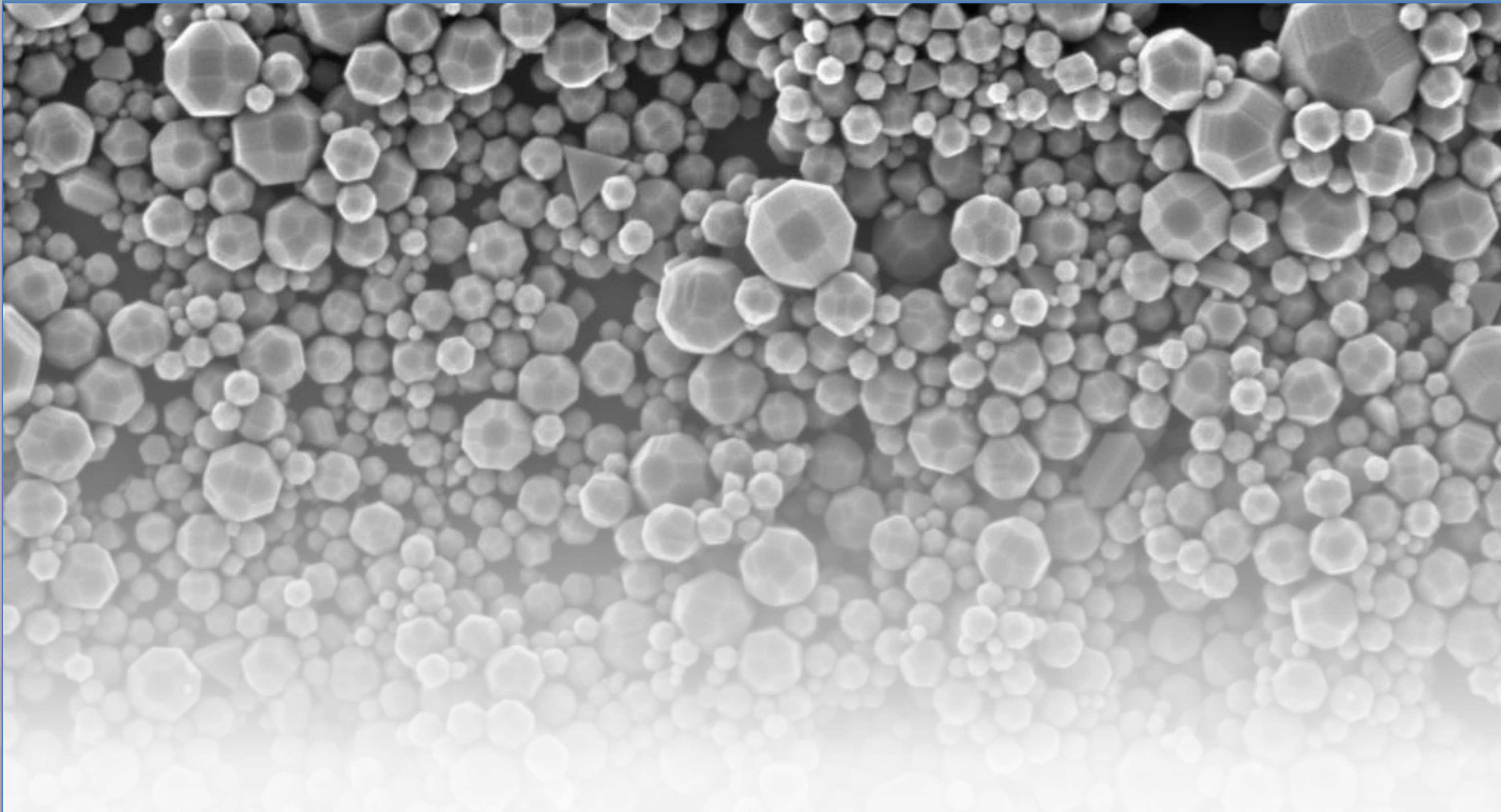
QUESTIONS?



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Possible Sources of printer emissions

Paper

$CaCO_3$

Organic compounds deriving from the degradation of cellulose.

Toner

Plastic (Styrene acrylate copolymer, polyester resin): 65-85% or 55-65%.

Iron oxide: 6-12% or 30-40%.

Wax, ground sand: 1-5%.

Amorphous silica: 1-3 %.

Carbon black: 1-10%.

Other metals.

Casing/Structure

Plastic.

Flame retardants (brominated).

Fillers (silica).

Micron → nano ?

References: Paper (Gaspar et al., 2010, Ramalho et al., 2009), Toner (Dell MSDS), Casing (Barthel et al., 2011).