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

HARVARD T.H. CHAN
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- 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 Nanototechnology 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.*)

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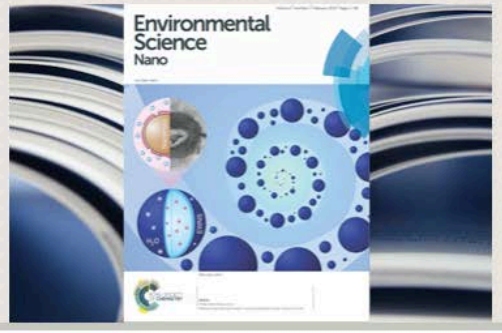

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Harvard NanoCenter draws on decades of experience with environmental pollutants and the health effects of particles to address the unique environmental health and safety (EHS) concerns raised by engineered nanomaterials (ENM) and nanotechnology applications.

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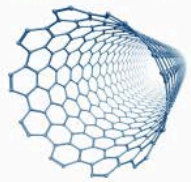


Title: *Commercialization of CNT-enabled Products:*

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
- Number of home offices in US households: 26 million (1999) → 38 million (2010)

Exposure studies

- Laser printers release both particulate matter (PM) and gaseous pollutants during their use [1]
- Particle release from board cooler, rear of printer, paper tray, fan and toner waste [2]

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)

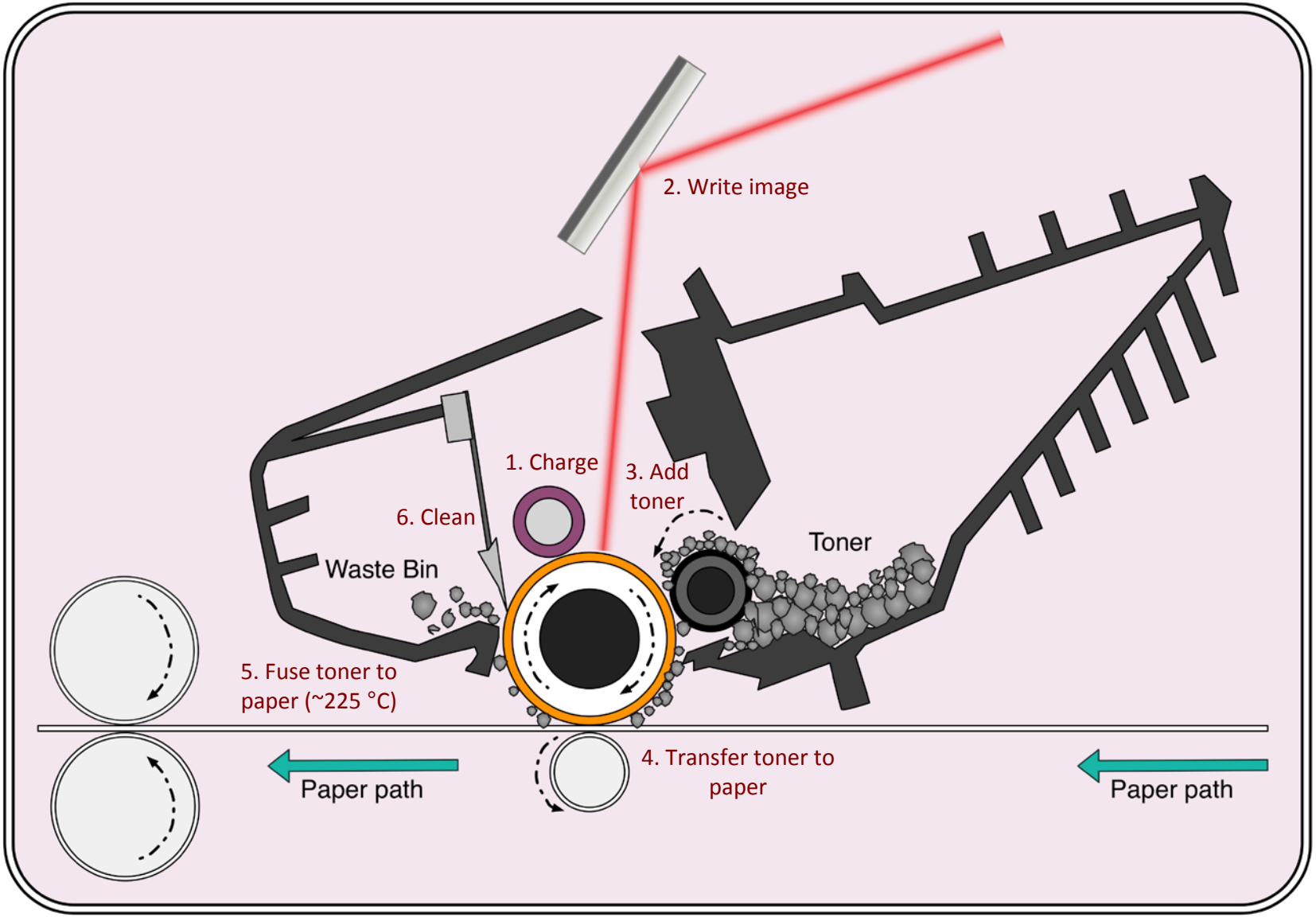
Physicochemical and morphological assessment

in vitro

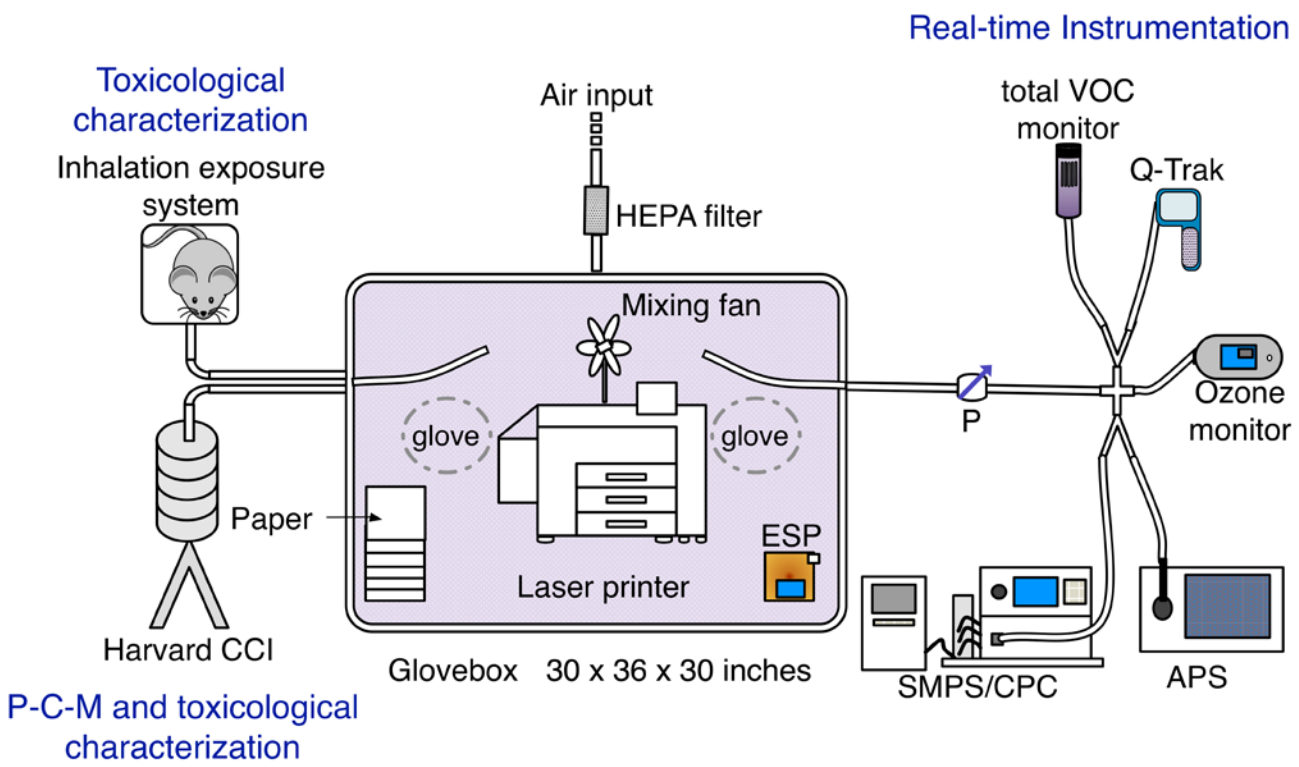
in vivo

Toxicological assessment

Background: How do laser printers work?



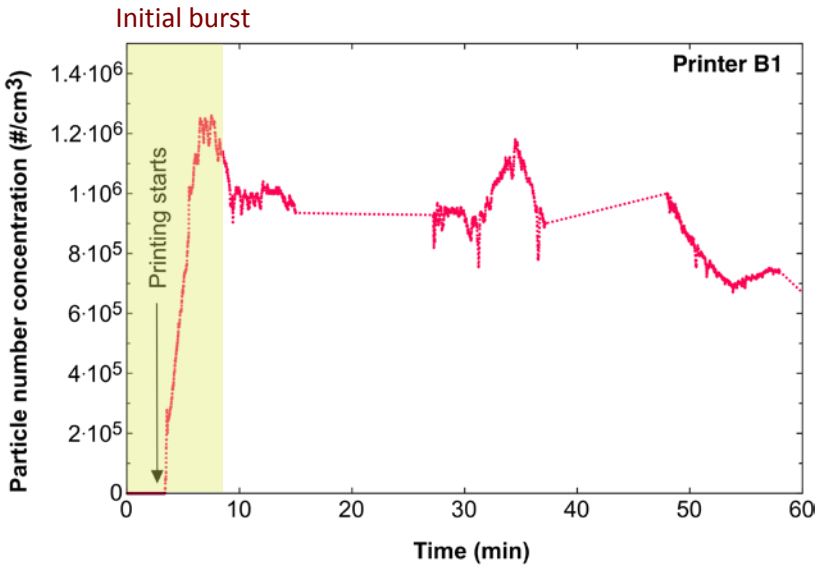
Development of Printer Exposure Generation System (PEGS)

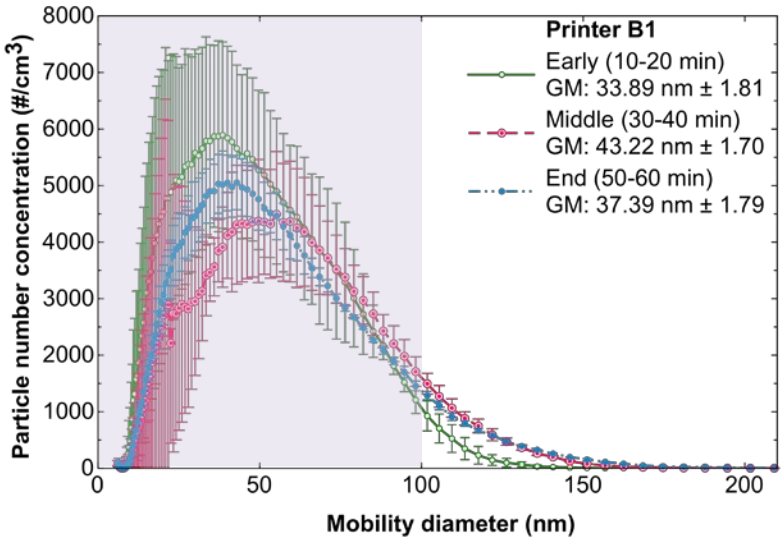


Features:

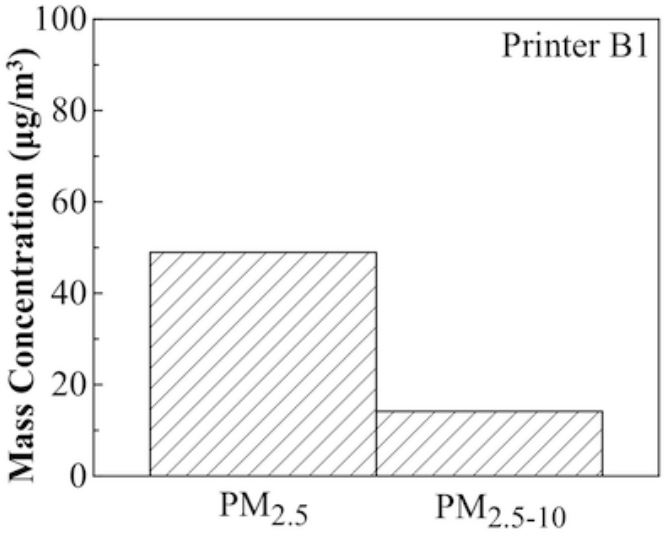
- Uninterrupted operation
- Real time aerosol and gaseous emission monitoring
- Particle generation and collection
- Animal exposures
- Simulation of different exposure scenarios (ACH)
- Versatile: can be used for characterization of particle released from various NEPs

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/cm^3
  - Initial burst within 10-12 min
  - Mean diameters: 39 - 122 nm, majority of particles by number < 100 nm
  - Mass concentrations: up to 100 µg/m^3
- Emission profiles identified for printers → rank them based on maximum particle released

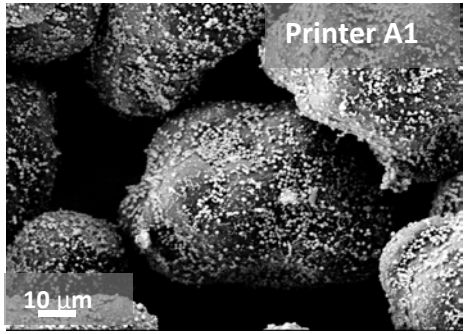


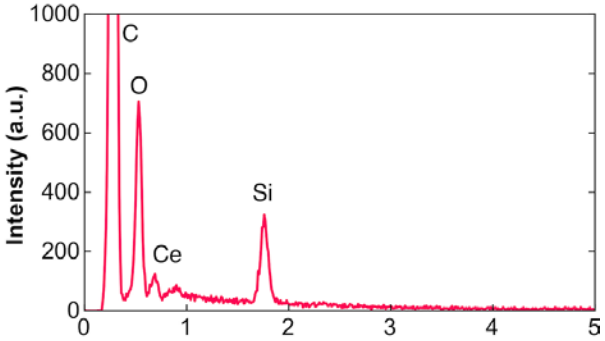
Ranking of commonly used laser printers

Ranking	Printer	Maximum particle number concentration (#/cm^3)
1	A1	1.27×10^6
2	B1	1.26×10^6
3	B2	6.78×10^5
4	C1	2.62×10^5
5	C2	2.12×10^5
6	C3	1.70×10^5
7	C4	1.52×10^5
8	C5	1.02×10^5
9	C6	3.27×10^4
10	D1	5.27×10^3
11	A2	2.99×10^3

Physicochemical and morphological assessment of toner powder and PEPs

Toner powder

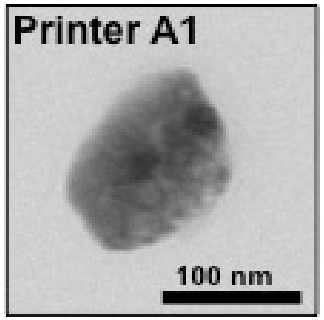


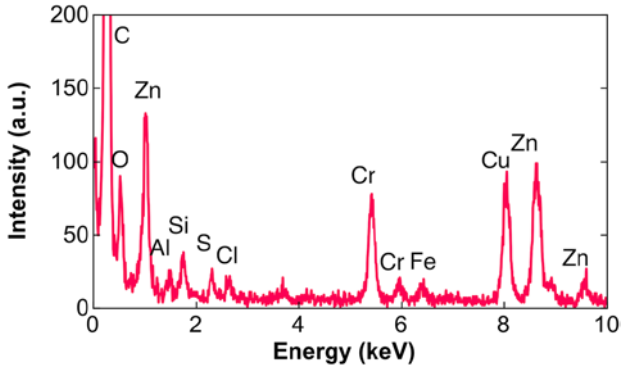


- Diameter 10-15 µm
- ENMs on the surface and embedded in the toner particle
- EDX: traces of carbon, oxygen, aluminum, silicon, cerium, iron, Mn, among others
- Chemistry matched that of MSDS sheet

Confirmation: toner formulations are nano-enabled products

PEPs





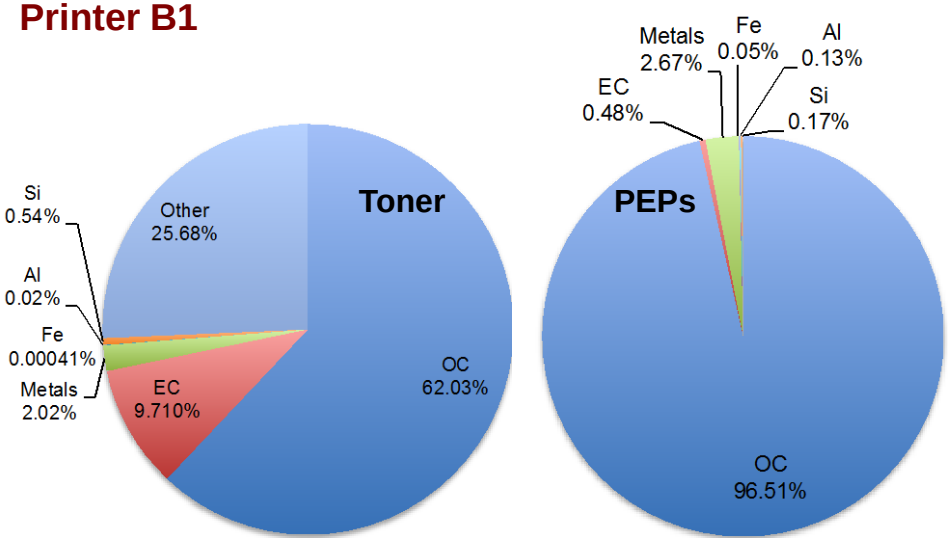
- Different aggregate shapes/sizes of ~ 20 – 200 nm
  - Consistent with RT monitoring data
- EDX: traces of carbon, oxygen, aluminum, silicon, zinc, iron, cerium, copper, tellerium, titanium, sulfur, among others

Confirmation: ENMs become airborne during consumer use of laser printer

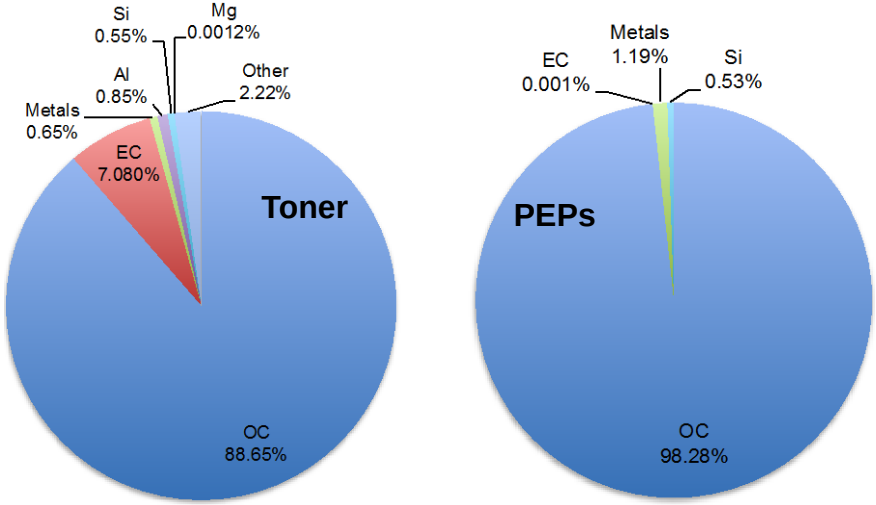
Pirela et al., *Inhalation Toxicology*, 2014
Pirela et al., *Nanotoxicology*, 2014

Complex Chemical composition of PEPs and toner powder

Printer B1

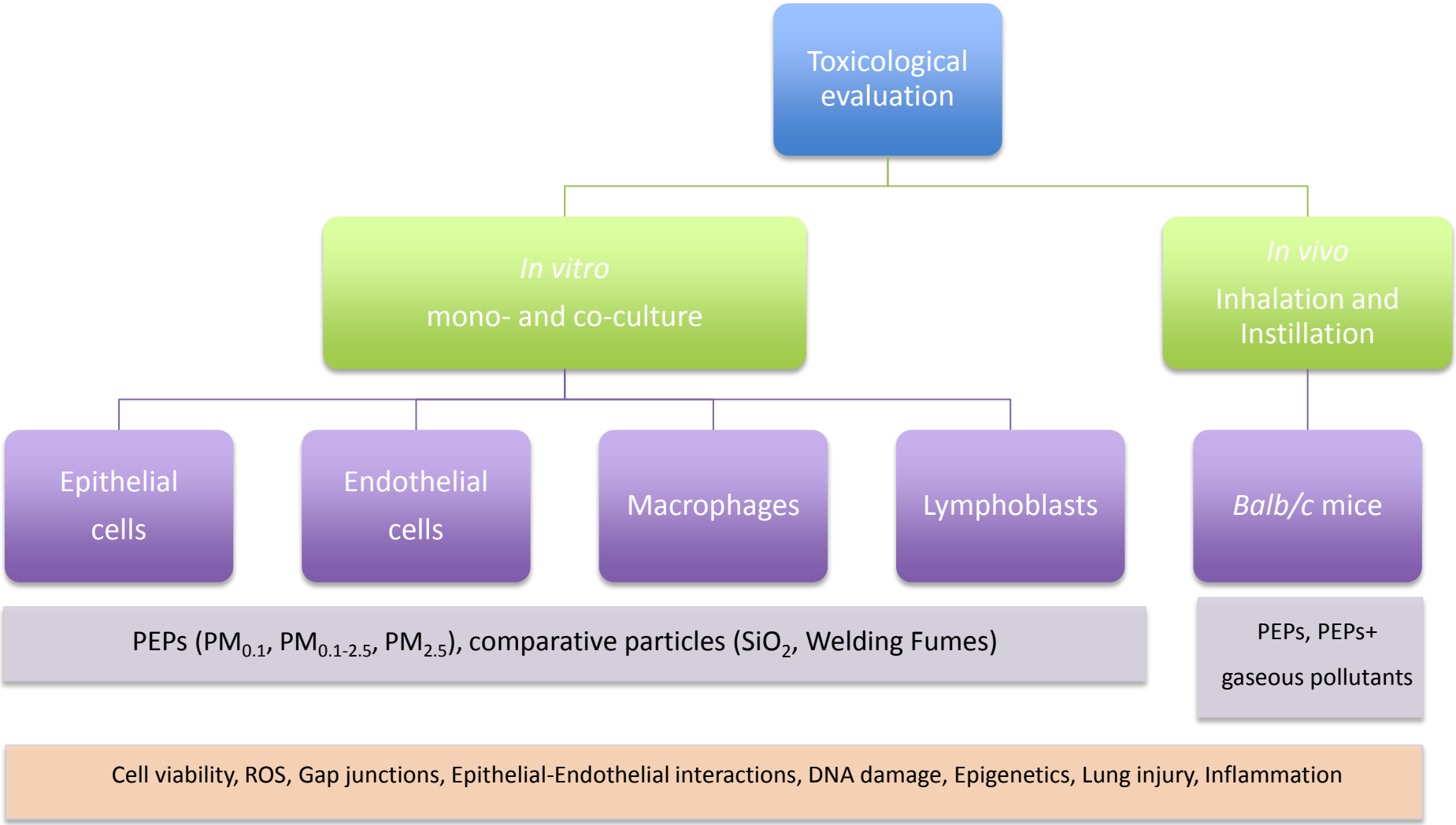


Printer C1



- 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%. CeO_2, ZnO, CuO, SiO_2
- Other elements: …

Toxicology Study Design



[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-culture[3] systems
- SAEC, HMVEC, THP-1

Test Particles

- PEPs ($PM_{0.1}$, $PM_{0.1\text{-}2.5}$, $PM_{2.5}$)
- Comparative particles (SiO_2, Welding Fumes)

Exposure/Doses

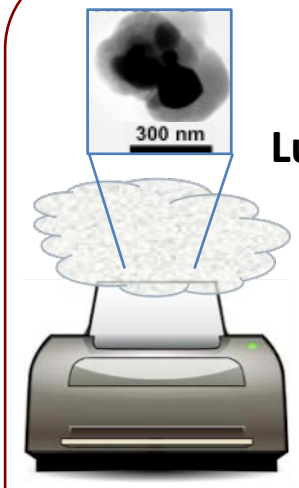
- Duration: 24 hours
- Doses: 0.5, 1, 5, 20, 30, 40, 100 µg/ml

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

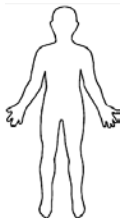


Source

Lung deposition model [1]

Estimating lung deposited mass of inhaled particles

Deposited mass In the lung

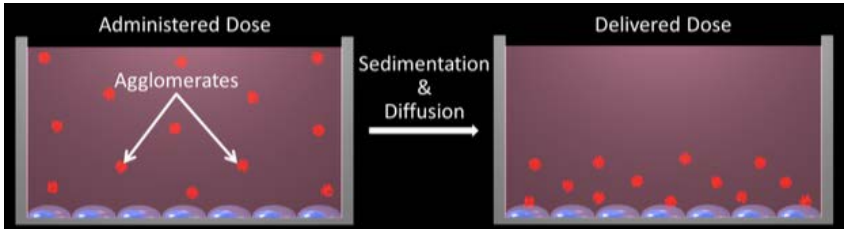


Breathing parameters **+** Airborne PEPs properties

Deposited mass *in vitro*

Media **+** Cell line + Particle

Estimating *In vitro* Administered dose using the Harvard *In vitro* dosimetric platform [2-4]



[1] Angilvel, 1995 | [2] Demokritou et al., 2013 | [3] Cohen et al., 2014 | [4] DeLoid et al., 2014

Dosimetric considerations for toxicological assessment

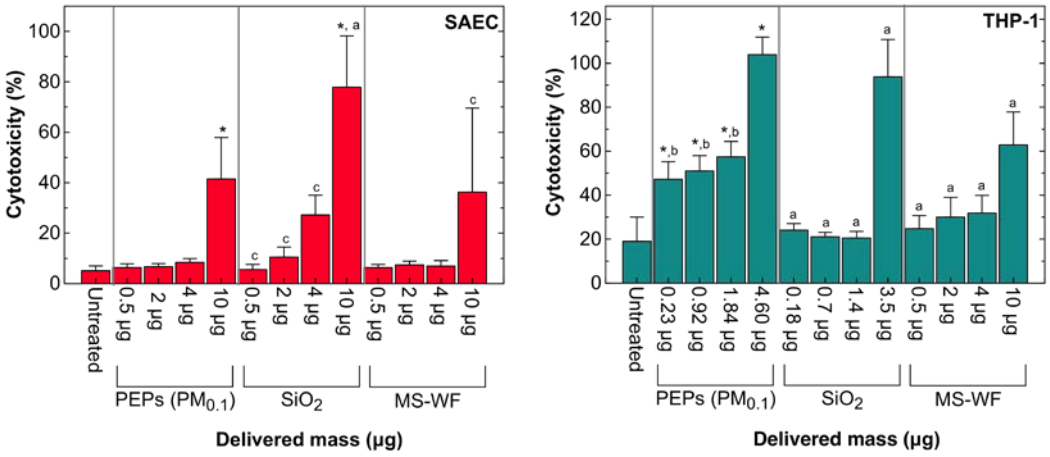
Duration of exposure to PEPs (inhalation)	Mass deposited in human lungs	*in vitro*			
		Cell delivered mass		Cell administered mass	
		SAEC	THP-1	SAEC	THP-1
24 hours	174.6 µg	0.08 µg	0.08 µg	0.08 µ	0.15 µg
Volumetric dose (µg/ml)		0.8 µg/ml			1.5 µg/ml

Real world exposure at consumer level	*in vivo*		*in vitro*		
Human Inhalation (hours)	Rodent Inhalation (hours)	Rodent Instillation (mg/kg)	*in vitro* administered dose (µg/ml)	*in vitro* delivered dose (µg/ml)	
				SAEC	THP-1
15	6.5	0.4	0.5	0.5	0.25
150	65	4	5	5	2.5
300	129	8	10	10	5
601	259	17	20	20	10
901	389	25	30	30	15
1202	518	33	40	40	20
3006	1295	83	100	100	50

[1] Angilvel, 1995 | [2] Demokritou et al., 2013 | [3] Cohen et al., 2014 | [4] DeLoid et al., 2014

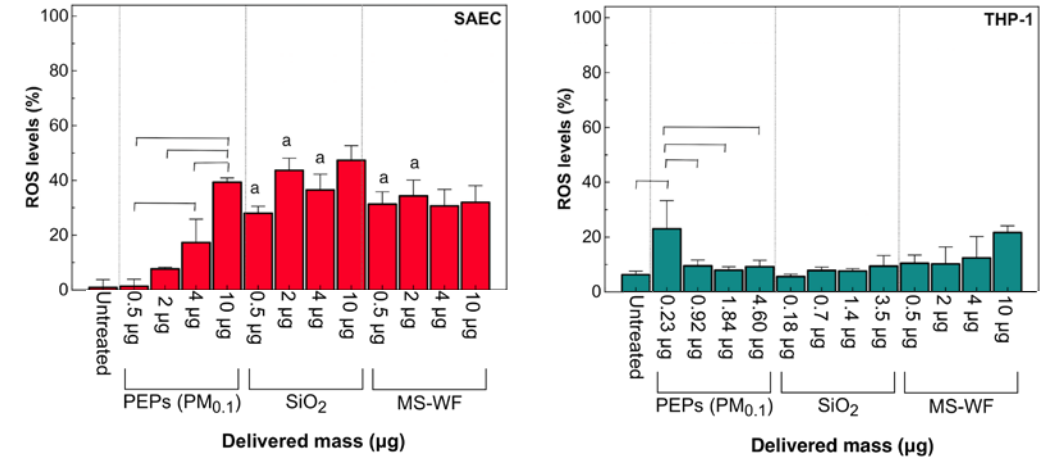
in vitro: effect of exposure to PEPs on cell viability and ROS production

Cytotoxicity



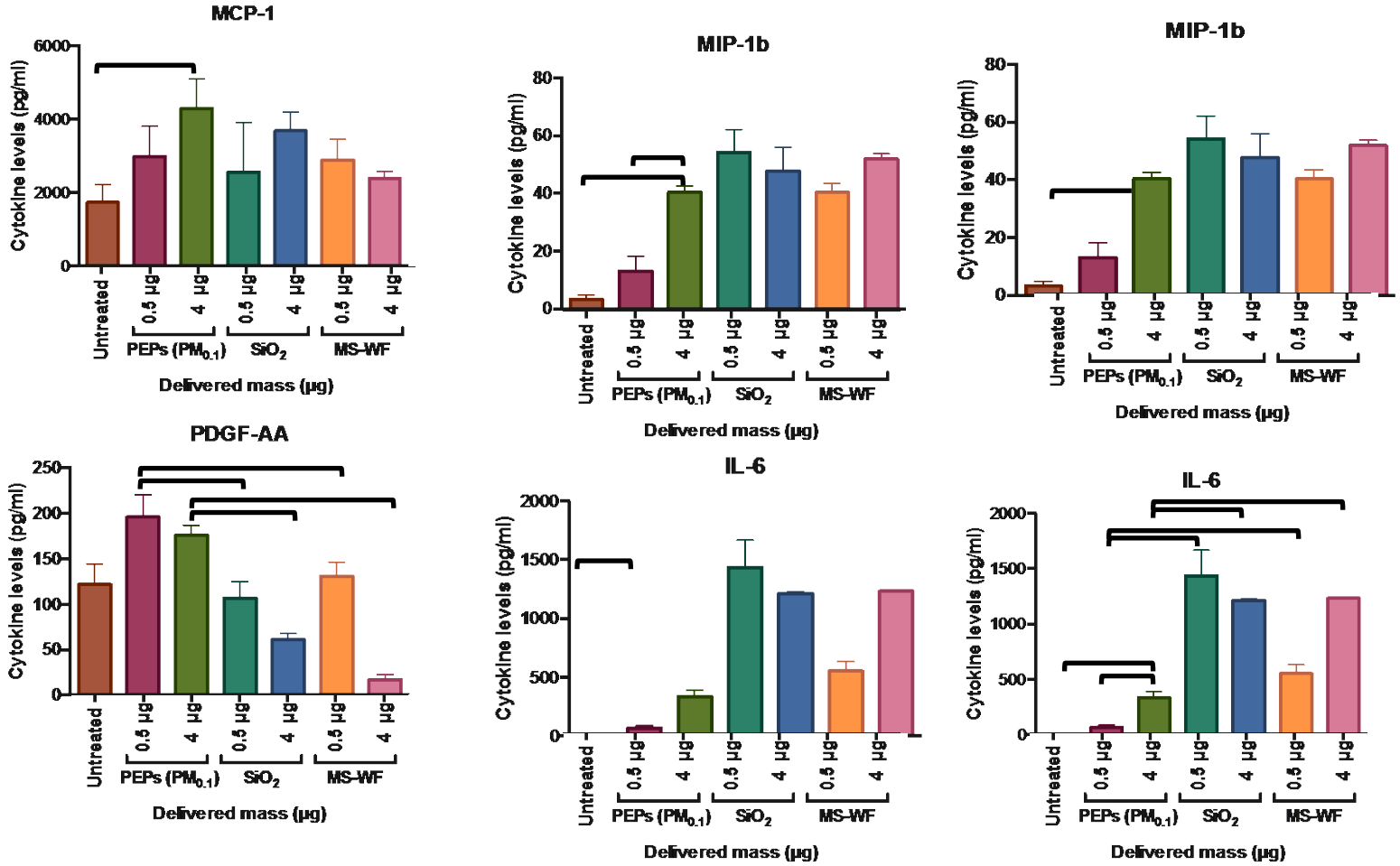
- PEPs led to significant cell death in epithelial cells (at highest delivered mass) and in macrophages in a dose-dependent pattern
  - THP-1 more responsive than SAEC

Reactive oxygen species



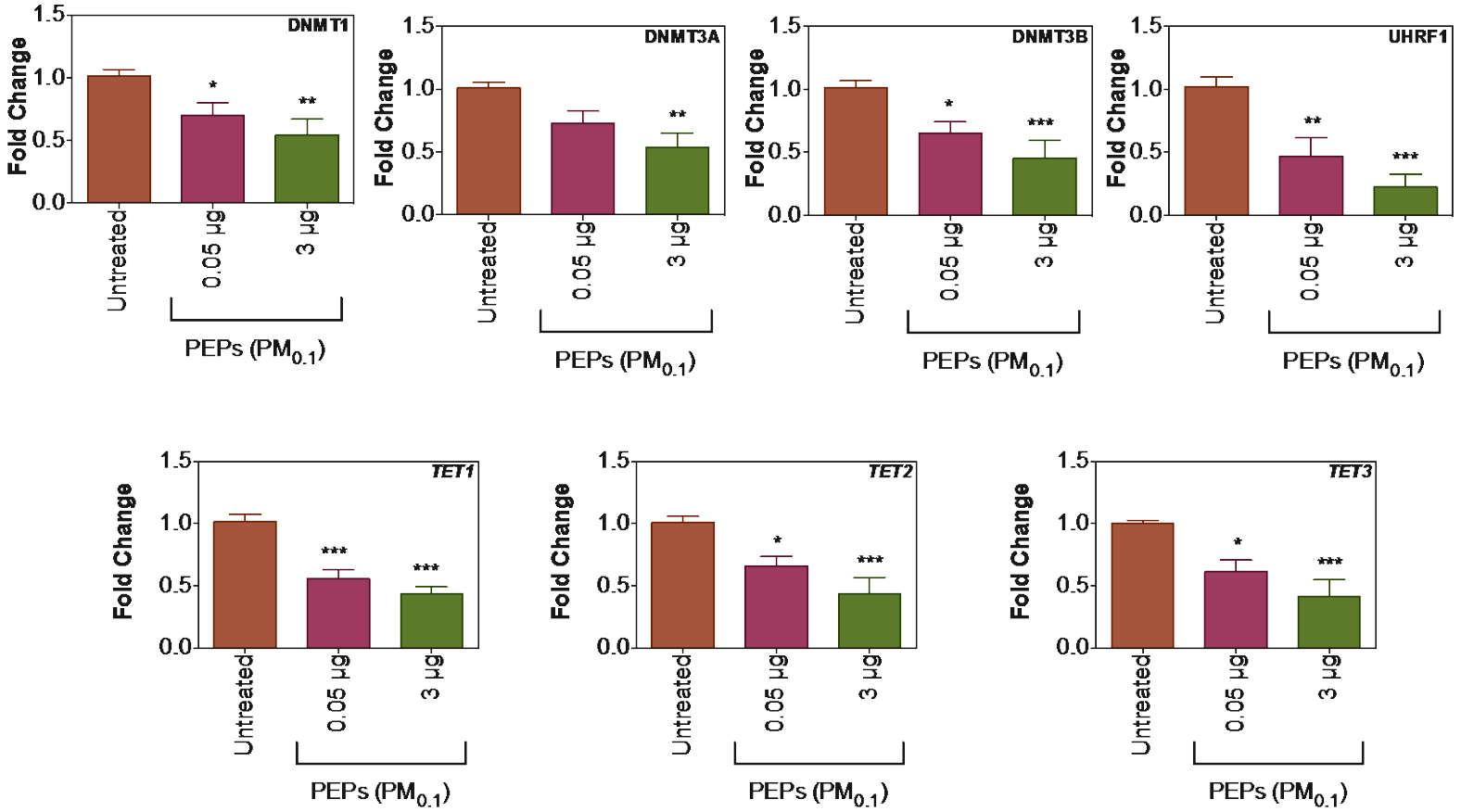
- PEPs led to a dose dependent increase in ROS production in epithelial cells and in macrophages
  - SAEC more responsive than THP-1

in vitro: effect of exposure to PEPs on SAEC cytokine expression



- PEPs affect cytokines associated with cell division and immune responses (recruitment of leukocytes to injury site, immune response stimulation, neutrophil production)

in vitro: expression levels of DNA methylation machinery components in SAECs following exposure to PEPs

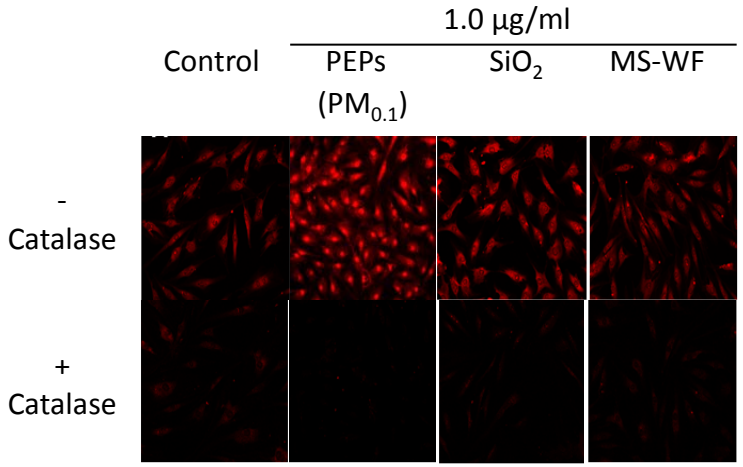


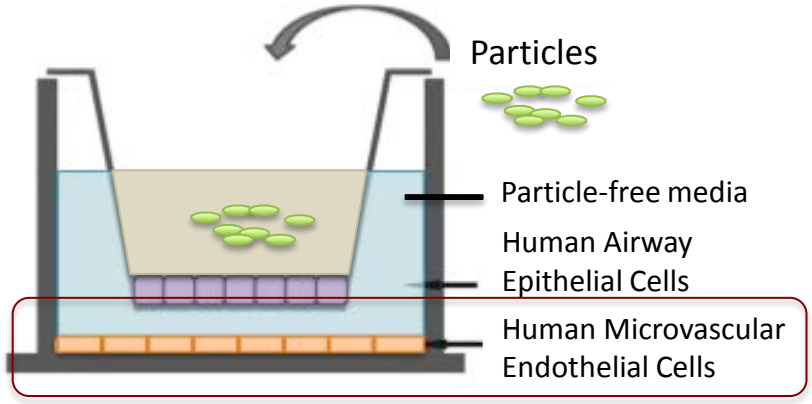
- PEPs decreased expression levels of DNA methyltransferases (DNMTs) and TET in a dose-response pattern → possible change in methylation patterns affecting overall gene expression

in vitro (co-culture): effect of exposure to PEPs on endothelial cells

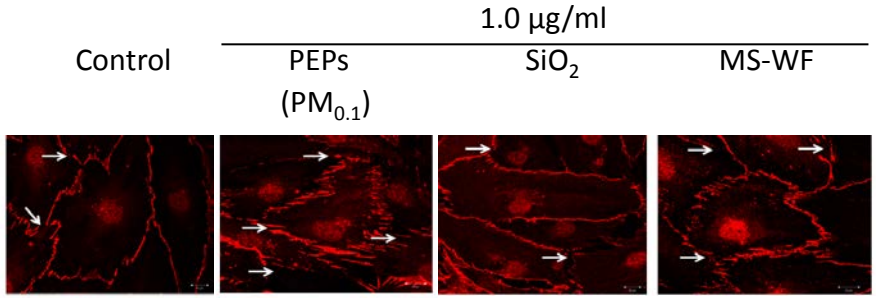
- Co-culture system allows for investigation of alveolar-capillary interaction
- Following epithelial cell treatment with PEPs, endothelial cells exhibited:
  - Increased reactive oxygen species
  - Substantial gap formation (arrows)
  - Elevated cytokines levels: IL-1β, IL-8, IP-10, FGF-basic, IL-1RA, IL-6, MCP-1, MIP-1b, RANTES

Reactive oxygen species

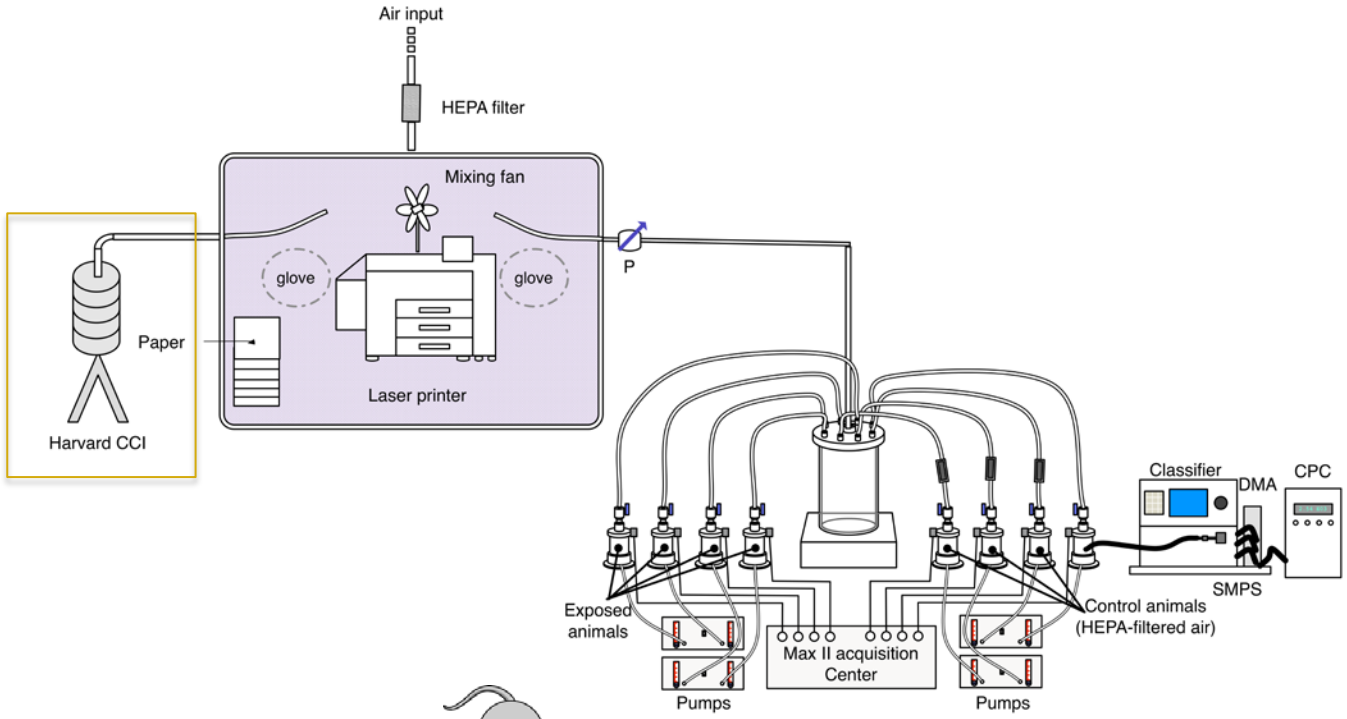




Gap formation



in vivo exposure to PEPs *via* inhalation and instillation



Intratracheal instillation: effect of PEPs

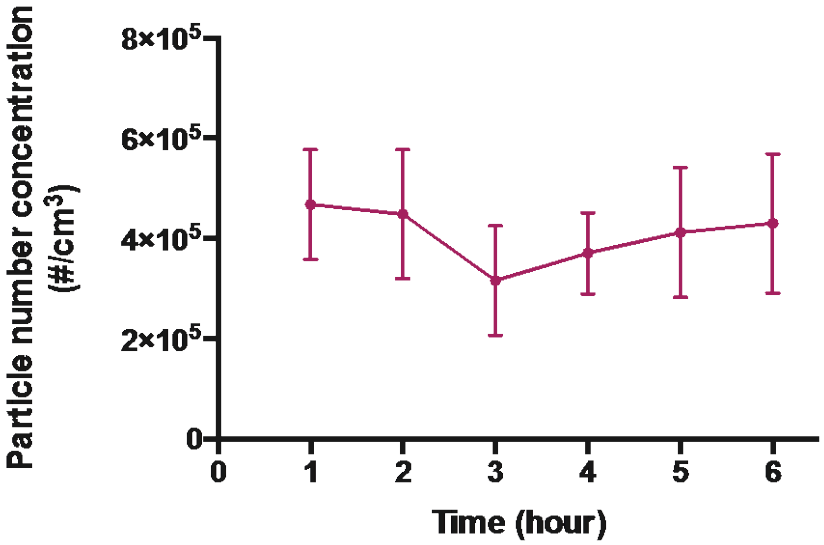
- Male *Balb/c* mice
- PEPs ($PM_{0.1}$) extracted from CCI
- Dose: 0.5, 2.5 and 5.0 mg/kg bw
- Samples obtained: blood, heart, liver, spleen, lungs, bronchoalveolar lavage
- Parameters examined: lung injury and inflammation, epigenetics, reactive oxygen species

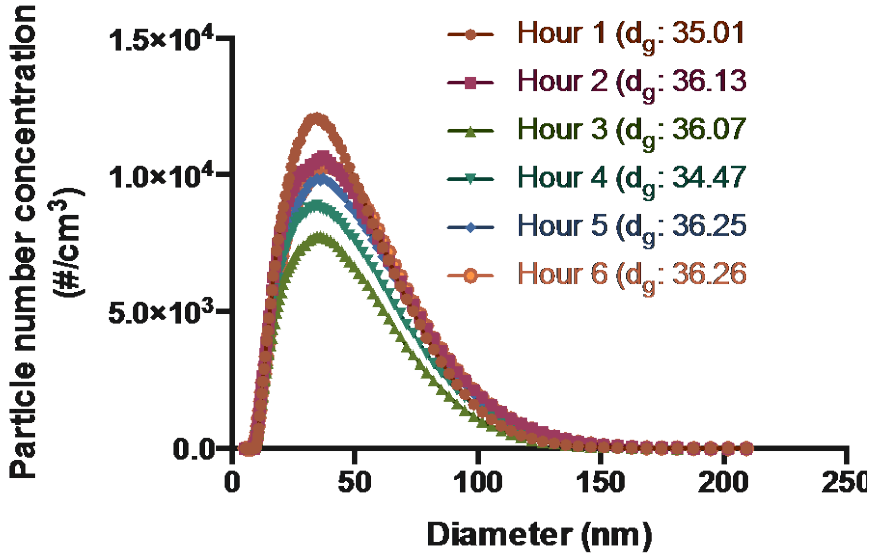
Whole-body inhalation: effect of VOCs and PEPs

- 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

Inhalation: snapshot of particle size and concentration during exposure

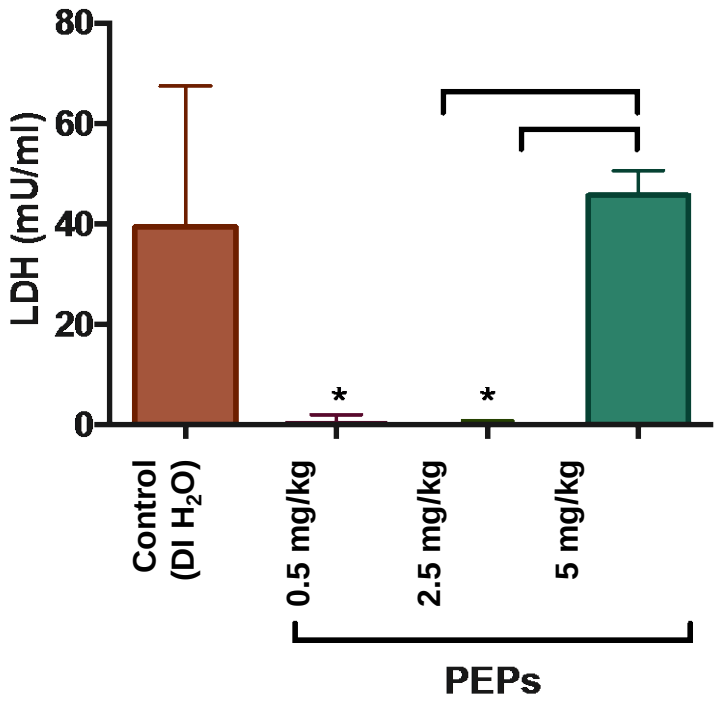
What are the effects of the PEPs + gaseous pollutants emitted by laser printers? Is there a synergistic effect?





- 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

Instillation: Evaluating lung injury and inflammation following PEPs exposure (1/2)

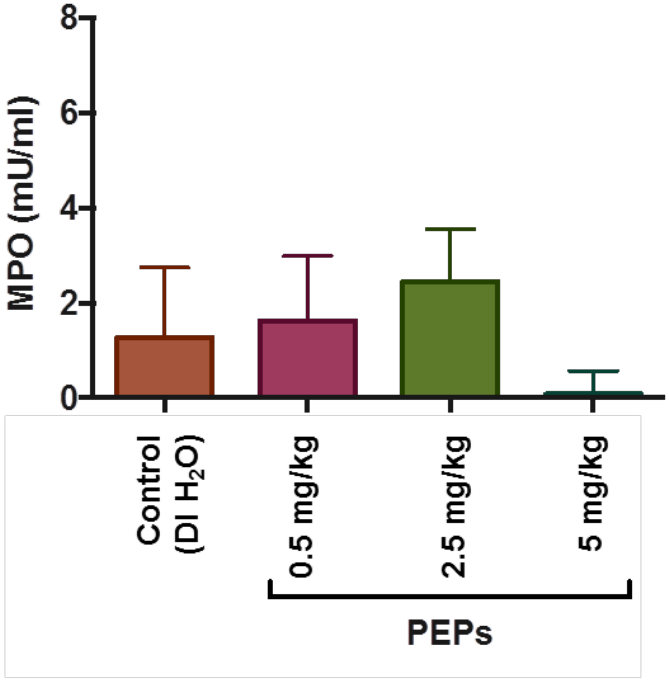


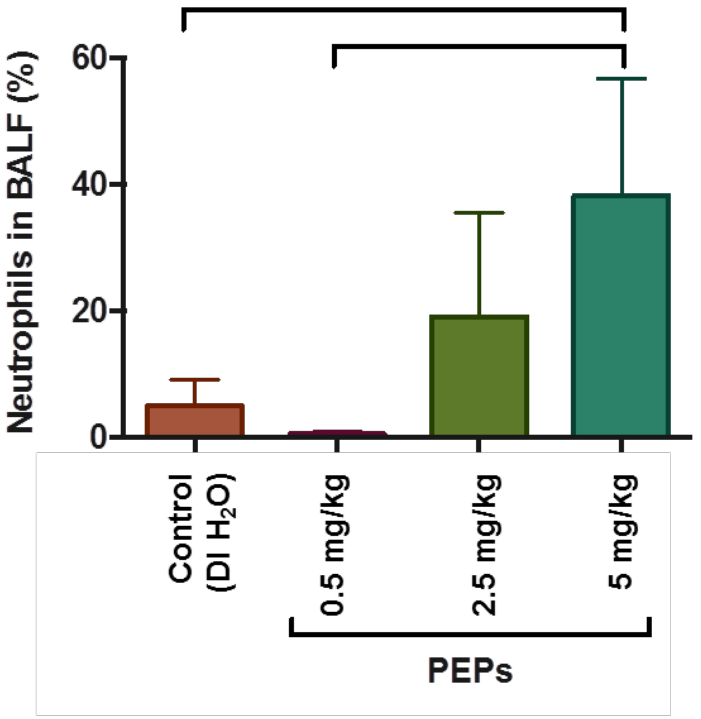
Dose comparisons (IT *vs.* inhalation)

- 0.5 mg/kg = 8.13 hours
- 2.5 mg/kg = 40.63 hours
- 5 mg/kg = 81.25 hours

- No change in lactate dehydrogenase (LDH) following instillation of PEPs
  - Agreement with results from epithelial cell cytotoxicity experiments

Instillation: evaluating lung injury and inflammation following PEPs exposure (2/2)

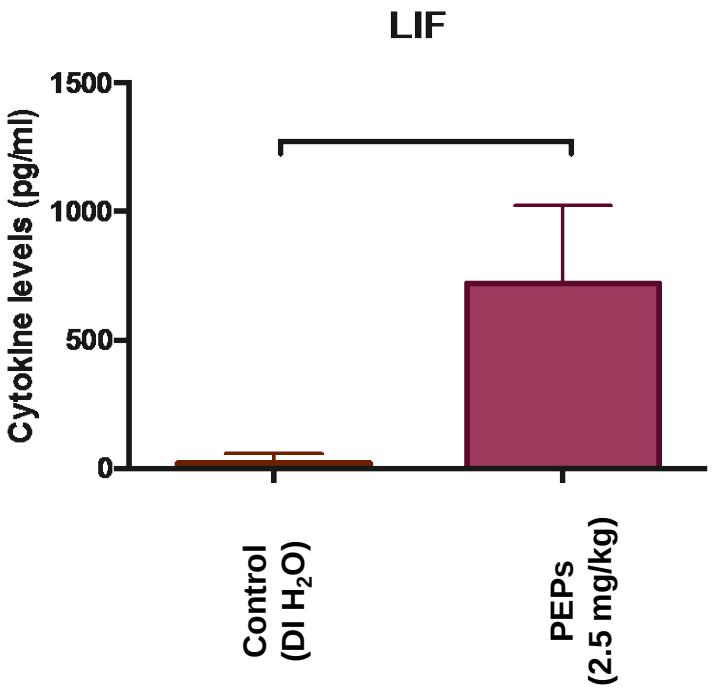




Exposure to PEPs led to:

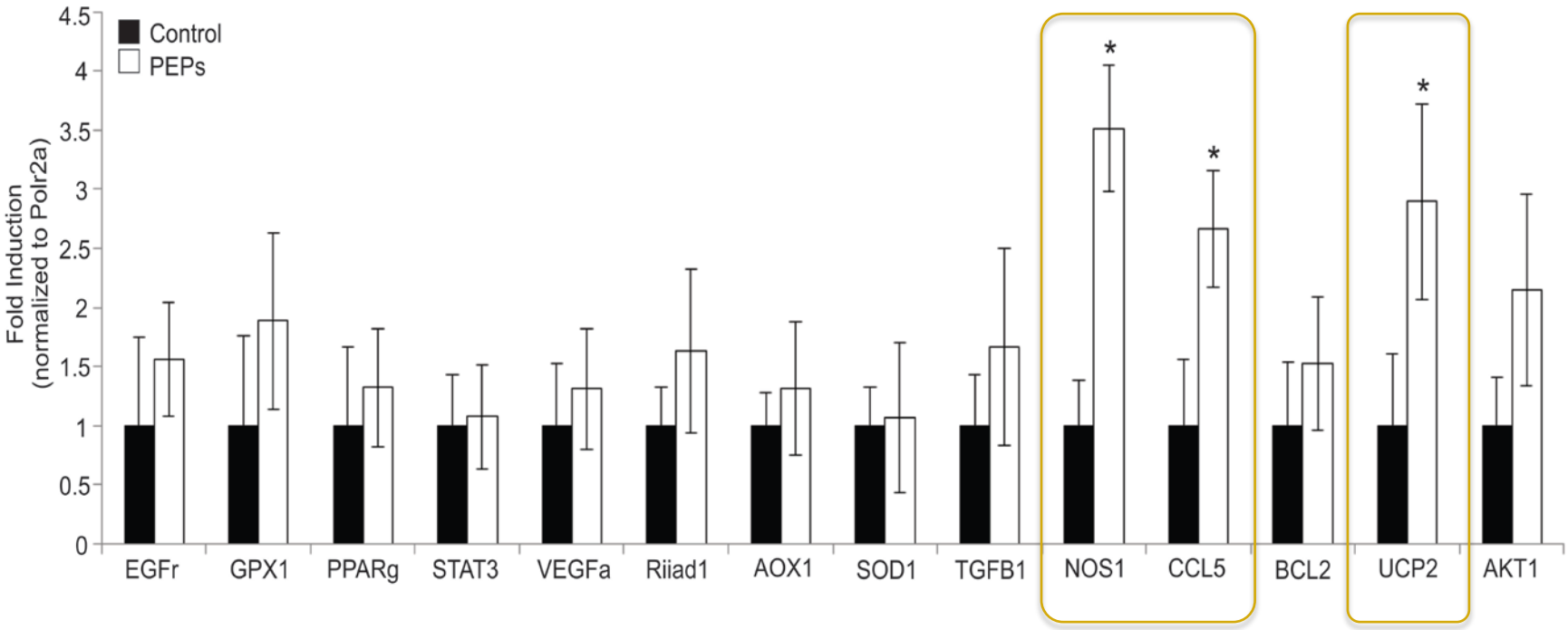
- No effect in neutrophil degranulation after instillation
- Significant elevation in percent of lavaged neutrophils at 5.0 mg/kg

Instillation: evaluating lung injury and inflammation following PEPs exposure (4/4)



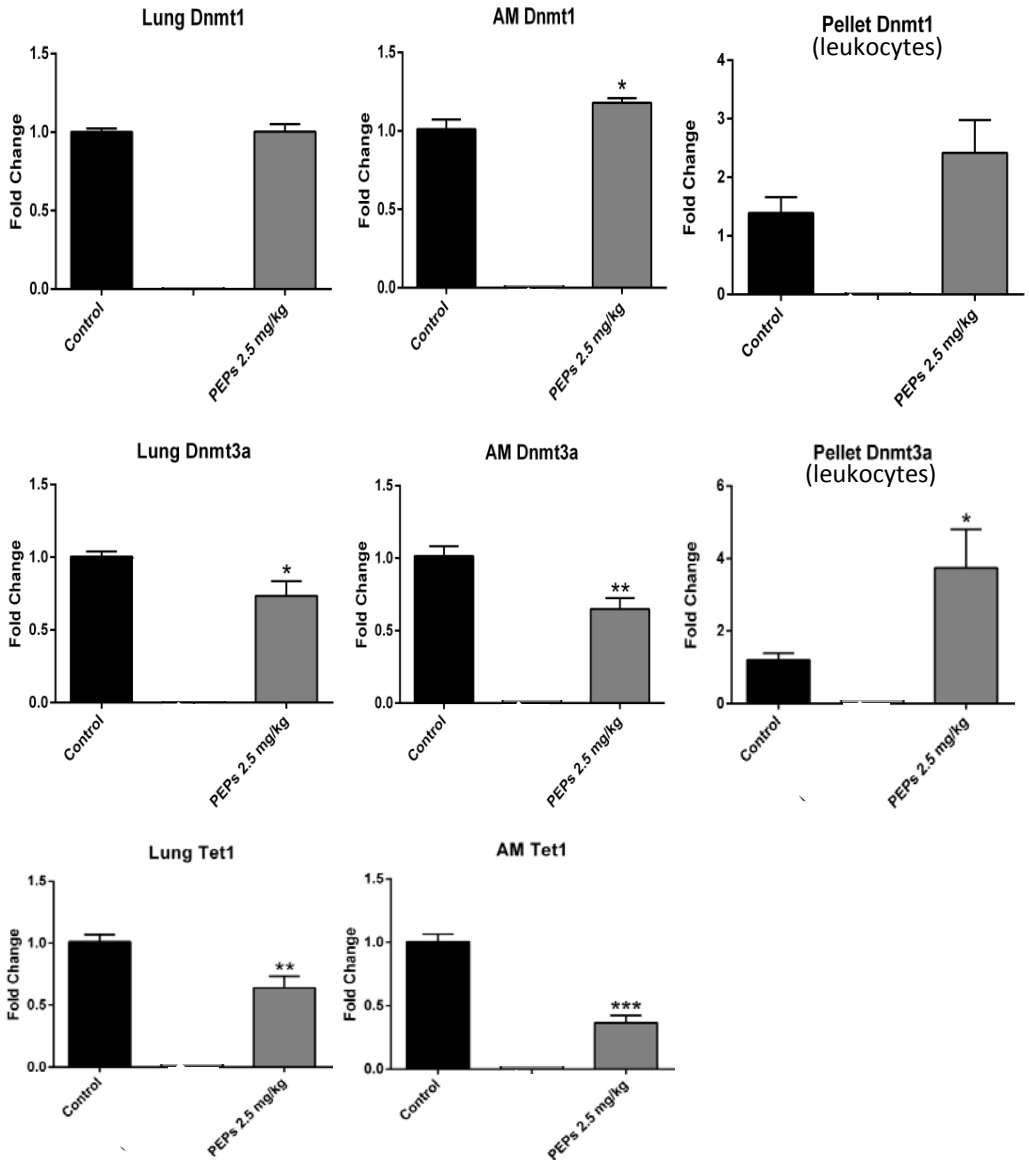
- Significantly increased levels of LIF post-PEPs exposure *vs*. control group
  - Involved in pulmonary response to inflammation (*e.g.*, repair processes, airway responsiveness)

Instillation: evaluation of gene expression following exposure to PEPs



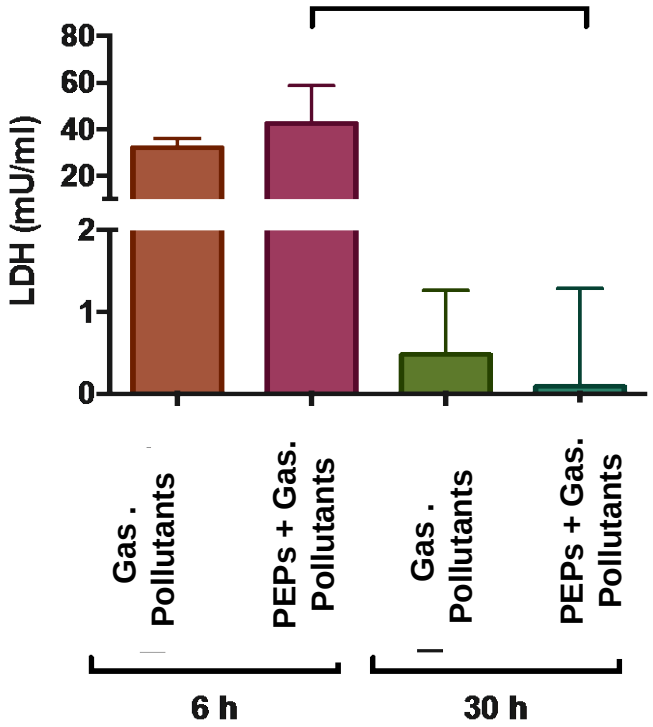
- Upregulated expression of genes due to exposure to PEPs (2.5 mg/kg)
  - Cell survival, inflammatory responses
- CCL5 (RANTES) also significantly elevated *in vitro* → consistency in results from both experimental platforms

Instillation: investigation of epigenetic modifications following exposure to PEPs



- 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)?

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/cm^3)
- In both *in vitro* and *in vivo* experimental conditions, PEPs had 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 hour s of exposure)

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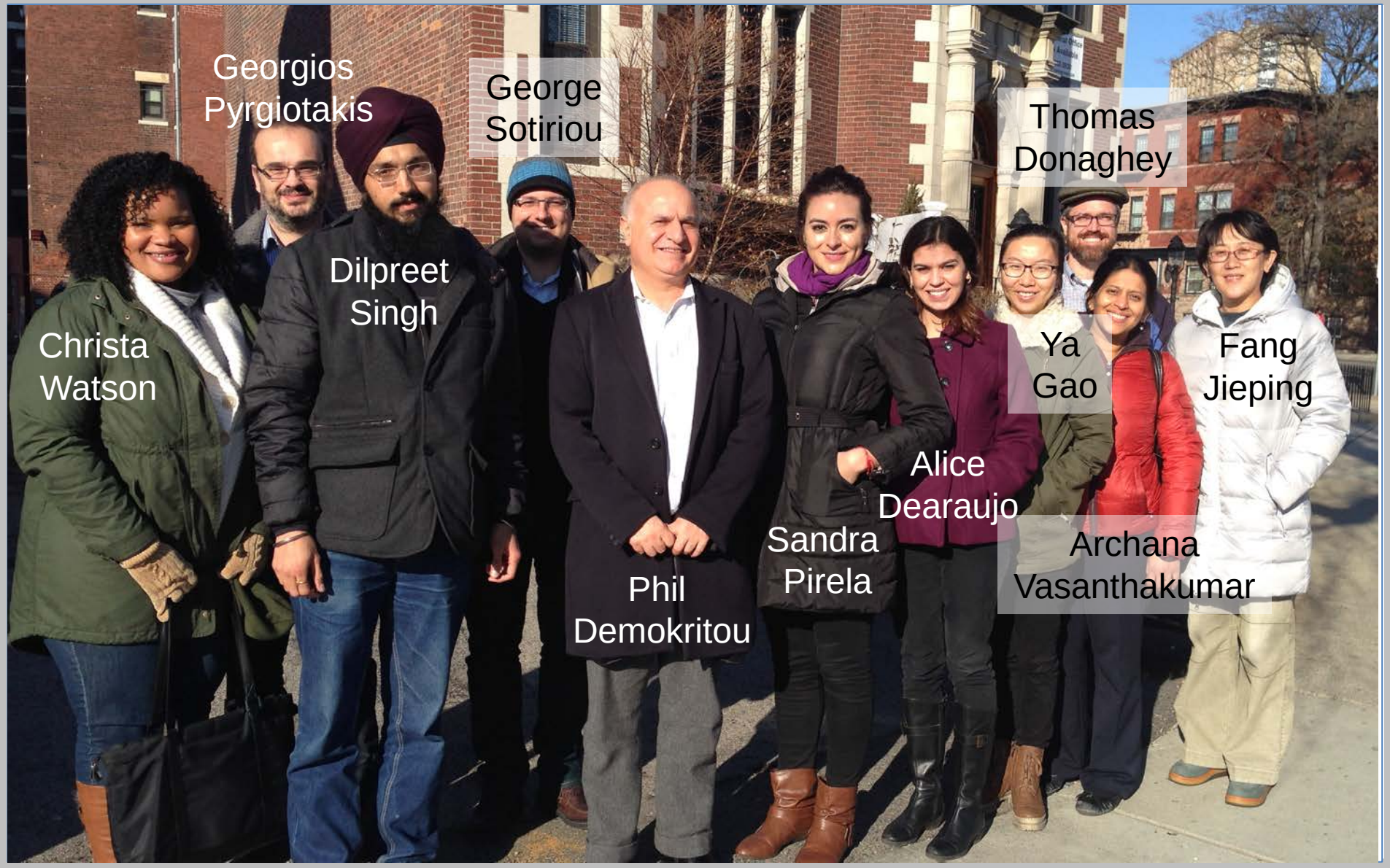
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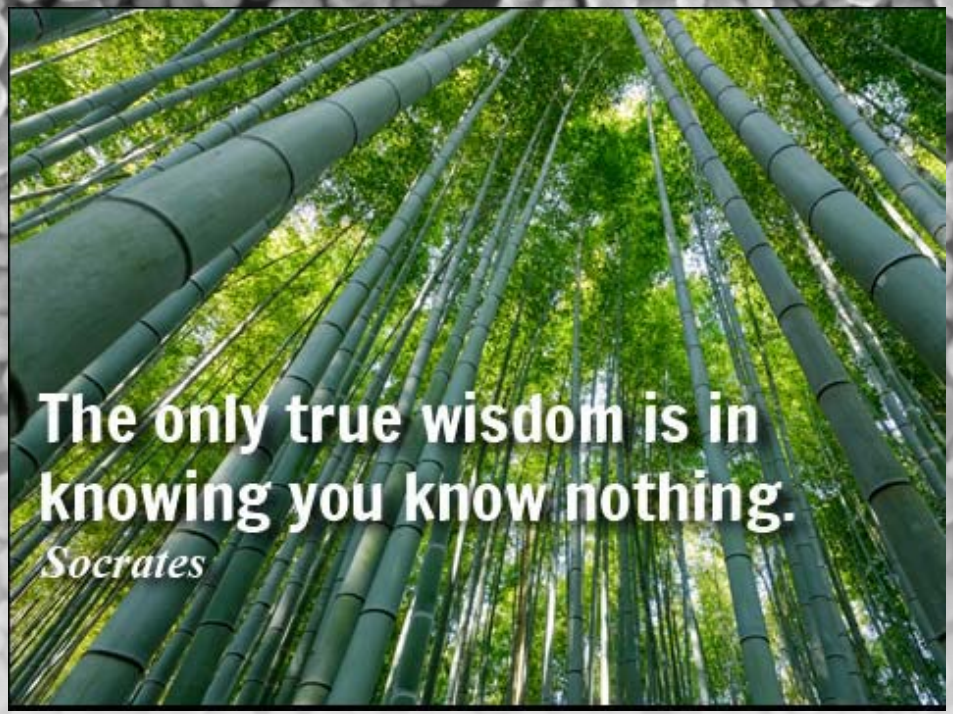

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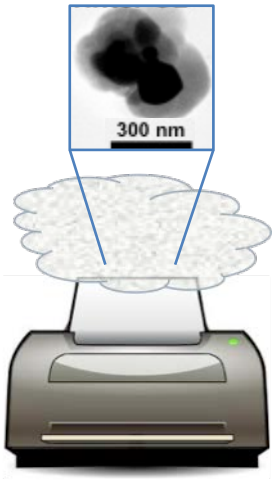
Dosimetric considerations for toxicological assessment – Dose table

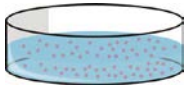


MPPD2 Model [1]

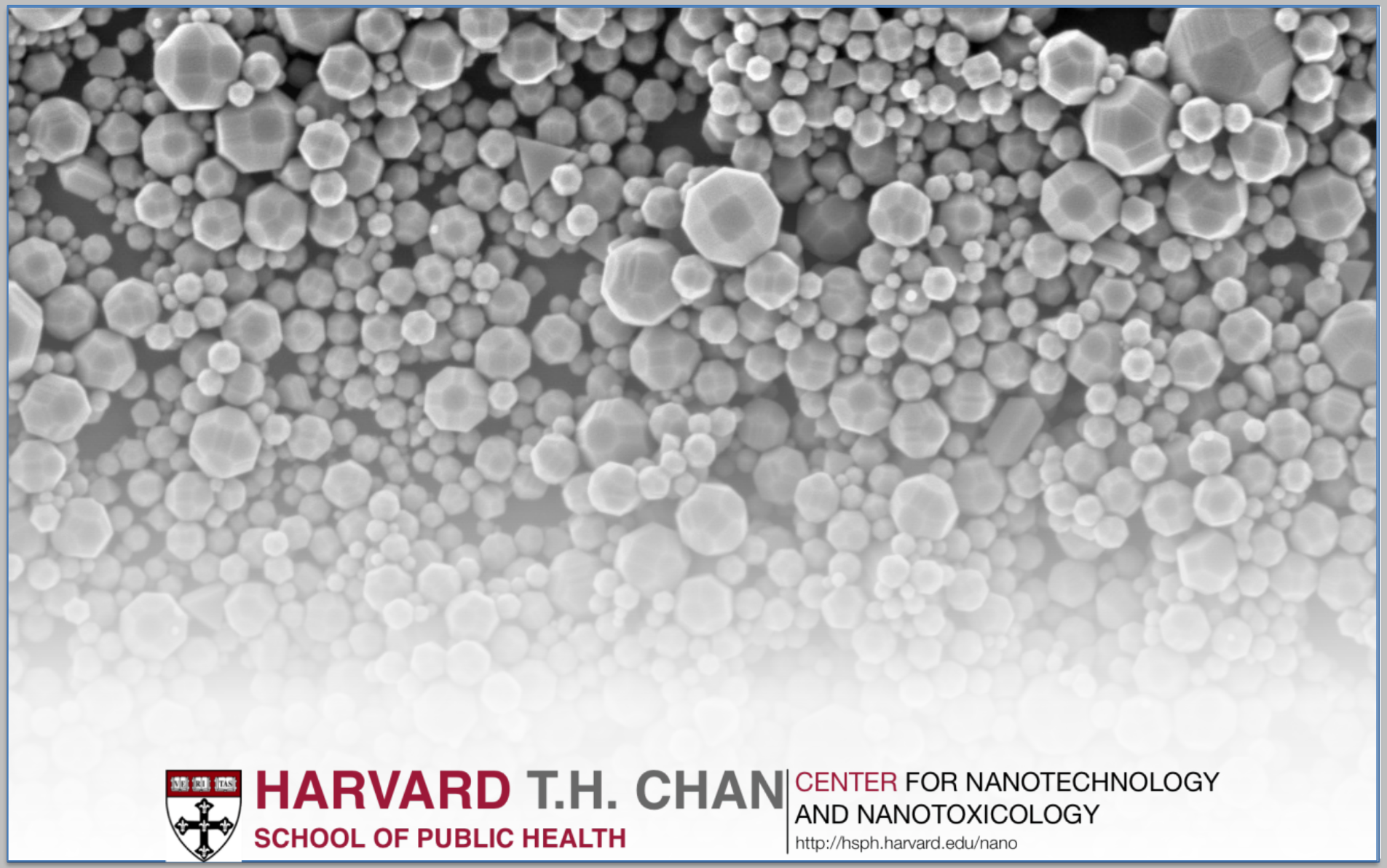
Deposited mass In the lung

VCM-ISDD Model [2-4]

Deposited mass *in vitro*



Real world exposure at consumer level	*in vivo*		*in vitro*		
Human Inhalation (hours)	Rodent Inhalation (hours)	Rodent Instillation (mg/kg)	*in vitro* administered dose (µg/ml)	*in vitro* delivered dose (µg/ml)	
				SAEC	THP-1
15	6.5	0.4	0.5	0.5	0.25
150	65	4	5	5	2.5
300	129	8	10	10	5
601	259	17	20	20	10
901	389	25	30	30	15
1202	518	33	40	40	20
3006	1295	83	100	100	50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