Exposure to engineered nanoparticles emitted from laser printers

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Session B | Exposure Scenario: Consumer Exposure (General Products)

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SCHOOL OF PUBLIC HEALTH

CENTER FOR NANOTECHNOLOGY
AND NANOTOXICOLOGY
http://hsph.harvard.edu/nano

Presentation Outline

- Background
  - Nanotechnology
  - Case study: laser printers
- Project design and Research objectives
- Results
  - Physicochemical, morphological and toxicological properties of laser printer-emitted particles (PEPs)
- Concluding remarks

Background: Knowledge gap

NANOTECHNOLOGY

- Superior physical, chemical and optical performance of nanoparticles in comparison to micron-sized components
- Thousands of nano-enabled products (NEPs) introduced to the market (textiles, paints, cosmetics, pharmaceutical, personal care products)

Exposure at the consumer level is inevitable

RESEARCH GAPS

- Risk assessment requires both exposure data as well as toxicological data
  - Exposure evidence is critical to understand adverse health effects from exposures across the life cycle of NEP
- No standardized methodology for the systematic investigation of real world exposures of particulate matter released across life cycle of NEPs (LCPM)
  - No link from LCPM exposure during consumer use or end-of-life to toxicology
  - Limited exposure data beyond manufacturing stage
  - Life cycle perspective toxicology

Case Study: Laser printer-emitted particles

Exposure studies

- Release both particulate matter (PM) and gaseous pollutants during their use

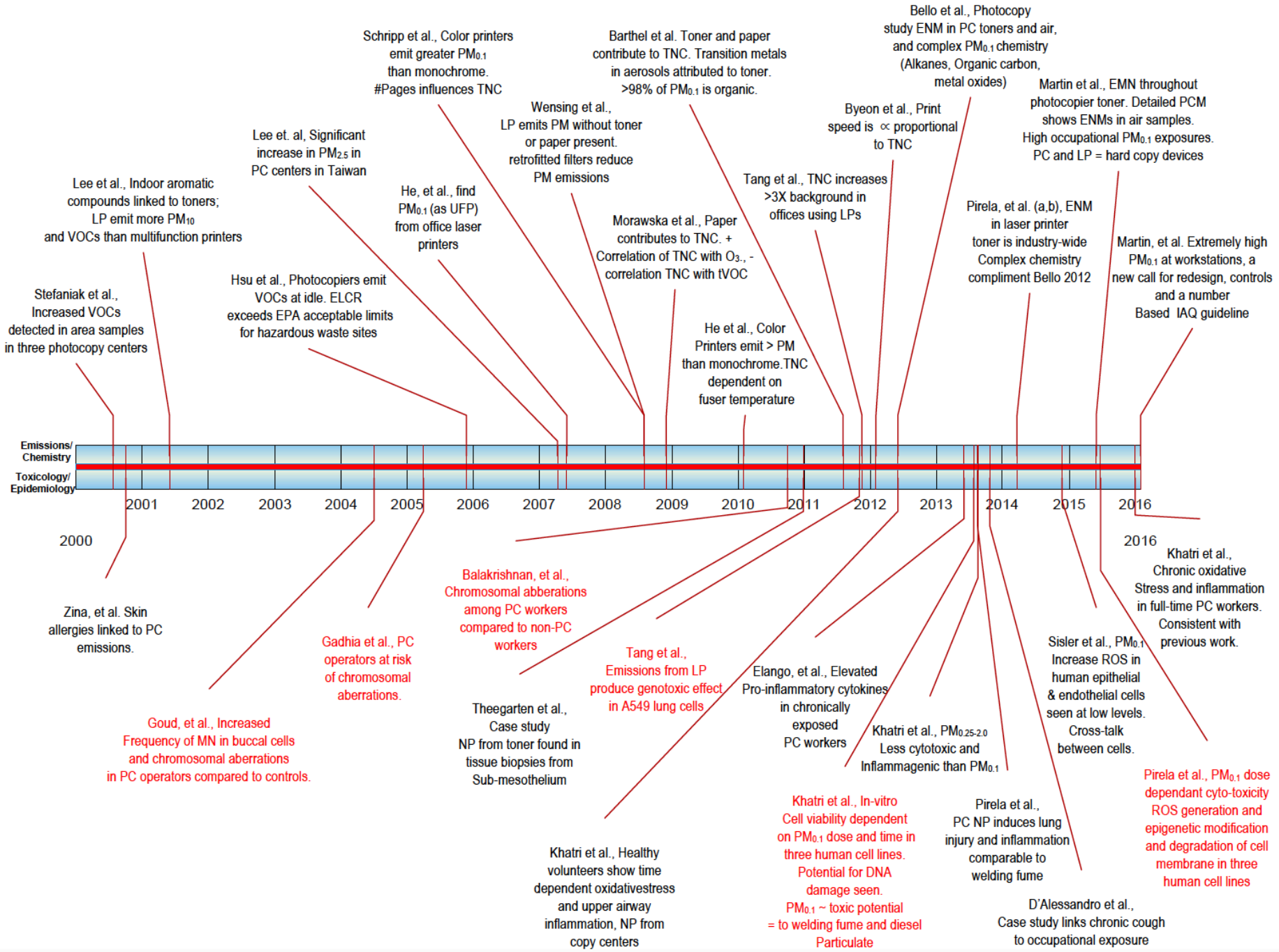
Has the laser-based printing industry incorporated ENMs in toners? If yes, are those ENMs released during printing? What are the properties (PCM) of the LCPM particle.

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

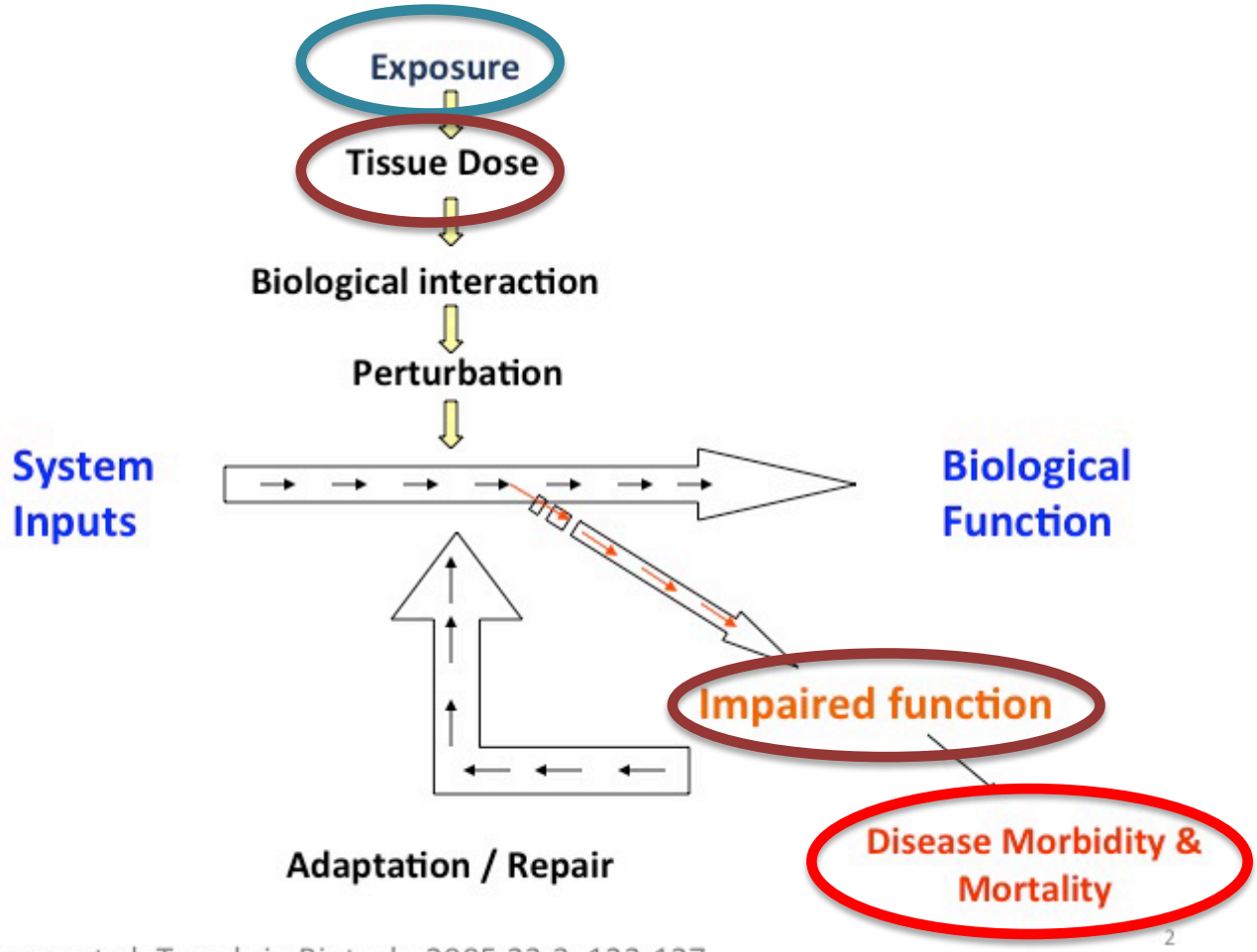
Not enough data for adequate science-based risk assessment of consumer exposure scenarios and no link between real word exposure to toxicology

Barthel *et al.*, 2011; He *et al.*, 2010; Morawska *et al.*, 2009; Lee *et al.*, 2009
Wensing *et al.*, 2008 | [3] Bai *et al.*, 2010



Pirela *et al.*, CRT 2017

Conceptual Framework

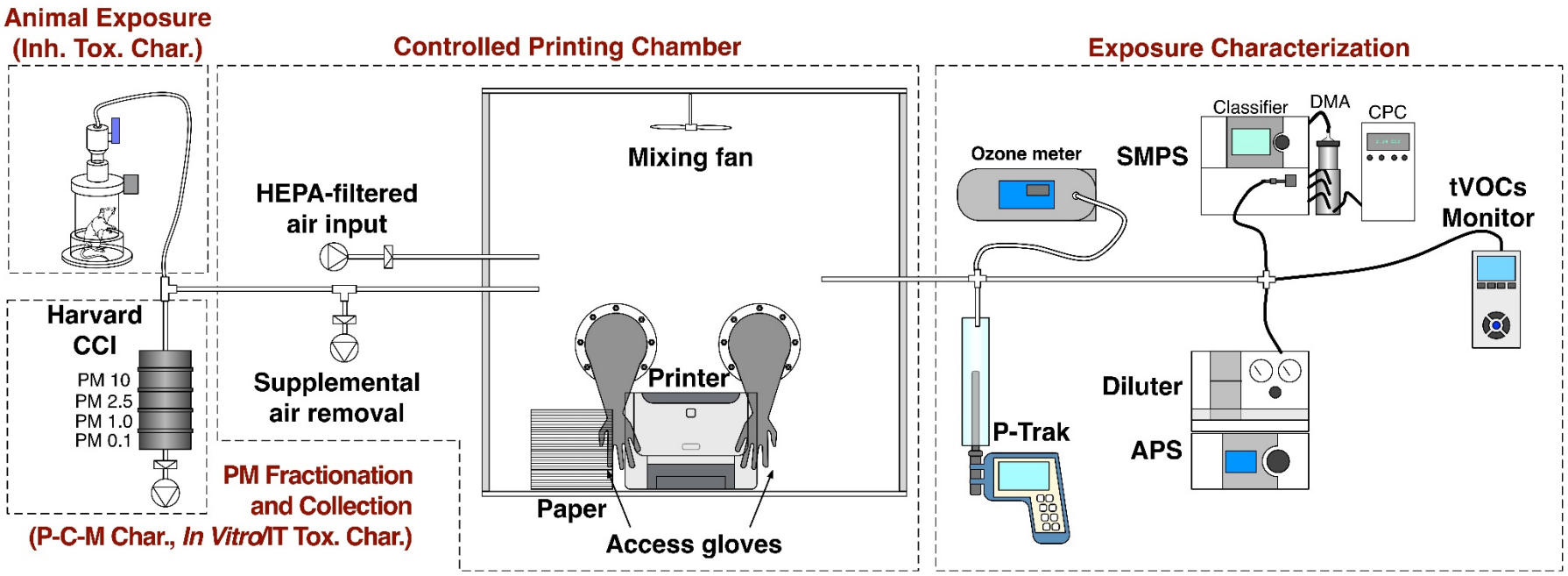


Andersen et al, Trends in Biotech, 2005,23,3, 122-127

Research Objectives

- Develop lab-based exposure platform to generate real-world PEPs
- Utilization of developed platform to evaluate PEPs and gaseous co-pollutants released by laser printers currently in the market
  - Is the toner a nano-enabled product (NEP)? Physico-chemical and morphological characterization of toner powders and PEPs
  - Are ENMs emitted during a print job? Assess emission profile of laser printers (*i.e.*, PM and gaseous co-pollutants)
  - Are there operational parameters that affect the emission profile of laser printers?
- Toxicological evaluation of PEPs
  - *In vitro*: mono- and co-culture systems
  - *In vivo*: whole-body inhalation and intratracheal instillation of PEPs

Development of Printer Exposure Generation System (PEGS)

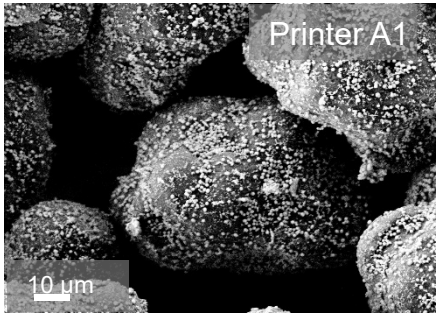


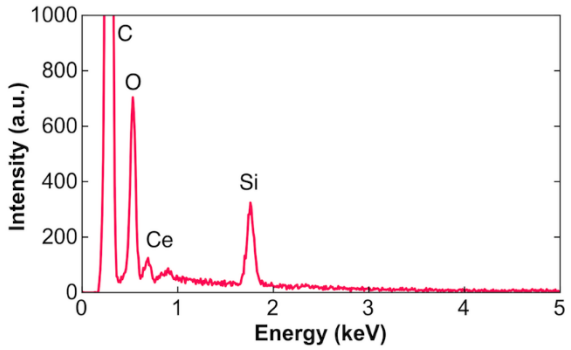
Features

- 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

Physicochemical and morphological assessment of toner powder and PEPs

Toner powder

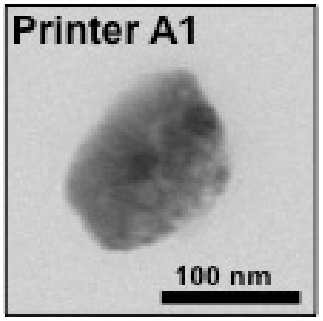


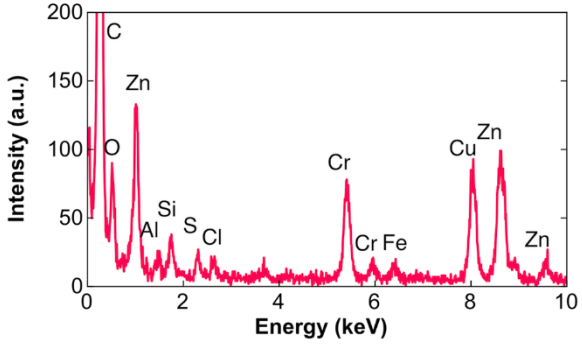


- Diameter 10-15 µm
- ENMs on the surface and embedded in the toner particle
- *EDX:* traces of carbon, oxygen, aluminum, silicon, cerium, iron, among others

Toner formulations are nano-enabled products

PEPs



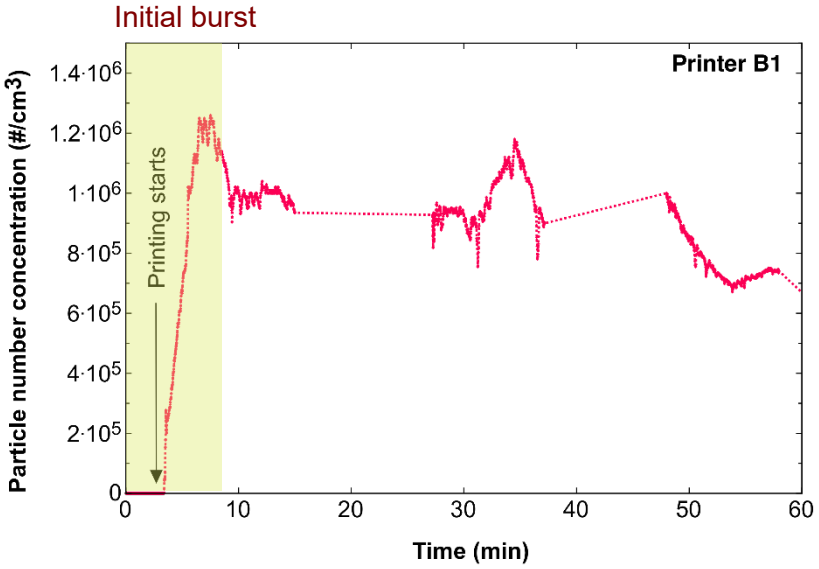


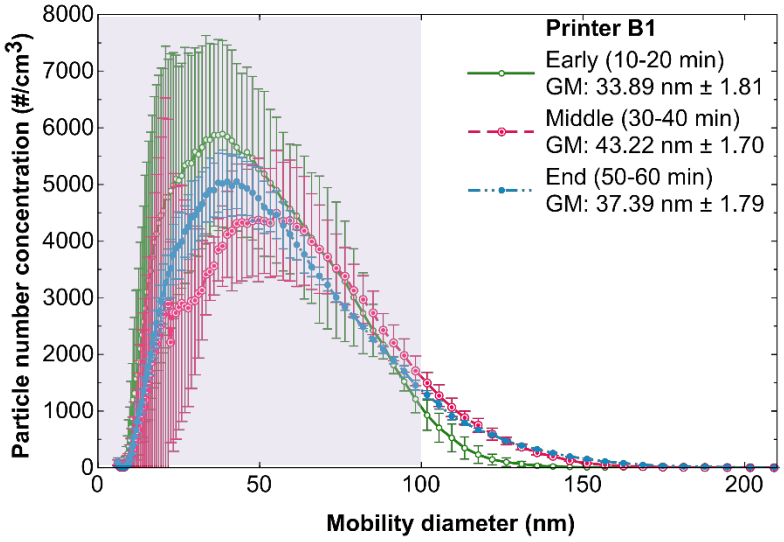
- Different aggregate shapes/sizes of ~ 20 – 200 nm
  - Consistent with RT monitoring data
- *EDX:* traces of carbon, oxygen, aluminum, silicon, zinc, iron, cerium, copper, tellerium, titanium, sulfur, among others

ENMs become airborne during consumer use of laser printer

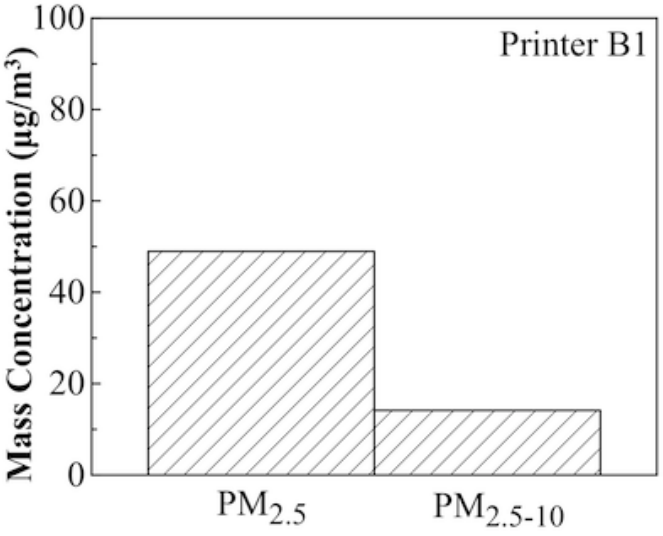
Pirela et al., Inhalation Toxicology, 2014
Pirela et al., Nanotoxicology, 2014

Assessment of laser printer emission profiles: Size distribution and number concentration of PEPs

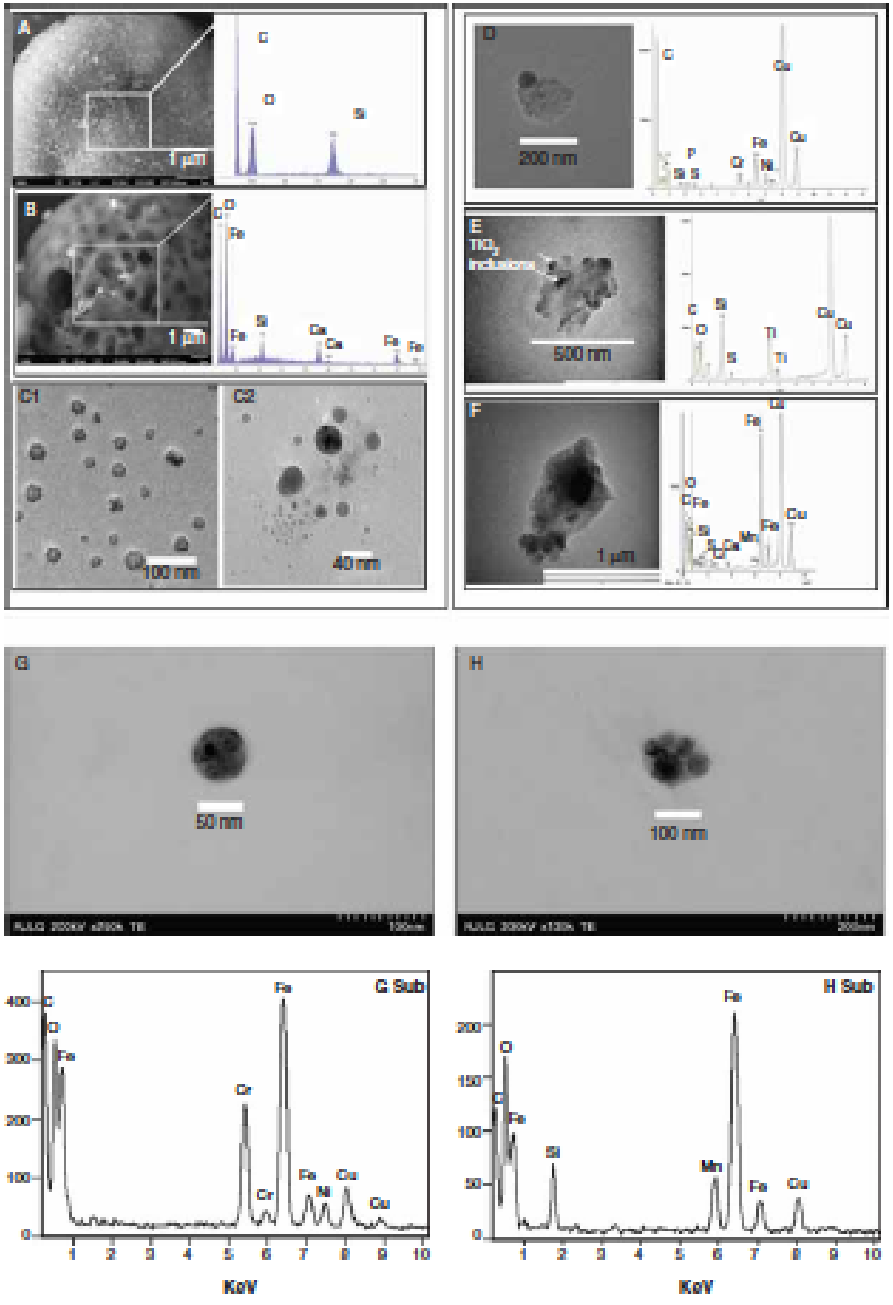


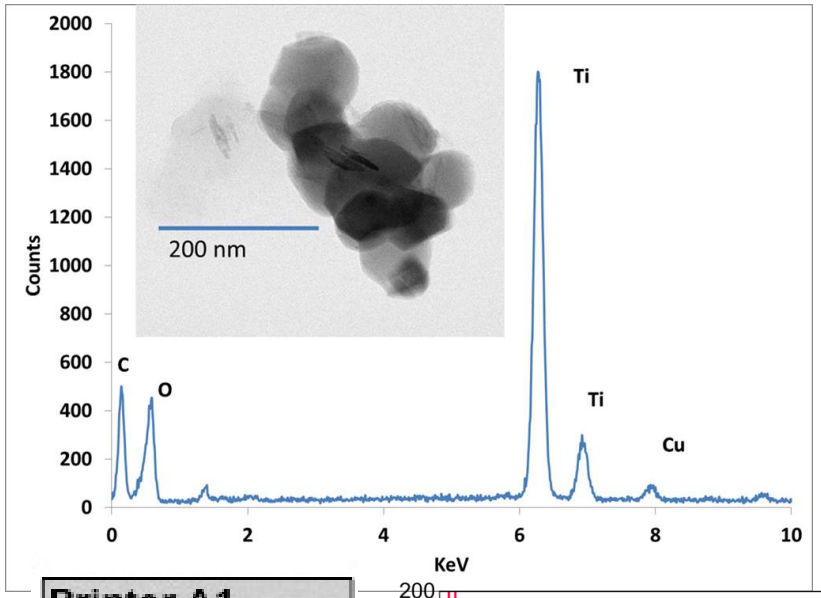


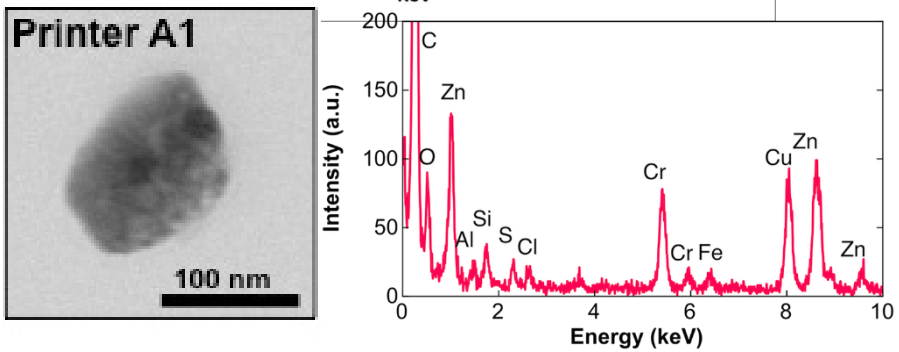
- Emission profiles of 11 laser printers (4 manufacturers)
  - No association between emission profile and brand/model
  - Peak emissions: 2,990 - 1.27 million particles/cm^3
  - Initial burst within 10-12 min
  - Mean diameters: 39 - 122 nm, majority < 100 nm
  - Mass concentrations of up to 100 µg/m^3
- Emission profiles identified for printers → rank them based on maximum particle released



ENM in the breathing zone



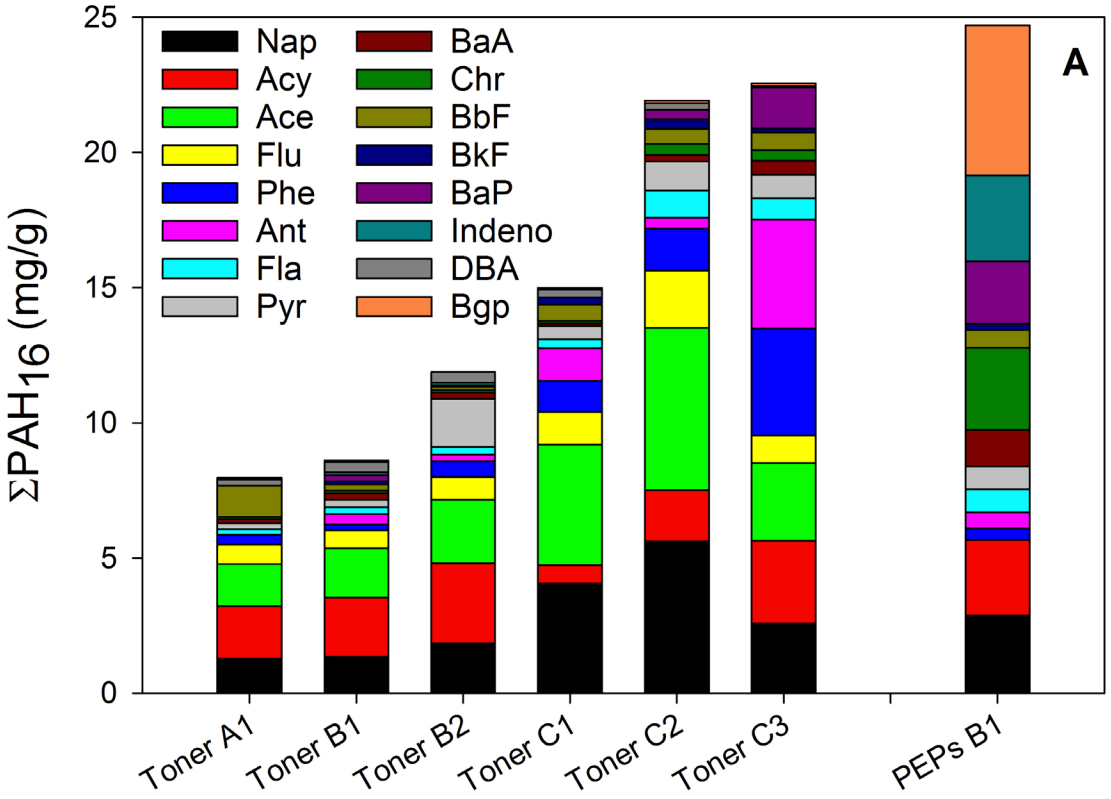




Fe, Mn, Cu, Si, Cr, Ti, Al, C, Zn, Fe, Ce, Te, S, Ni, & others

Pollen et al Nature Nanotechnology 2013; Martin et la 2015, J Hazardous Materials; Pirela et al 2014/2015

Chemical speciation of PAHs in toner powder and PEPs



PEPs (B1)

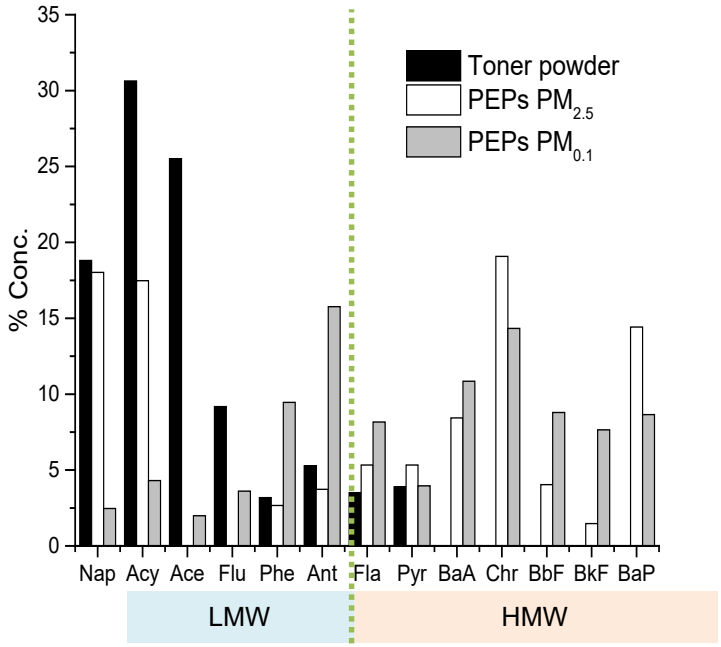
- Concentration of PAHs: 24.71 ng/mg
- Major contribution to the PAHS were high molecular weights

Toner powders (A1, B1, B2, C1, C2, C3)

- Concentration of PAHs: 22.5, 21.93, 14.99, 11.88, 8.62 and 7.97 ng/mg.
- Relatively high fraction of low molecular weight PAH compounds that made up 73-85% of the sample
- 1.86 fold increase of PAHs concentration in PEPs compared to toner (B1)

Chemical speciation of PAHs in toner powder and PEPs

Relative distribution of PAHs



❖Relative distribution of PAHs changes from low to high molecular weight PAHs from toner to high molecular weight in PEPs

❖PEPs $PM_{0.1}$ appears to have a higher concentration of high molecular weight PAHs than PEPs $PM_{2.5}$

❖Higher PEF associated with high molecular weight PAHs found mainly in the PEPs rather than the toner → toxicological implications?

Mean PAHs and BaP-equivalent concentrations estimated using cancer potency-equivalent factor (PEF)

	Compound	PEF	Concentration (ng/g) Toner powder	PEPs $PM_{0.1}$	PEPs $PM_{2.5}$
LMW	Naphthalene	0.001	1.3	2.9	1.7
	Acenaphthylene	0.001	2.2	2.8	2.9
	Acenaphthene	0.001	1.8	0.0	1.3
	Fluorene	0.001	0.7	0.0	2.4
	Phenanthrene	0.001	0.2	0.4	6.3
	Anthracene	0.001	0.4	0.6	10.6
	Fluoranthene	0.001	0.3	0.9	5.5
	Pyrene	0.001	0.3	0.9	2.6
HMW	Benzo[a]anthracene	0.1	0.0	1.3	7.3
	Chrysene	0.01	0.0	3.0	9.6
	Benzo[b/j]Fluoranthene	0.1	0.0	0.6	5.9
	Benzo[k]Fluoranthene	0.1	0.0	0.2	5.1
	Benzo[a]pyrene	1	0.0	2.3	5.8
	Total PAHs conc.		7.2	16.0	67.0
	Total PEF-equivalent conc.		0.0	2.6	7.8
	% PEF-equivalent/total conc.		0%	16%	12%

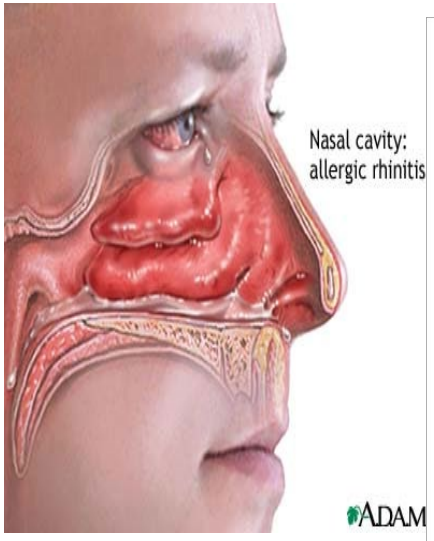
Substantial Deposition and Retention in the Lungs

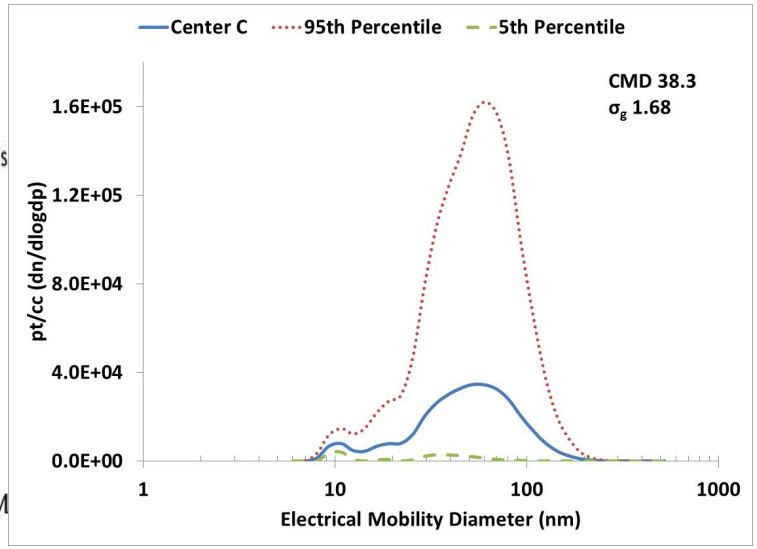
MPPD2 Lung Deposition Model

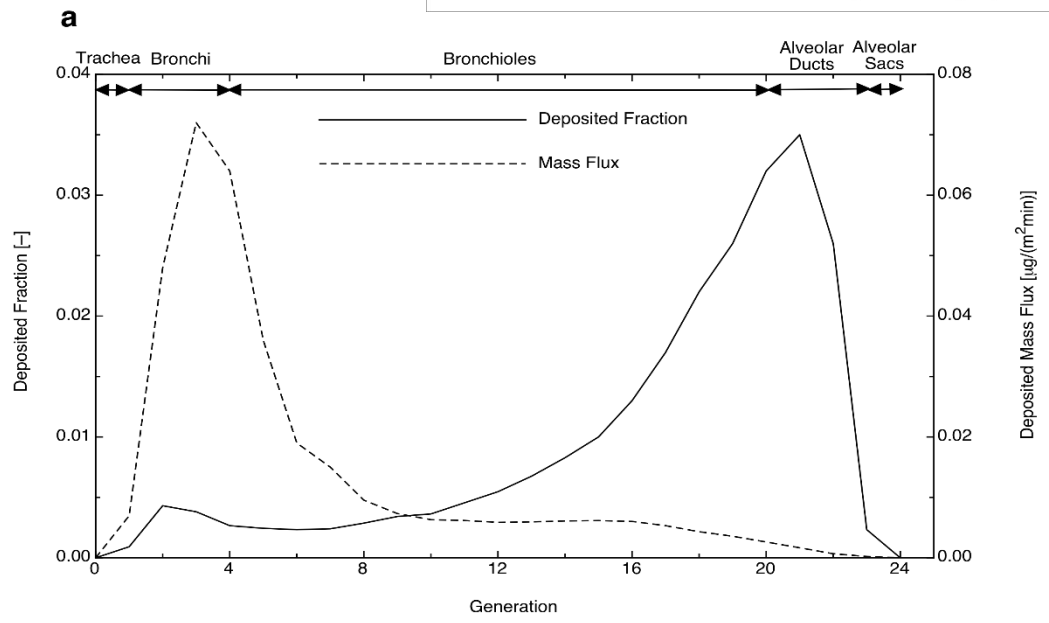
Center ID	CMD (nm)	σ_g	MMD (nm)	$PM_{0.1}$ Mass Conc.[b] $\mu g/m^3$	% Deposition (number)			
					Total[a]	Head	Thoracic	Alveolar
1	35.1	1.9	123.9	4.5	33.7	5.7	11.1	17.0
2	23.1	2.1	113.2	2.2	35.9	6.2	12.1	17.9
3	28.0	2.01	121.1	1.9	32.1	6.1	8.6	17.4
4	38.3	1.7	86.48	2.2	39.8	6.4	13.2	20.2
5	32.4	2.04	148.7	3.6	29.4	5.9	7.7	15.7
6	36.2	2.07	177.7	4.6	28.2	6.7	7.1	14.5
7	28.2	1.96	109.5	1.8	33.4	6.1	9.0	18.2
8	34.9	1.75	89.7	6.4	36.4	6.4	9.9	20.1

Human Model	Breathing Parameters
Functional Residual Capacity: 3300.0 mL	*Tidal Volume*: 625 ml
Head Volume: 50 mL	*Breathing Frequency:* 12 breaths/ min
Breathing Route: Nasal	*Inspiratory Fraction*: 0.5
	Pause Fraction: 0.0

High Dose and Dose Rate in the Nasal Cavities







Mass Flux 0.072 μg/(m^{2}min)
Exposure time of 480 min (8 hr)

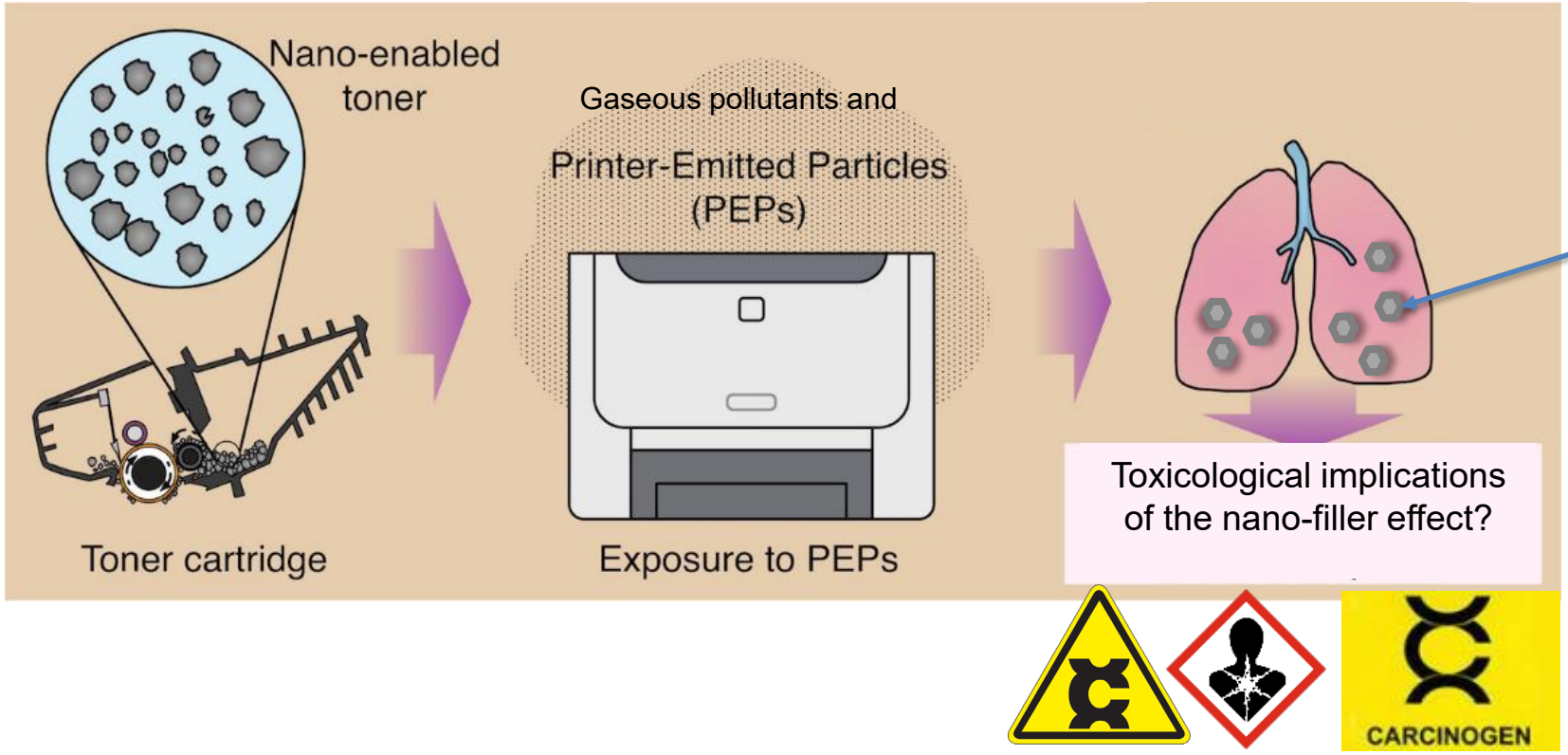
Estimated lung surface dose of 34.6 μg/m^2

Nasal Cavity: 150 cm^2
Deep Lungs: 120 m^2

Lungs/Nasal SA Ratio = ~8000
Deposited Fraction ~5x

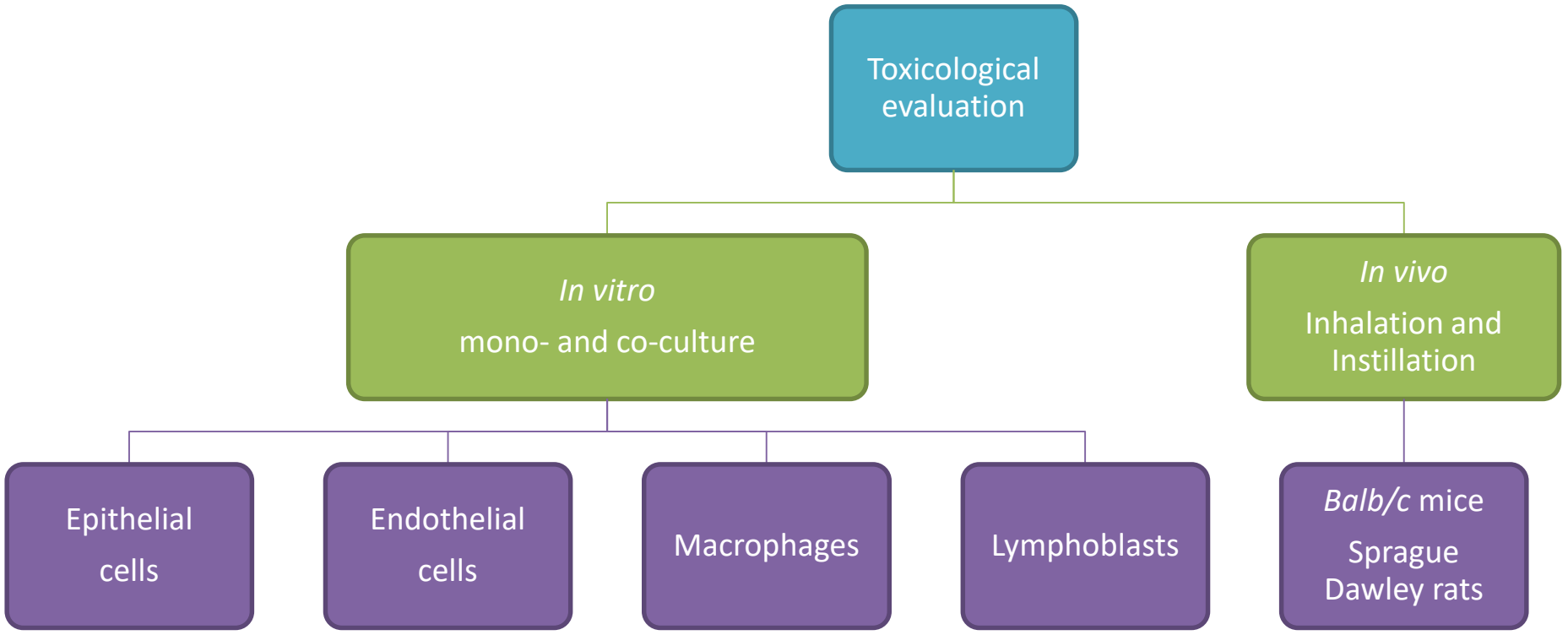
Nose/Alveolar Dose (cm^{-2}) ~ 2,500x

Chemical speciation of tVOCs present in toners and PEPs



Chalbot et al., Environmental Science: Nano, 2017

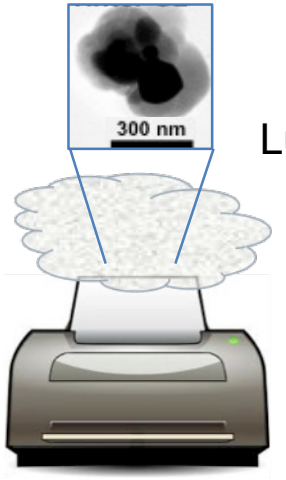
Toxicological assessment of PEPs – Study design



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

[1] Pirela et al., EHP 2015 | [2] Lu et al., Nanotoxicology, 2015 | [3] Sisler et al., Nanotoxicology, 2014

Dosimetric considerations for toxicological assessment

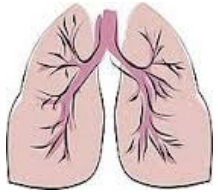


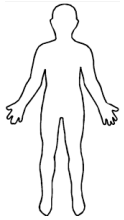
Source of emissions

Lung deposition model [1]

Estimating lung deposited mass of inhaled particles

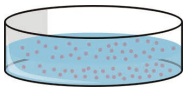
Deposited mass In the lung





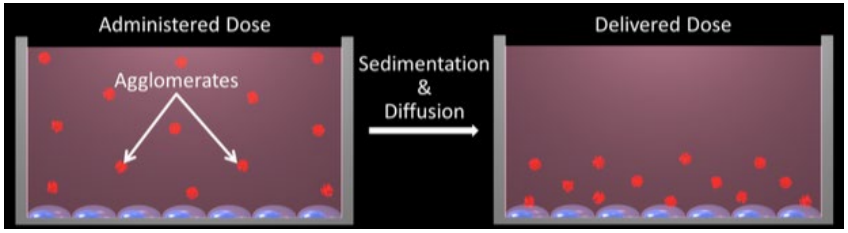
Breathing parameters + Airborne PEPs properties

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

Summary of results from *in vitro* toxicological assessment

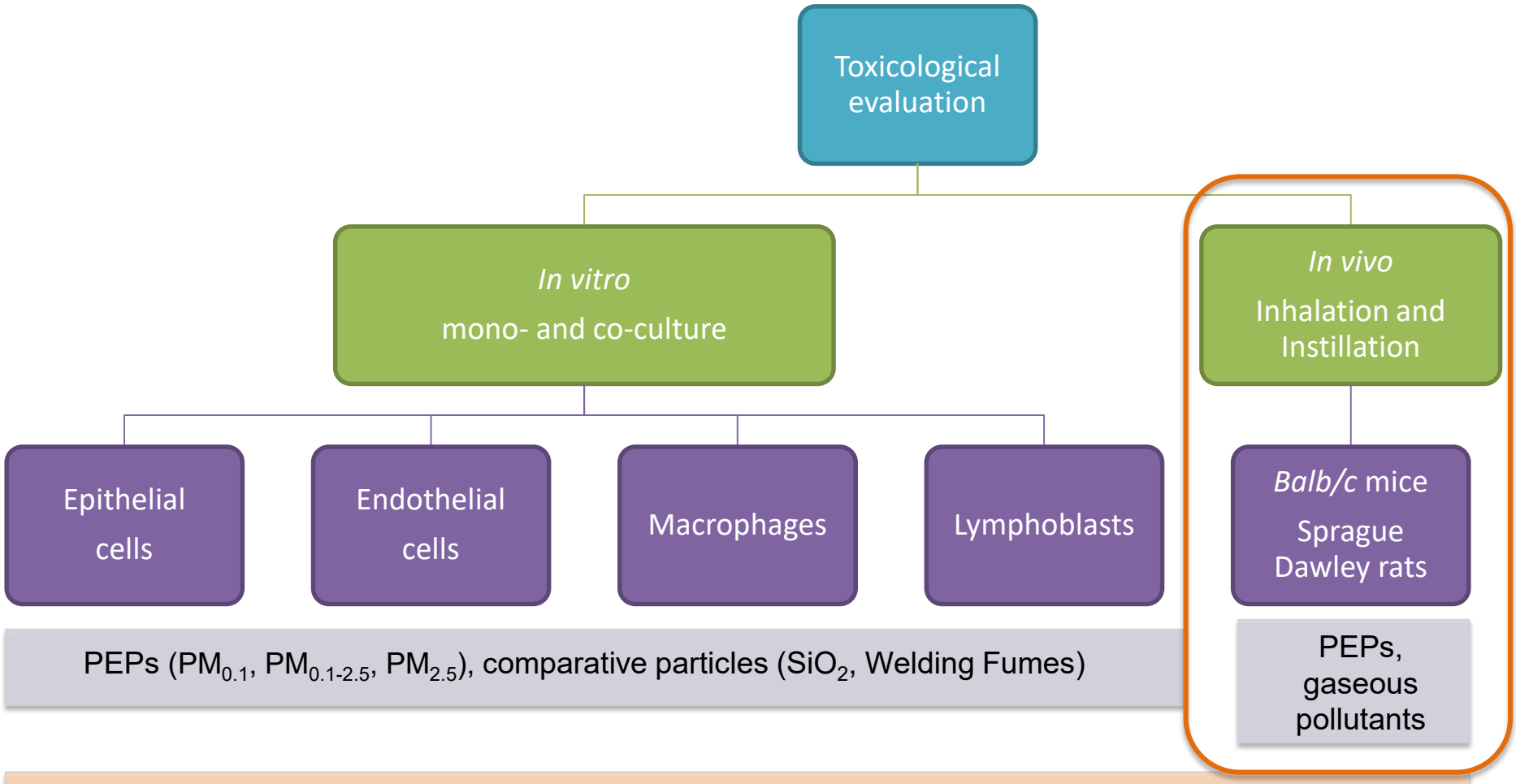
Mono-culture system

- PEPs led to significant cell death in epithelial cells (at highest delivered mass) and in macrophages in a dose-dependent pattern
- PEPs led to a dose dependent increase in ROS production in epithelial cells and in macrophages
- 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 in DNA methyltransferases (DNMTs) and TET in a dose-response pattern
  - Possible change in methylation patterns affecting overall gene expression

Co-culture system

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

Toxicological assessment of PEPs – Study design



[1] Pirela et al., EHP 2015 | [2] Lu et al., Nanotoxicology, 2015 | [3] Sisler et al., Nanotoxicology, 2014

In vivo toxicological assessment: Instillation exposure

Experimental Design

- Animals: male Balb/c mice
- Exposure by intratracheal instillation
  - $PM_{0.1}$ (sampled/extracted from CCI)
  - Control group: DI H_2O
- Doses: 0.5, 2.5 and 5.0 mg/kg bw
- Assessment done 24-hrs post exposure
- Samples collected: blood, heart, liver, spleen, lungs, bronchoalveolar lavage
- Parameters examined: lung injury and inflammation, epigenetics, oxidative damage

Summary of results from *in vivo* toxicological assessment

Intratracheal instillation

- No effect observed on pulmonary membrane integrity and neutrophil degranulation.
- Significant differences in white blood cell population (neutrophils, macrophages and lymphocytes) after PEPs exposure (5 mg/kg).
- Expression of a number of genes (*Nos1*, *Ccl5* and *Ucp2*) involved in inflammatory and oxidative damage responses was elevated after PEPs exposure.
- Leukemia inhibitory factor (LIF) was considerably upregulated by exposure to PEPs.
- Significant loss of DNA methyltransferase Dnmt3a and an elevated expression of TE LINE-1 observed in the whole lung tissue of mice instilled with PEPs.

Inhalation study design (1/2)

PEPs exposure

Study repeated 2016 and 2017

9 weeks old Sprague-Dawley rats

HEPA exposure (Control)

Exposed for 5 hours a day

Exposure Days	1	5	9	13	17	21

Animals Sacrificed

Real-time exposure measurement

Multiple pathway particle dosimetry modelling

Multivariate statistical analysis

Nasal lavage fluid collection

- LDH release
- Peroxidase activity
- GSH levels
- Multiplex cytokine and chemokine analysis

Bronchoalveolar lavage fluid collection

- LDH release
- Peroxidase activity
- GSH levels
- Albumin levels
- Hemoglobin levels
- Total and differential WBC analysis
- Multiplex cytokine and chemokine analysis

Pulmonary and cardiac tissues analysis

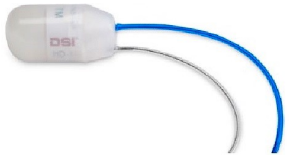
- Histopathological analysis
- In situ chemiluminescence based oxidative stress analysis

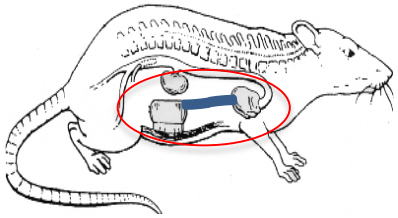
Blood serum collection

- Oxidative stress and inflammation markers.
- Metabolomics analysis.

Determination of dosimetry: NOAEL and LOAEL

Inhalation study design (2/3)





Animal assignment:
HR and Contractility

PEPs (n= 4)
HEPA filtered air (n= 4)

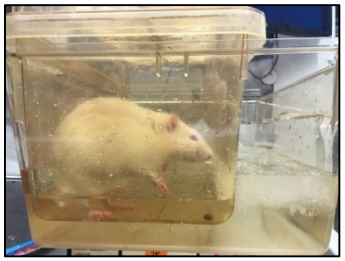
Baseline				Exposure (21d)										Post-Exposure Days (*: cold-water stress)					Sac
1	2	3	4	1	2 - 4	5	6 -8	9	10-12	13	14 - 16	17	18 - 20	21	23*	50 , 57	58*	65 , 86	91*	93

HEPA filtered air: All

5 hrs exposure to PEPs and HEPA filtered air (Control)

1h Monitoring

- 20 min pre
- 20 min Stress
- 20 min post

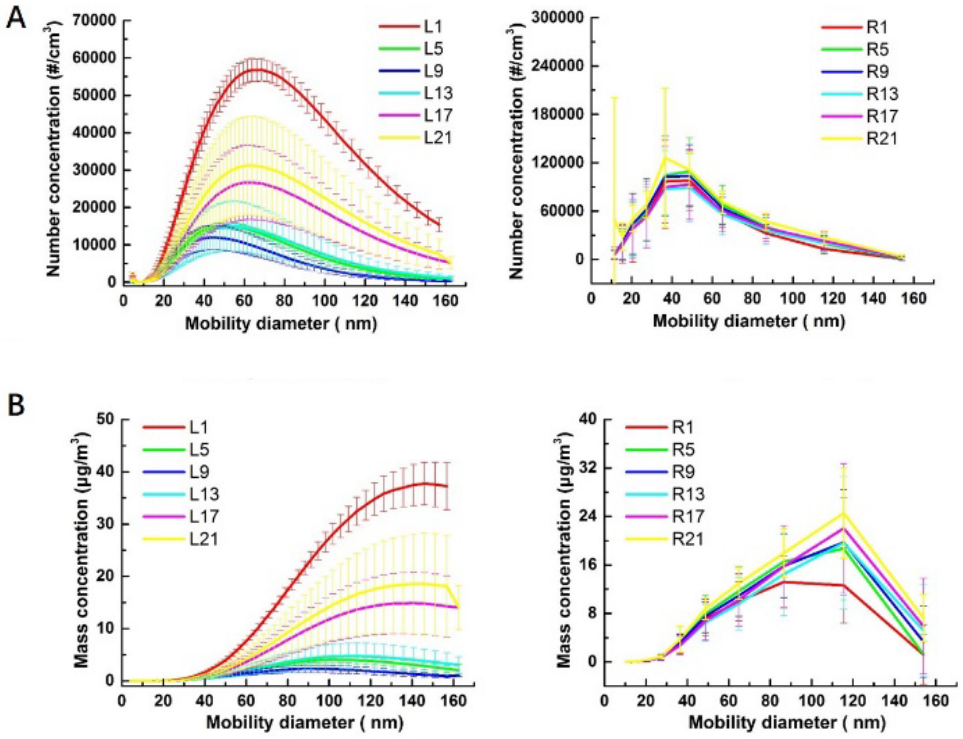


Effects of PEPs on Cardiac & Autonomic Responses to Stress

Detection of stress related metabolites in urine

Real-time exposure measurement

2016 2017



- Real-time mean particle diameter: ~45 nm
- Total particle number concentration: ~4-5 x10^5 #/cm^3
- Highest mean particle diameter: 67.62 nm
- Particle mass 737.90 µg/m^3
- Variation between exposure days was detected in the 2016 study
  - This was due to use of different printers, wear and tear.

Real-time exposure analysis

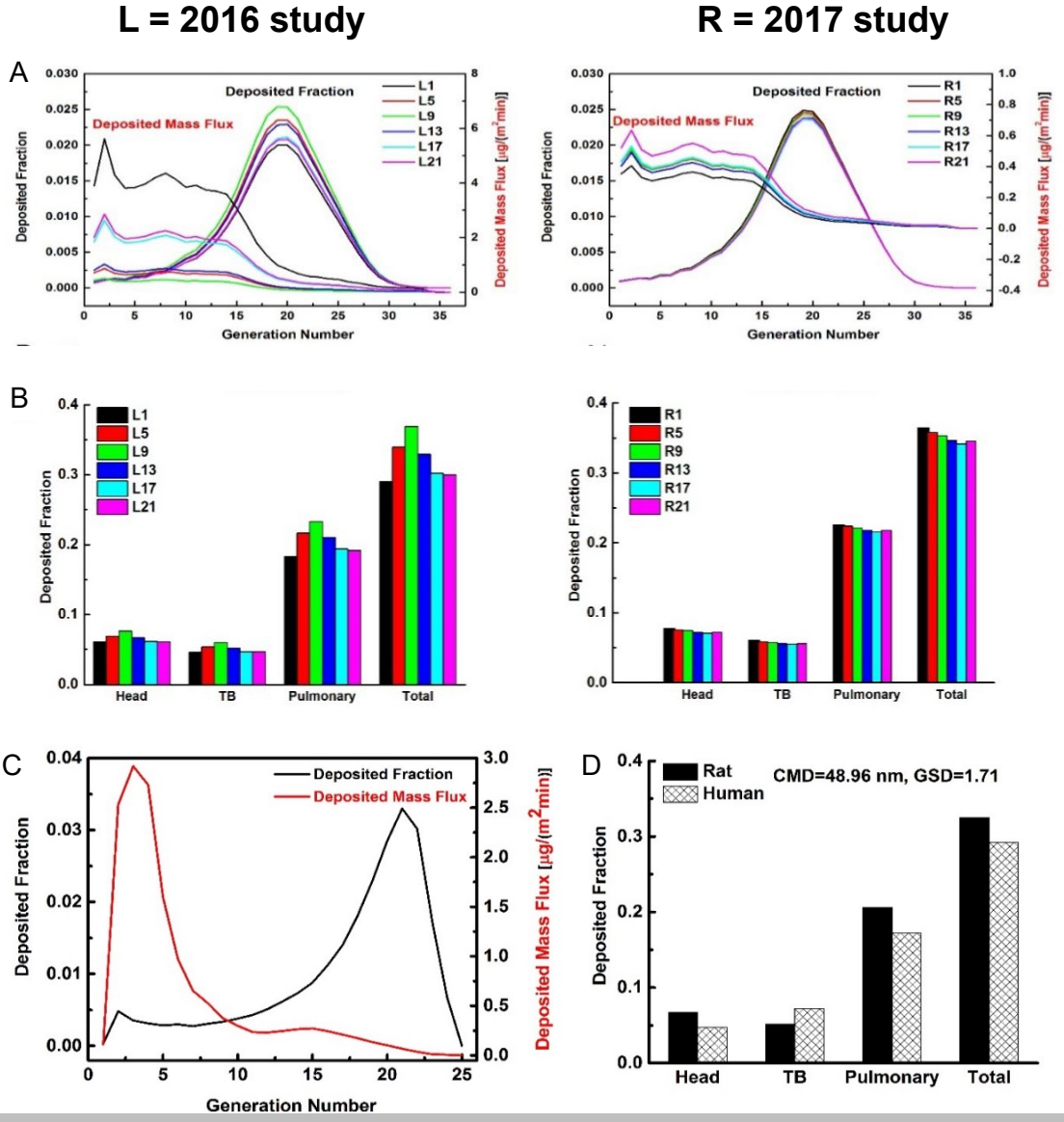
	Group	Mean particle diameter (nm)	Count median diameter (nm)	Geometric standard deviation	Particle number concentration ($10^5/cm^3$)	Particle mass concentration ($\mu g/m^3$)	VOCs
2016	L1	67.62±6.31	61.94±7.41	1.68±0.05	21.67±3.89	737.90±137.56	n/a
	L5	55.68±6.05	50.55±6.59	1.65±0.05	5.48±1.61	107.25±29.21	262.8±134.8
	L9	50.62±6.83	45.95±7.29	1.62±0.07	4.04±1.98	58.97±30.01	363.2±161.7
	L13	57.34±8.33	52.39±8.41	1.66±0.07	5.62±2.75	127.52±66.40	248.6±197.1
	L17	63.63±9.15	58.15±9.90	1.69±0.06	10.66±5.14	331.35±155.74	244.8±164.2
	L21	64.93±9.88	59.63±10.20	1.68±0.07	11.10±6.07	363.63±209.30	257.8±165.6
2017	R1	46.44±6.89	43.76±7.77	1.65±0.06	4.21±1.73	48.10±9.02	
	R5	47.69±6.20	44.78±6.95	1.66±0.07	4.63±1.76	60.31±18.17	
	R9	47.49±6.39	44.13±7.12	1.68±0.08	4.64±1.78	61.60±17.56	
	R13	48.25±7.02	44.64±7.93	1.70±1.10	4.06±1.85	58.96±24.77	
	R17	49.35±7.82	45.75±8.90	1.70±0.09	4.22±1.94	64.41±24.20	
	R21	48.96±8.18	44.00±9.05	1.71±0.13	5.84±6.79	76.43±34.08	

Unpublished data.

Multiple particle pathway analysis (1/2)

Rats

Human



Deposition:

~ 7%: head

~6%: TB region

21%: alveolar region

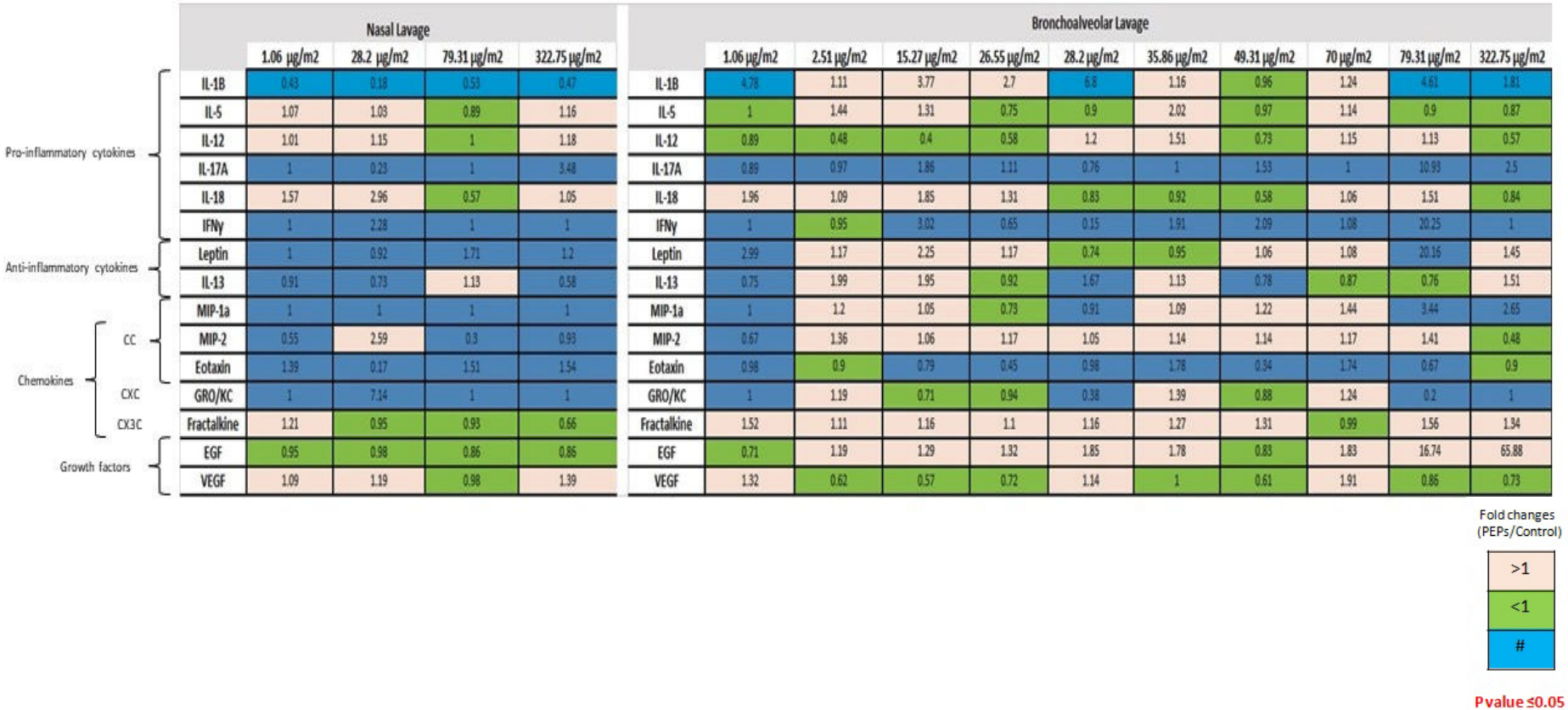
Multiple particle pathway analysis (2/2)

	Group	Retained dose (μg/m²)	Deposition rate (μg/hour)	Deposited mass (μg)			Retained mass (μg)			
				Total	TB	Pulmonary	Total	TB	Alveolar	Lymph nodes
2016	L1	1.06	2.34	11.71	2.36	9.35	0.31	0.00	0.30	0.01
	L5	28.2	0.43	10.83	2.15	8.68	8.18	0.05	8.11	0.03
	L9	28.24	0.25	11.45	2.33	9.12	8.19	0.04	8.10	0.04
	L13	79.31	0.50	32.74	6.46	26.27	23.00	0.15	22.70	0.17
	L17	252.06	1.23	104.51	20.35	84.15	73.10	0.45	71.90	0.71
	L21	322.75	1.31	137.25	26.90	110.35	93.60	0.53	91.90	1.13
2017	R1	2.51	0.19	0.93	0.20	0.74	0.73	0.00	0.73	0.00
	R5	15.27	0.24	5.95	1.23	4.72	4.43	0.03	4.39	0.01
	R9	26.55	0.24	10.80	2.24	8.56	7.70	0.05	7.61	0.04
	R13	35.86	0.23	15.24	3.10	12.14	10.40	0.07	10.30	0.08
	R17	49.31	0.25	21.67	4.39	17.29	14.30	0.09	14.10	0.14
	R21	70	0.30	31.66	6.47	25.19	20.30	0.11	19.90	0.25
	Human[b]	36.33	0.383	2760	810	1950	2278.36	8.36	1810	460

Rats: 0.29 m^2 alveolar surface area in rat

Human: 62.7 m^2 alveolar surface area in human

Pulmonary Region: Inflammatory response



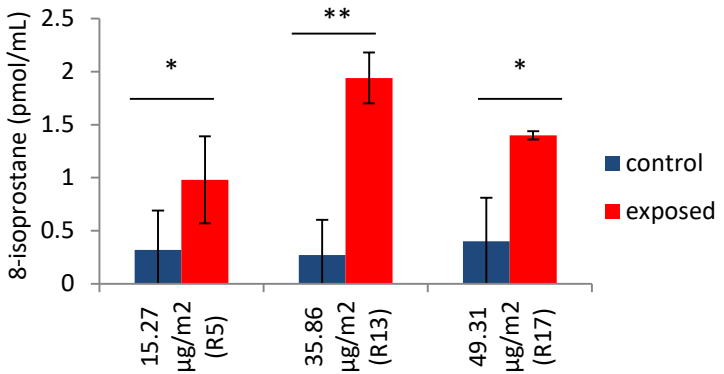
		Nasal Lavage			
		1.06 μg/m2	28.2 μg/m2	79.31 μg/m2	322.75 μg/m2
Pro-inflammatory cytokines	IL-1B	0.43	0.18	0.53	0.47
	IL-5	1.07	1.03	0.89	1.16
	IL-12	1.01	1.15	1	1.18
	IL-17A	1	0.23	1	3.48
	IL-18	1.57	2.96	0.57	1.05
	IFNγ	1	2.28	1	1
Anti-inflammatory cytokines	Leptin	1	0.92	1.71	1.2
	IL-13	0.91	0.73	1.13	0.58
Chemokines CC	MIP-1a	1	1	1	1
	MIP-2	0.55	2.59	0.3	0.93
	Eotaxin	1.39	0.17	1.51	1.54
CXC	GRO/KC	1	7.14	1	1
CX3C	Fractalkine	1.21	0.95	0.93	0.66
Growth factors	EGF	0.95	0.98	0.86	0.86
	VEGF	1.09	1.19	0.98	1.39

	Bronchoalveolar Lavage									
	1.06 μg/m2	2.51 μg/m2	15.27 μg/m2	26.55 μg/m2	28.2 μg/m2	35.86 μg/m2	49.31 μg/m2	70 μg/m2	79.31 μg/m2	322.75 μg/m2
IL-1B	4.78	1.11	3.77	2.7	6.8	1.16	0.96	1.24	4.61	1.81
IL-5	1	1.44	1.31	0.75	0.9	2.02	0.97	1.14	0.9	0.87
IL-12	0.89	0.48	0.4	0.58	1.2	1.51	0.73	1.15	1.13	0.57
IL-17A	0.89	0.97	1.86	1.11	0.76	1	1.53	1	10.93	2.5
IL-18	1.96	1.09	1.85	1.31	0.83	0.92	0.58	1.06	1.51	0.84
IFNγ	1	0.95	3.02	0.65	0.15	1.91	2.09	1.08	20.25	1
Leptin	2.99	1.17	2.25	1.17	0.74	0.95	1.06	1.08	20.16	1.45
IL-13	0.75	1.99	1.95	0.92	1.67	1.13	0.78	0.87	0.76	1.51
MIP-1a	1	1.2	1.05	0.73	0.91	1.09	1.22	1.44	3.44	2.65
MIP-2	0.67	1.36	1.06	1.17	1.05	1.14	1.14	1.17	1.41	0.48
Eotaxin	0.98	0.9	0.79	0.45	0.98	1.76	0.34	1.74	0.67	0.9
GRO/KC	1	1.19	0.71	0.94	0.38	1.39	0.88	1.24	0.2	1
Fractalkine	1.52	1.11	1.16	1.1	1.16	1.27	1.31	0.99	1.56	1.34
EGF	0.71	1.19	1.29	1.32	1.85	1.78	0.83	1.83	16.74	65.88
VEGF	1.32	0.62	0.57	0.72	1.14	1	0.61	1.91	0.86	0.73

- Only IL-18 up-regulation was found to be statistically significant in the BALF at the retention dose of 28.2 μg/m^2 (L5).

Blood serum biomarker analysis

A



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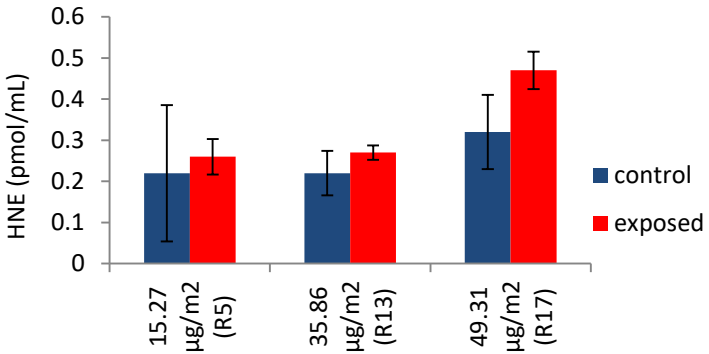

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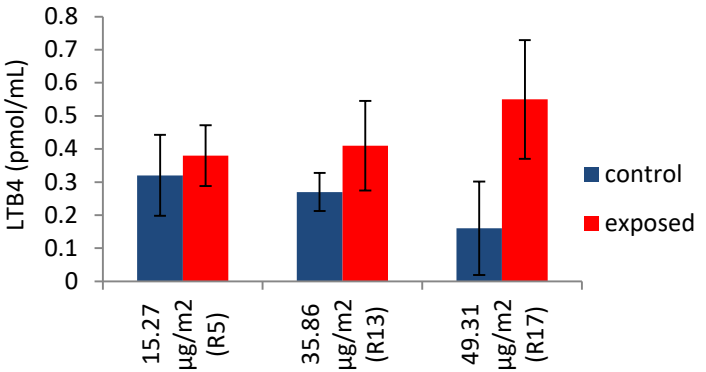


- 8-Isoprostane and 4-HNE are well established markers of oxidative stress originating from free radical oxidation of arachidonic acid *in vivo.*
- Leukotriene B4 (LTB4) is an important, well-established inflammatory mediator generated from activated innate immune cells such as neutrophils and macrophages, and mast cells.
- Serum markers for oxidative stress and inflammation showed upregulation in response to PEPs exposure.

HNE= 4-hydroxynonemal

LTB4= Leukotriene B4

Rats dose-response analysis relationship

Exposure day	Deposition rate (μg/hour)	Retained mass dose (μg/m²)	Biological outcomes for control versus PEPs exposed Sprague-Dawley rats (p value ≤0.05)
L1	2.34	1.06	----
R1	0.19	2.51	----
R5	0.24	15.27	----
R9	0.24	26.55	----
L5	0.43	28.2	BALF LDH ↑
L9	0.25	28.24	----
R13	0.23	35.86	----
R17	0.25	49.31	IL-18 ↓
R21	0.30	70	BALF Hemoglobin ↑; BALF IL-2 ↑
L13	0.50	79.31	BALF LDH ↑
L17	1.23	252.06	----
L21	1.31	322.75	----

NOAEL

LOAEL

NOAEL= No adverse effect levels LOAEL= Low adverse effects levels

0.29 m^2 alveolar surface area in rat

Unpublished data.

EPA, 1995

Extrapolated human dose-response analysis relationship

Exposure day	Retention mass dose
NOAEL	4.71 mg/m^2
LOAEL	7.53 mg/m^2
Worst case scenario (Martin et al., 2015)	3.14 mg/m^2

- Exposure NOAEL, LOAEL and Worst case scenario to PEPs exposure for 8 hrs/day, 5 days a week for 21 days.
- Human: 62.7 m^2 alveolar surface area in human (Oller and Oberdorster, 2010, Regulatory Toxicol Pharma.)
- Worst case scenario based on measurements at Boston, MA photocopier center 8 printing >11,000 copies per day (Martin et al., 2015. J Hazard Mater.).

Summary of results from *in vivo* toxicological assessment

Inhalation – *Work in progress*

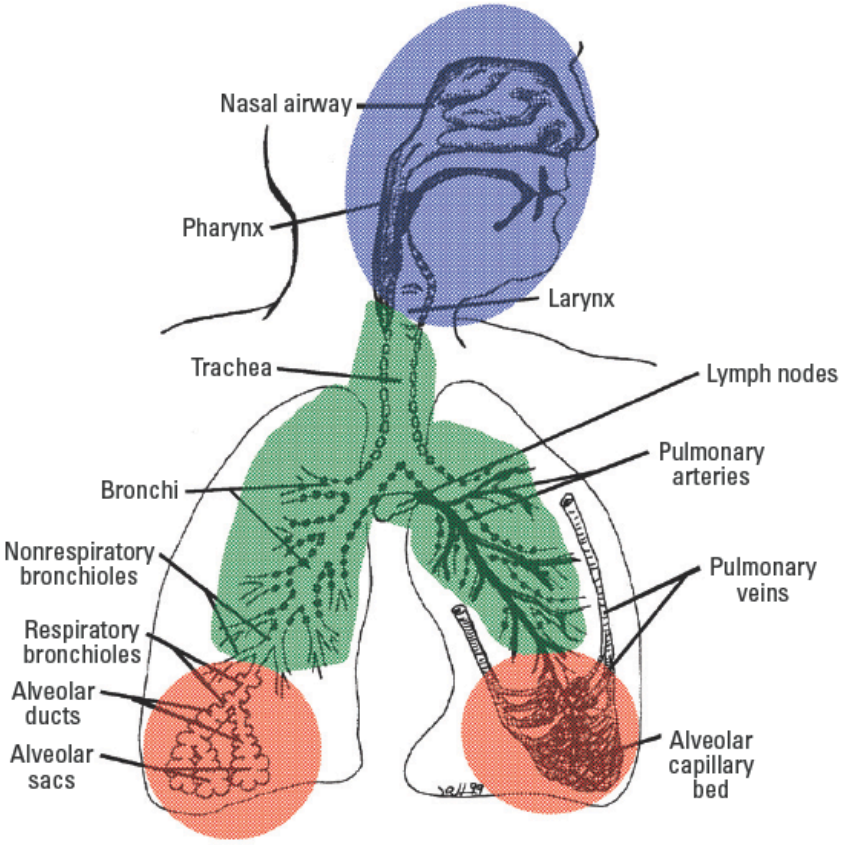
- PEPs induced mild cytotoxicity, inflammation and oxidative stress in the respiratory region of the Sprague-Dawley rats.
- These responses were in the form of modest release of pro-inflammatory cytokines and chemokines, influx of immune cells and modest increase in peroxidase activity and glutathione levels both in the NLF and BALF of the exposed animals.
- Histological and in situ ROS studies demonstrated no negative and pathological effects from PEPs exposure to both pulmonary and cardiac region of the exposed animals.
- Serum samples analysis indicated upregulation of oxidative stress and inflammatory metabolic biomarkers.
- Repeated PEPs exposure causes hypertension and sympathetic excitation.

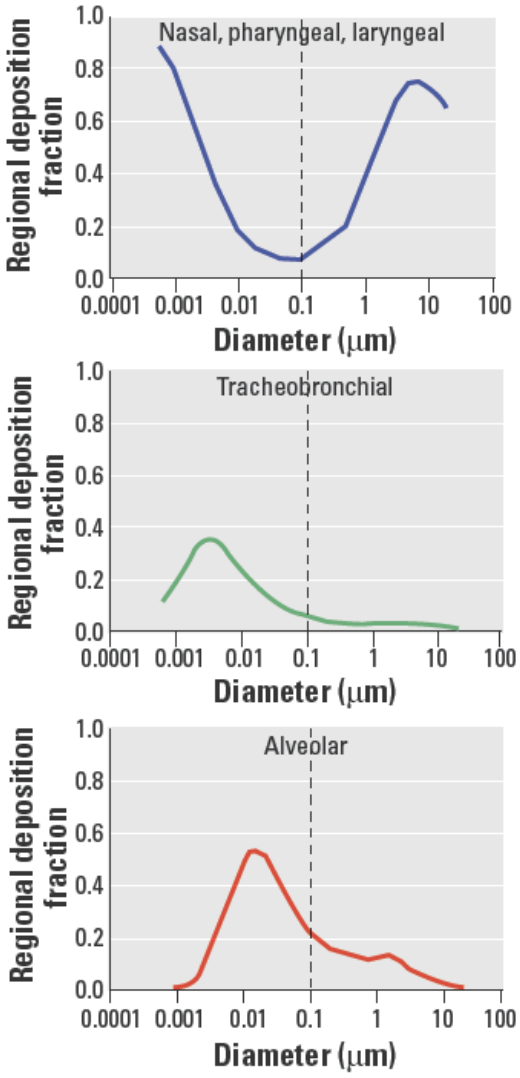
- Based on the measured biological responses the PEPs concentration of 28.2 $\mu g/m^2$ was found to be the transition point from NOAEL to LOAEL.
- Extrapolating the obtained results to human exposure to PEPs for 8 hrs/day, 5 days per week and 3 weeks the NOAEL and LOAEL after pulmonary clearance was determined at 4.71 mg/m^2 and 7.53 mg/m^2.

Future Directions - Objectives

- To establish a prospective cohort
  - To serve as a model for assessing the risks to exposures from engineered nanoparticles released from nano-enabled products.
  - Develop integrated methodologies that can be used along the exposure-disease continuum.
  - Develop research driven by mechanistic hypothesis.
  - Develop novel effect biomarkers.
  - Develop intervention strategies.
  - Safer by design product reformulations to minimize risks.
  - Promote sustainable nanotechnology efforts in this field.

Inhalation – Primary Exposure Pathway





Central Hypothesis

- Nanoparticles from toner-based printing equipment induce inflammation and oxidative stress, leading to respiratory disorders of the upper and lower airways, immune system activation, cardiovascular health risk, and possibly genotoxicity, in exposed individuals.
  - Oxidative Stress
  - Pro-inflammatory responses
  - Respiratory
  - Cardiovascular
  - Genotoxicity

Impact of the study

- Addressed the importance of evaluating life-cycle implications of NEPs.
- Assessing real world exposures and their associated toxicological properties rather than focusing on “raw” materials used in NEP synthesis.
- Multidisciplinary approach and methodology to investigate toxicological implications of consumer exposures to released PM from NEPs.

Major Knowledge Gaps

- Estimates of the disease burden in workers and consumers are lacking.
  - Respiratory, cardiovascular and immune system, and genotoxicity.
  - Carcinogenicity, neurological and reproductive toxicity.
- Exposure-dose-effect relationships in cohorts have to be established.
- Exposure bio/markers for routine exposure monitoring purposes are currently lacking.
- Exact molecular mechanisms not fully elucidated.

Thank you for your attention!
Questions?

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In vitro doses of PEPs and corresponding consumer inhalation exposure duration

Table 2. *In vitro* doses of PEPs and the corresponding consumer inhalation exposure duration.

	SAEC		THP-1	
Administered dose (cells)[a] (μg/mL)	Delivered dose (cells)[a] (μg/mL)	Corresponding consumer inhalation exposure duration to PEPs (hr)[b]	Delivered dose (cells)[a] (μg/mL)	Corresponding consumer inhalation exposure duration to PEPs (hr)[b]
0.5	0.5	15.0	0.26	7.8
5	5	75.2	2.6	39.0
10	10	150.4	5.2	77.9
20	20	300.7	10.4	155.8
30	30	451.1	15.6	233.7
40	40	601.4	20.8	311.5
100	100	1503.6	52.0	778.9

[a]*In vitro*–administered and delivered doses were based on a 24-hr *in vitro* exposure. [b]Calculations of the corresponding consumer inhalation exposure duration (hours) were based on the added values of deposition mass flux (μg/m^2 • min) in the various human airways, excluding head airways: the conducting zone (generations 0 to 16) and the transitional and respiratory zones (generations 17 through 23).

In vivo doses of PEPs and corresponding consumer inhalation exposure duration

Table 2
Comparison of doses of murine PEP exposures used in the study by intratracheal instillation with comparable human inhalation exposures to PEPs.

PEP exposure by intratracheal instillation (mg/kg bw)	Duration of consumer inhalation exposure of PEPs (h)
0.5	13.7
2.5	70.9
5.0	141.9