

Integrated Approaches to Meet NanoEHS Challenges Along the Life Cycle of Nano-Enabled Products :

Nanoparticles from Photocopiers as an Example

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3. Biomedical Engineering and Biotechnology Program
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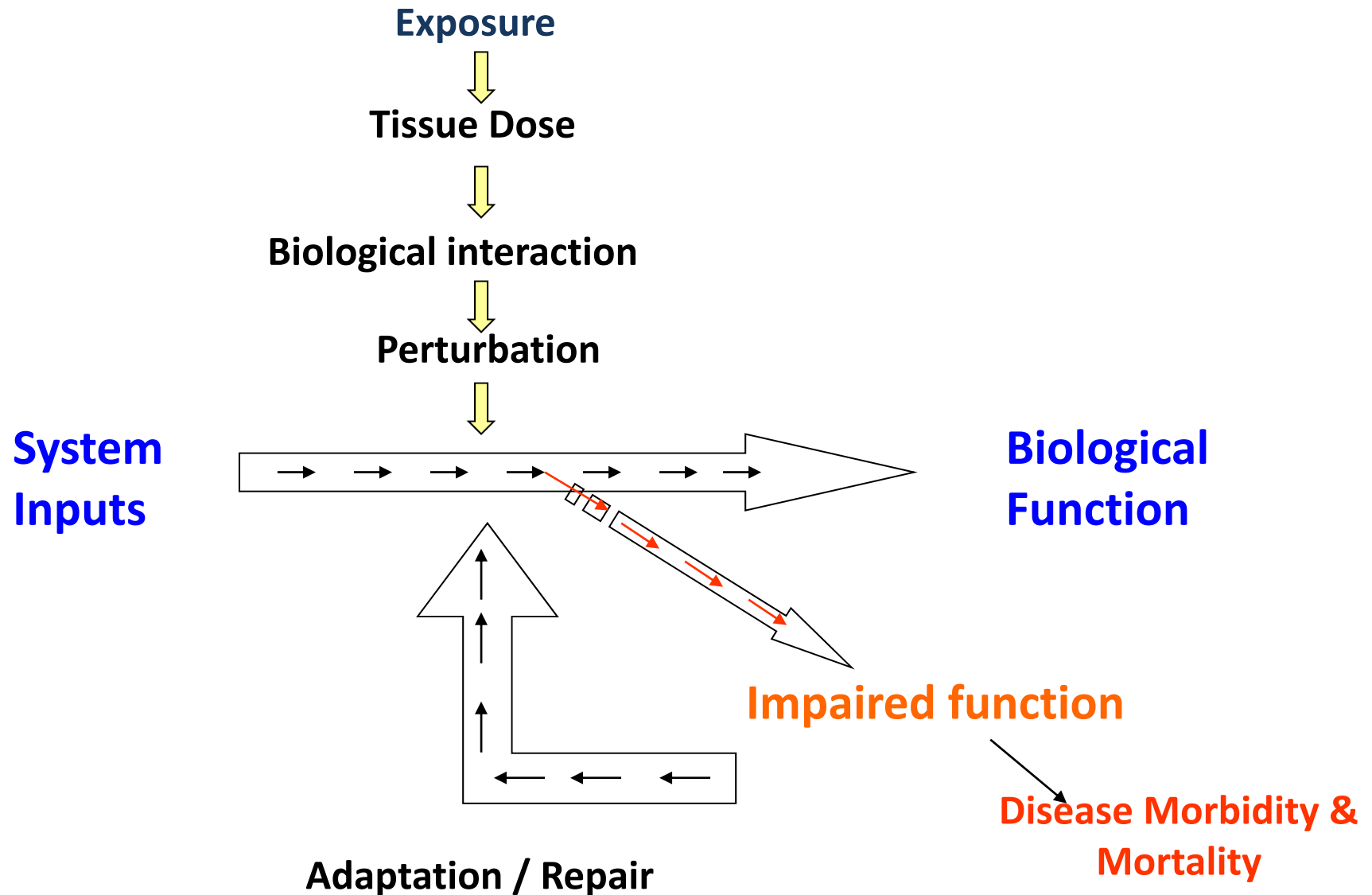
**Center for Nanotechnology and Nanotoxicology
at Harvard School of Public Health**



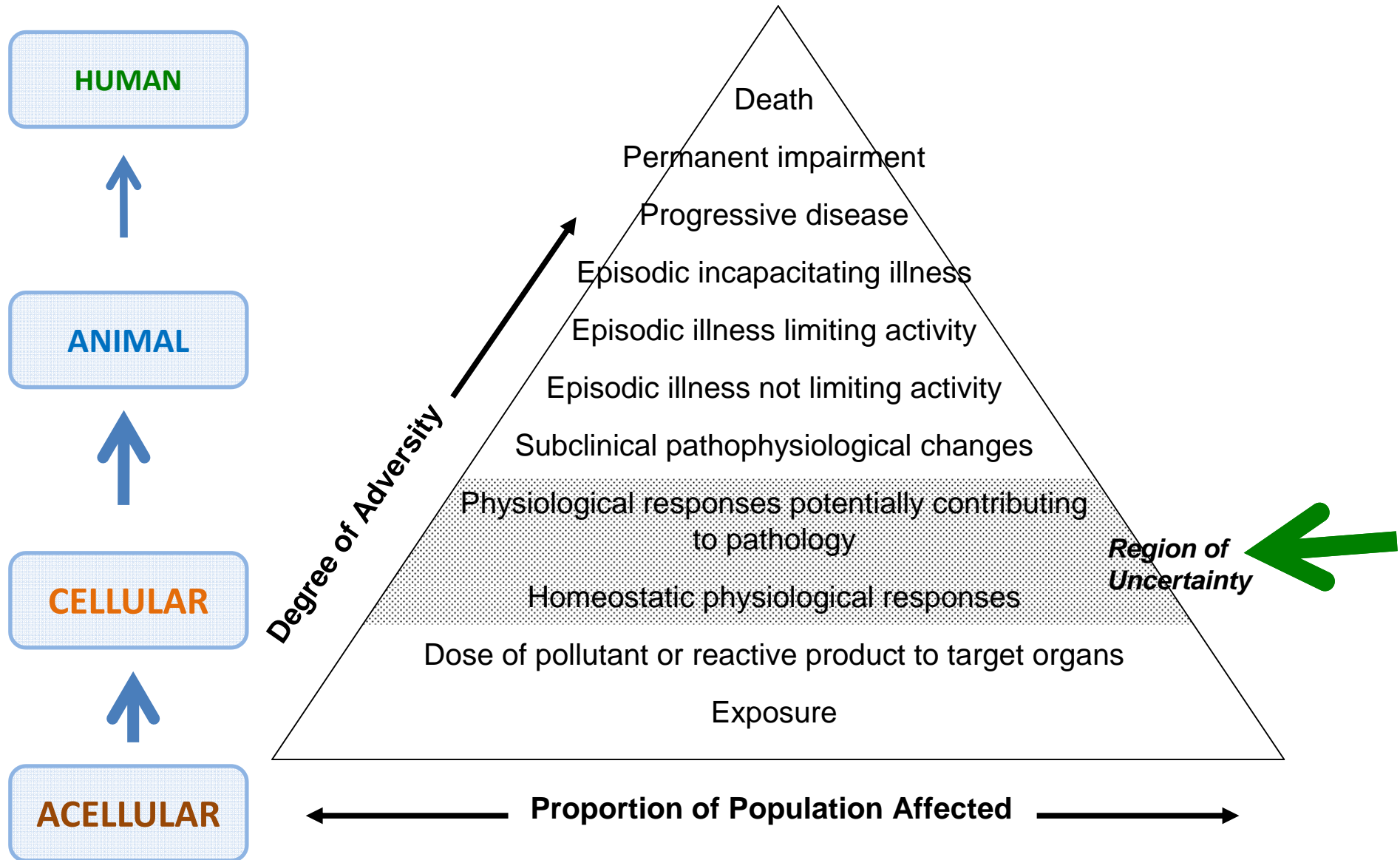
PRINCE LOBEL



Disease – A Complex Process



What Do We Want to Know? What Test Systems Should Be Used?



Mauderly et al., Chapt. 4 in: *Technical Aspects of Multipollutant air Quality Management*, Springer. 2011

Nanoparticle exposure at nanotechnology workplaces: A review

Thomas AJ Kuhlbusch^{1,3*}, Christof Asbach¹, Heinz Fissan^{1,3}, Daniel Göhler² and Michael Stintz²

- ~60 studies in all, 25 studies in workplaces
- Area Samplers
- Number concentration & size distribution + STEM/EDS
- Need for standardization & harmonization of measurements & approaches
- Collection of systematic contextual information
- Tiered Approach to understanding nature of exposures

Nanomanufacturing: Diverse, Dynamic & Challenging to Study

Sector	Functionality	Exposure situation	Market penetration (%)	Average number of companies per sector	Average Number of potentially exposed workers per company	Number potentially exposed workers
Production of wet concrete	Fumed silica for strength	- Mechanical mixing of nano-concentrates and - composites (powder and liquid) with relatively low energy levels	10	190	8	152
Production of cosmetics	TiO ₂ and ZnO for UV absorption		Not applicable	Not applicable	4	12¹
Paint production	TiO ₂ for absorption of pollutants		20	112	1.7	37
Paper production	Nano cellulose for strength and durability	Not available	1	29	2	1
Plastics/synthetics production	Nanoclay or nano silica for strength and durability, Ag for antibacterial effect, ZrO ₂ in scratch resistant coating	- Mechanical mixing of nano-concentrates (powder and granules) with relatively low energy levels - Manual application of liquid MNM-enabled end products	1	878	6.3	56
Tire production	Carbon black as filler and nano silica for strength and durability	- Mechanical mixing of nano-concentrates (powder) with relatively low energy levels	100	150	1	150
Toner production	Rheological properties	Not applicable	Not applicable	Not applicable	Not applicable	90²
Total						498

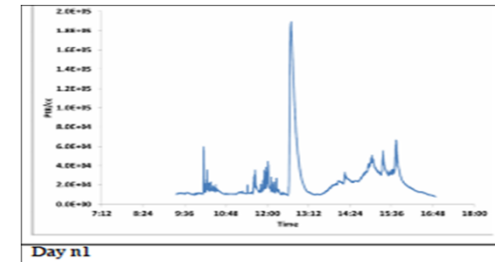
Bekker et al, AnnHyg 2012

An Integrated Approach to Fast Track NanoEHS

CHARACTERIZE REALISTIC EXPOSURES

Levels; PCM

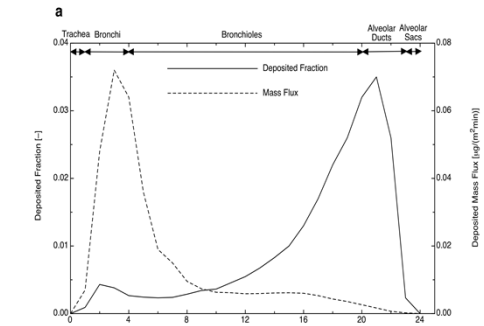
Variability



CALCULATE EQUIVALENT DOSE to HUMANS

Regional Deposition Model

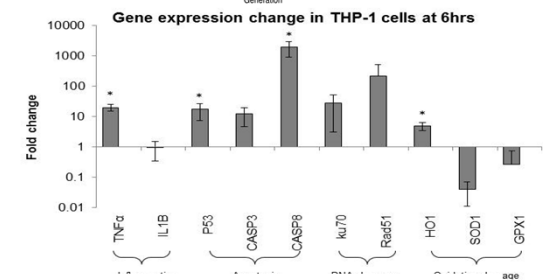
Sensitivity Analysis



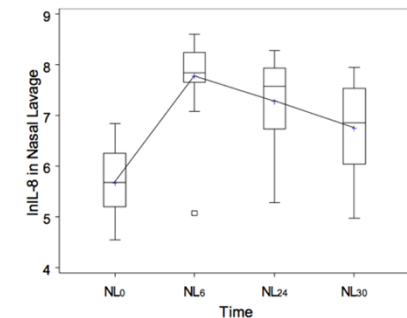
IN-VITRO Toxicity Assessment

Equivalent Dose Estimates

Mechanistically Endpoints



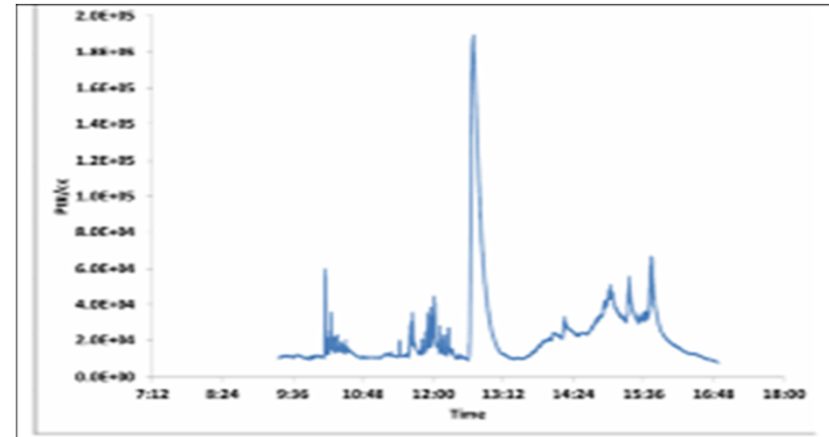
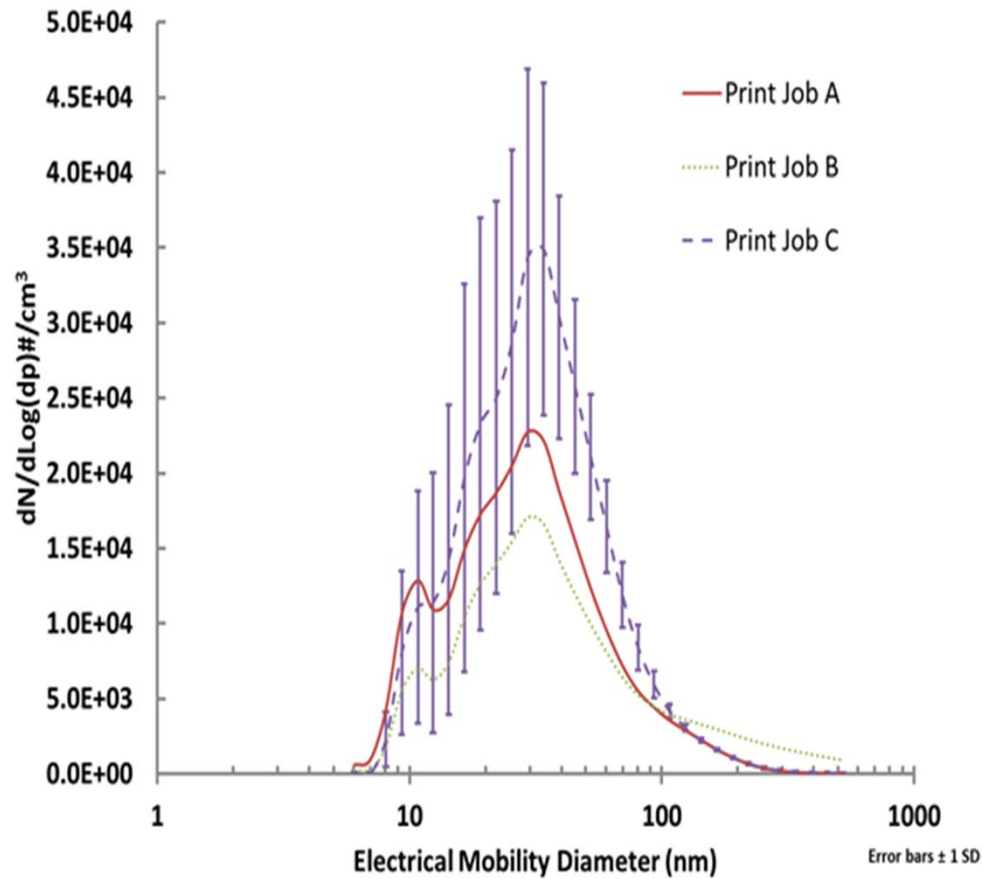
VALIDATE OBSERVATIONS in ANIMALS / HUMANS



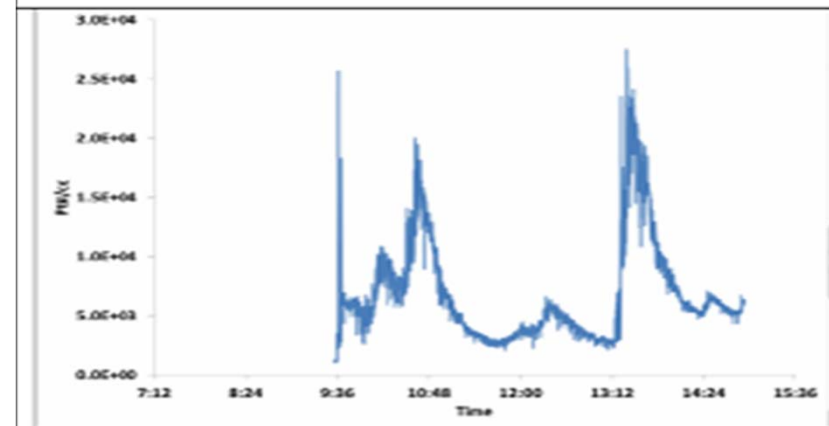
We started here....



Nanoparticle Emissions from Commercial Photocopiers

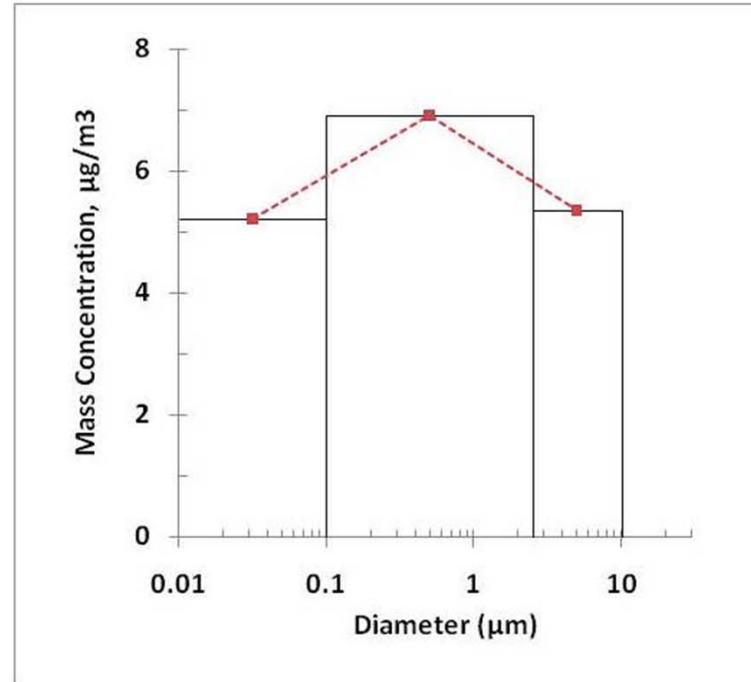
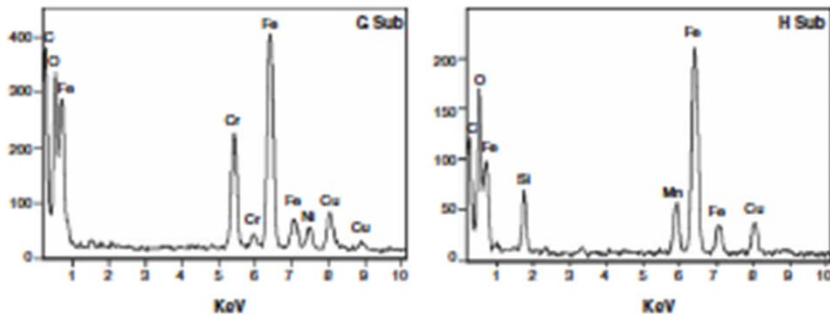
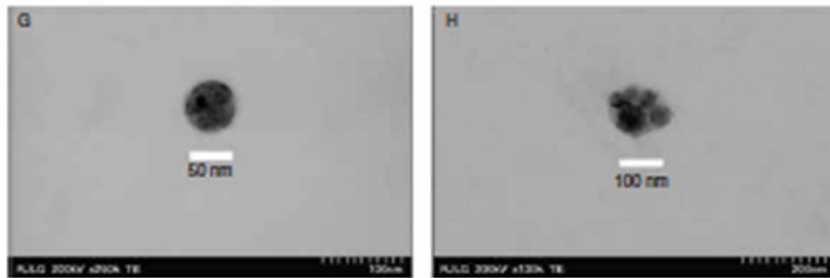
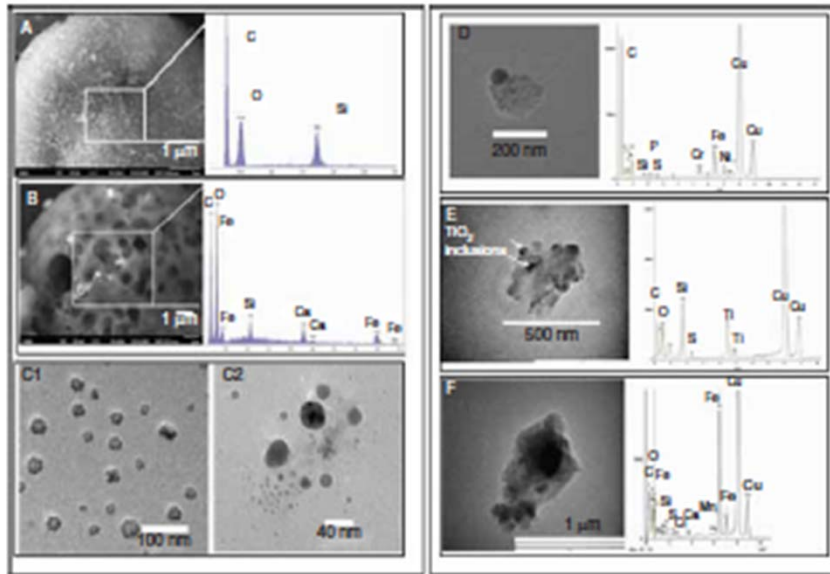


Day n1

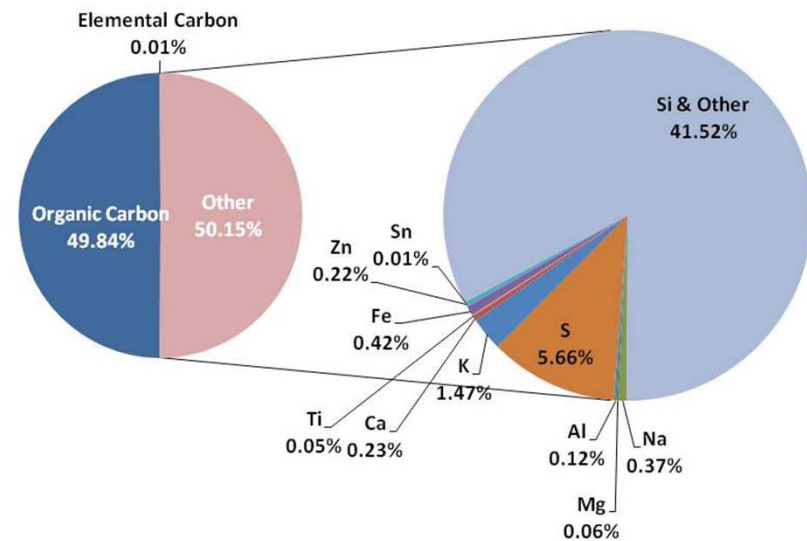


Day n3

Morphology & Chemistry

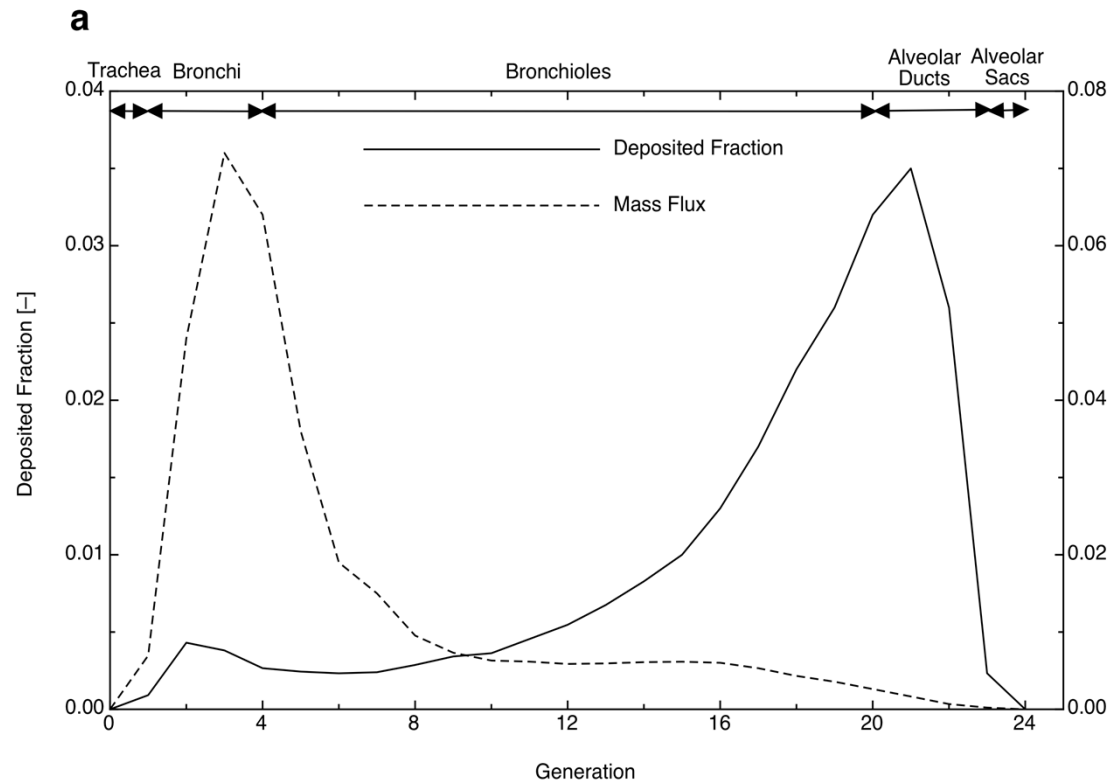


Ultrafine (PM <0.1 µm), 5.21 µg/m³
 Elements, 0.47 µg/m³



Bello et al Nanotoxicology 2012

Deposition Models & Dose: Linking *in Vivo* with *In Vitro* Dosimetry



Mass Flux $0.072 \mu\text{g}/(\text{m}^2\text{min})$
 &
 Exposure time of 480 min =
 8hr



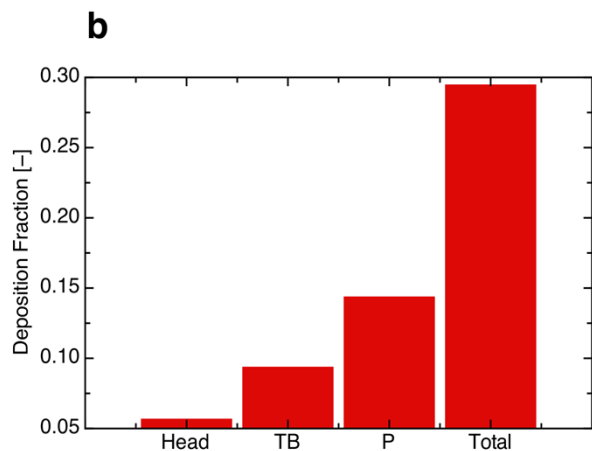
Estimated lung surface dose of $34.6 \mu\text{g}/\text{m}^2$

Nasal Cavity: 150 cm^2
 Deep Lungs: 140 m^2

SA Lungs/Nasal Ratio =
 $\sim 1.2 \times 10^4$

Deposited Fraction $\sim 5x$

Nose/Alveolar Dose (cm^{-2})
 $\sim 2,500x$



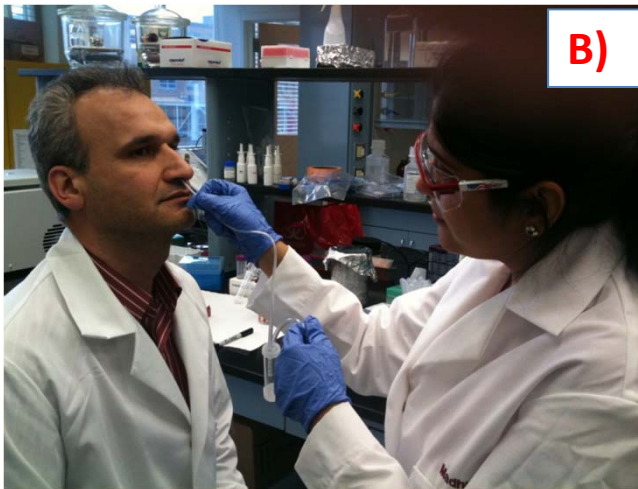
Khatri et al Part Fiber Tox 2013

Sample collection

A) The nasal cavity is misted over with saline using a disposable plastic sprayer



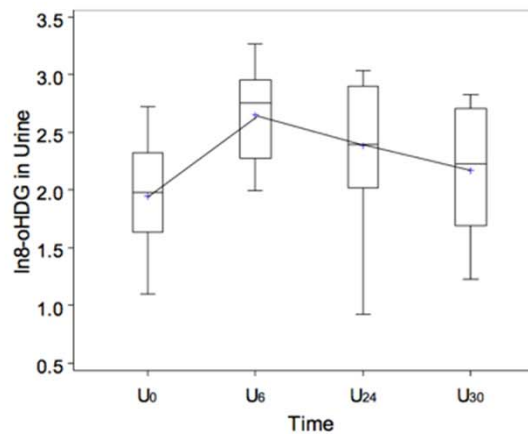
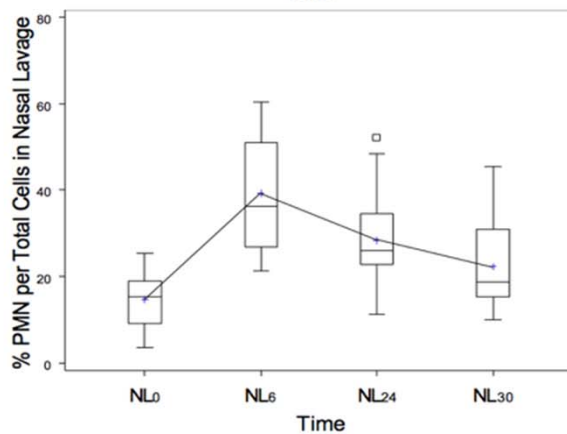
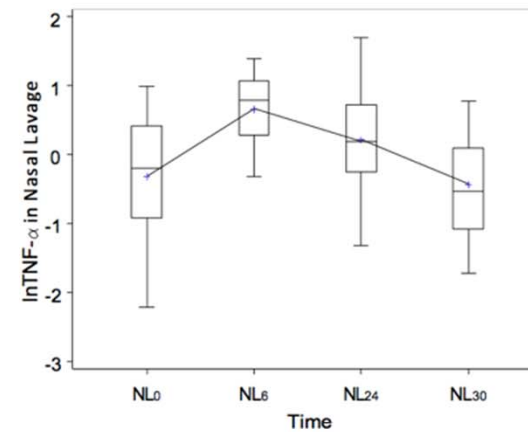
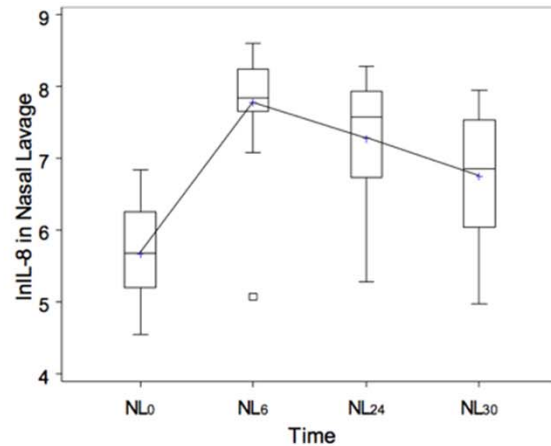
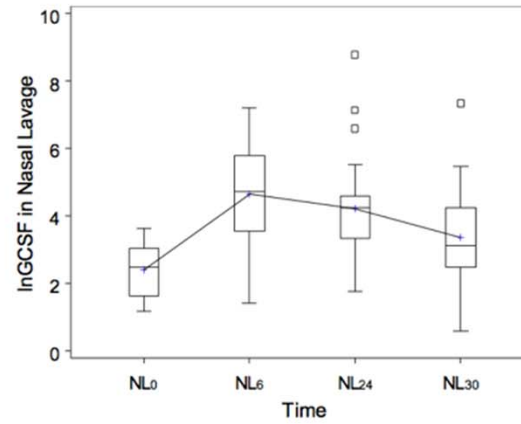
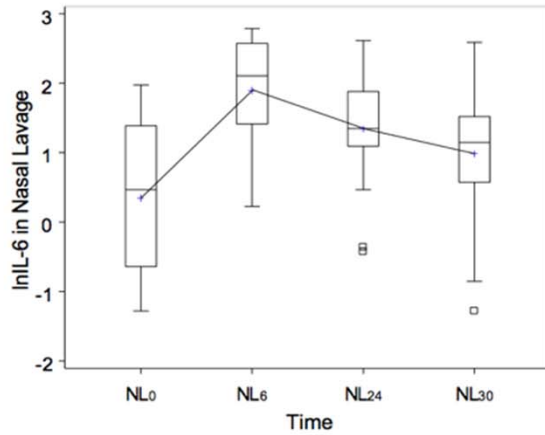
B) Nasal lavage fluid is collected by aspiration from both nostrils for 1 min each through a silicon tube 2.7 mm in diameter, and trapped in mucous trap



C) Traps are connected with the small portable evacuator



Biomarkers in Nasal lavage & Urine



↑ 10 cytokines overexpressed (10-2X):
 G-CSF, IL-8, VEGF, IL-6, IL-10, MCP1, Fractalkine, TNF- α , EGF, IL-1 β
~~IFN- γ , MCP-1, IL-1 α~~

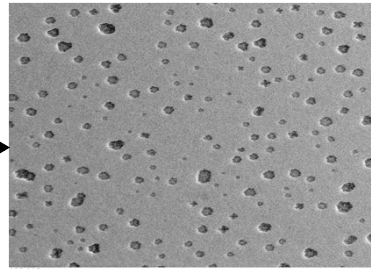
↑ Total Protein

↑ Inflammatory cells

↑ 8-OH-dG in urine UP

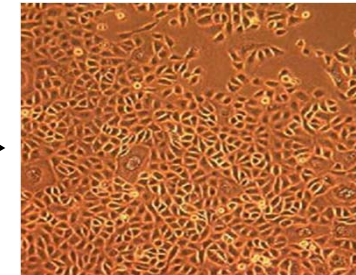
Khatri, Bello et al 2012
 Nanotoxicology

In vitro cytotoxicity and cytokine release



Particle collection
from copy centers

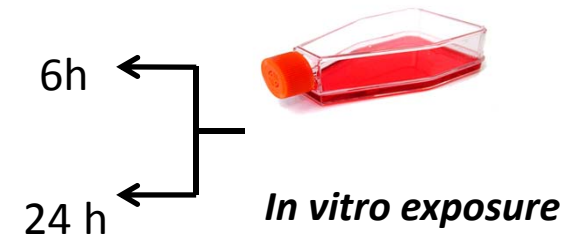
10ug/ml
polymyxine B
treatment for
60 min



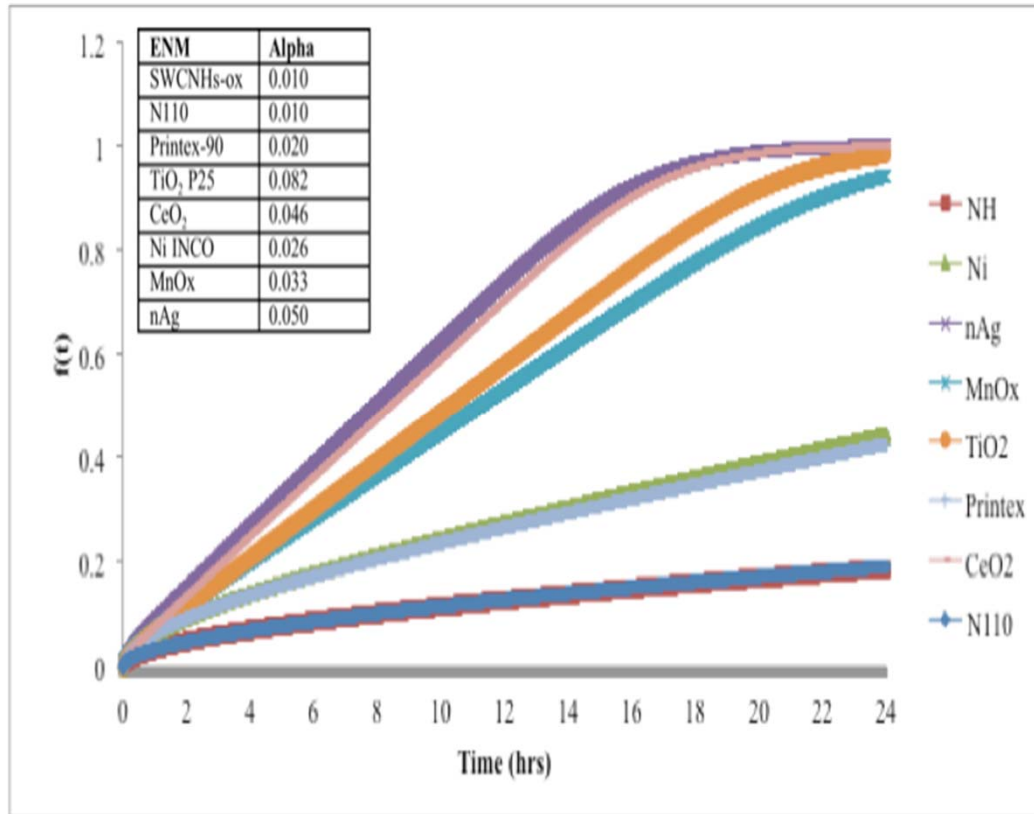
Human primary respiratory
epithelial cells
and THP-1 cell line exposed
PM_{0.1}, PM_{0.1-2.5}

30 ug/ml
100ug/ml
300ug/ml

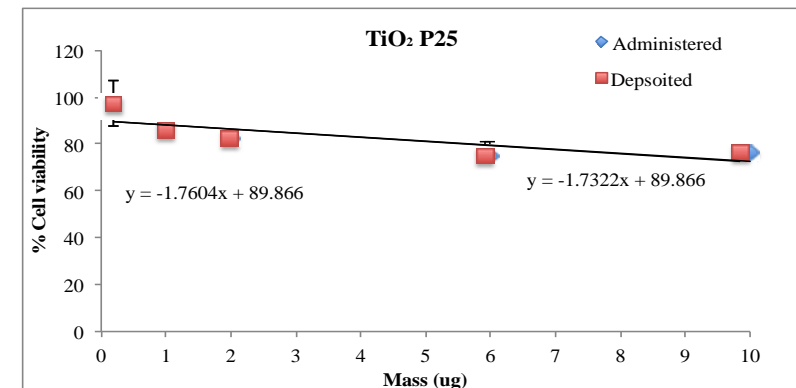
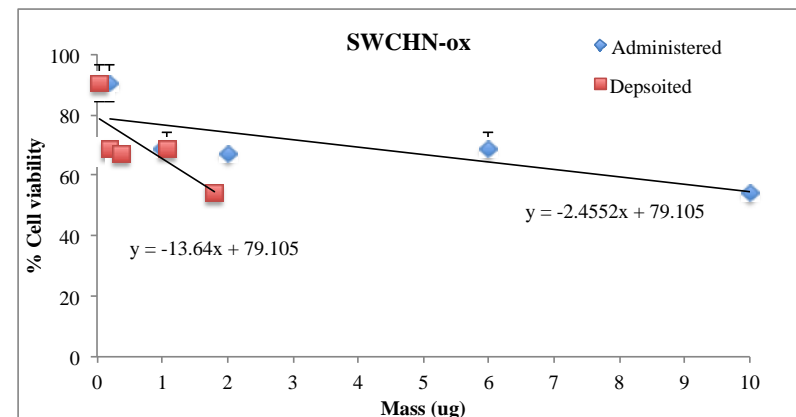
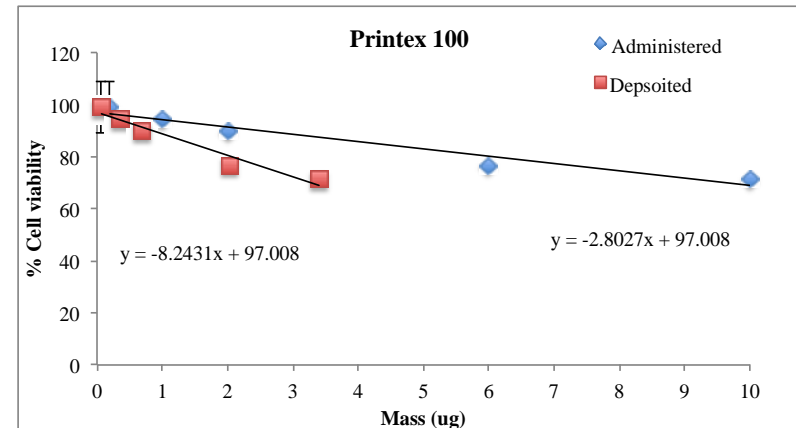
- Cell Viability
- Inflammatory cytokines
- DNA damage/ROS
- Apoptosis
- Gene Expression



Delivered Dose is Critical



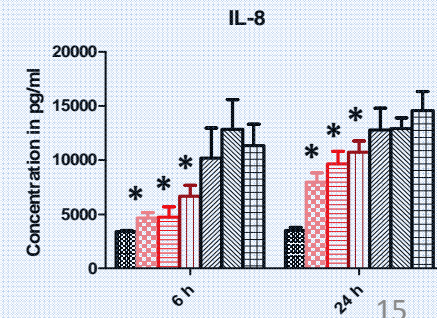
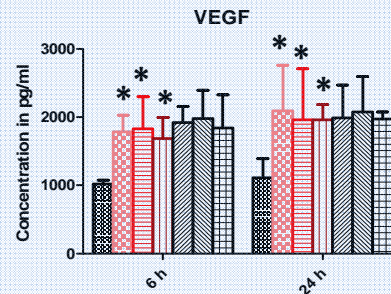
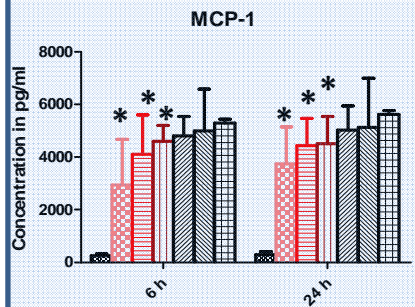
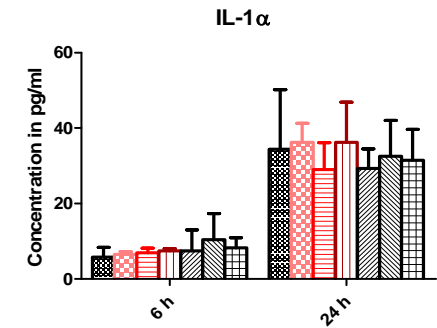
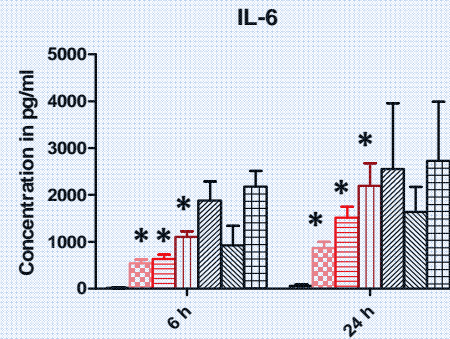
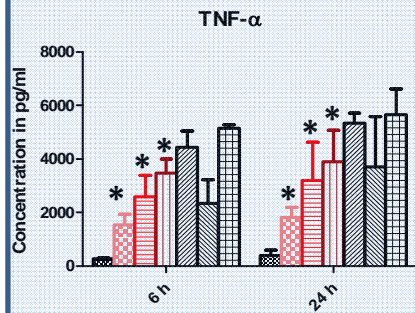
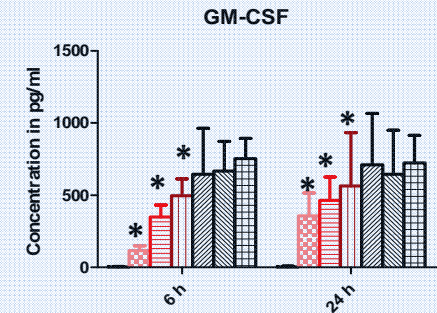
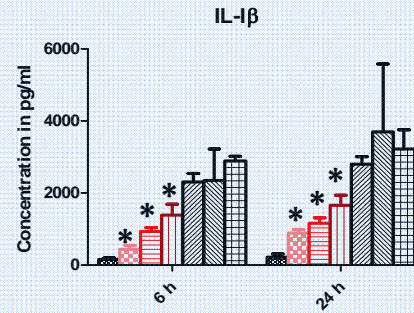
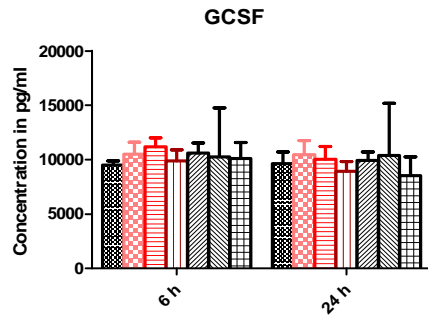
Pal et al (manuscript in preparation)



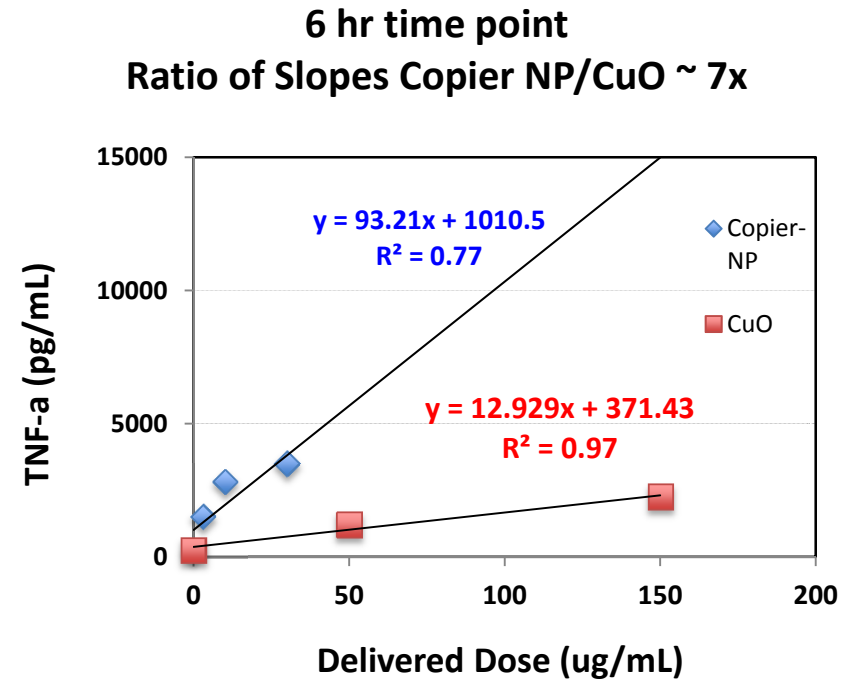
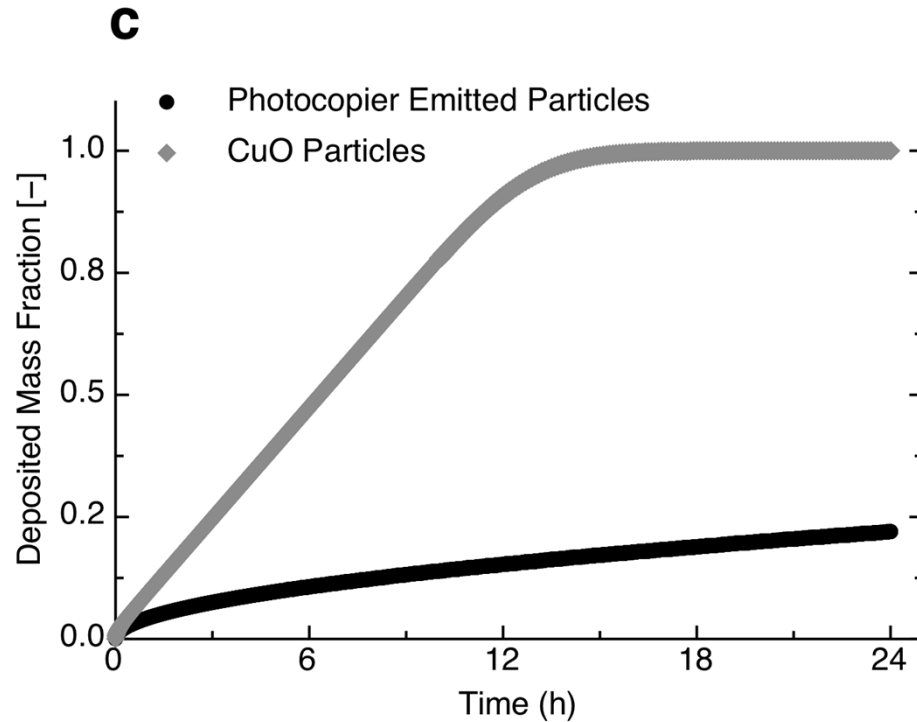


THP-1 Cell Line- Inflammatory Cytokines

-  PBS
-  30
-  100
-  300
-  300 endo
-  Cuo (300)
-  LPS

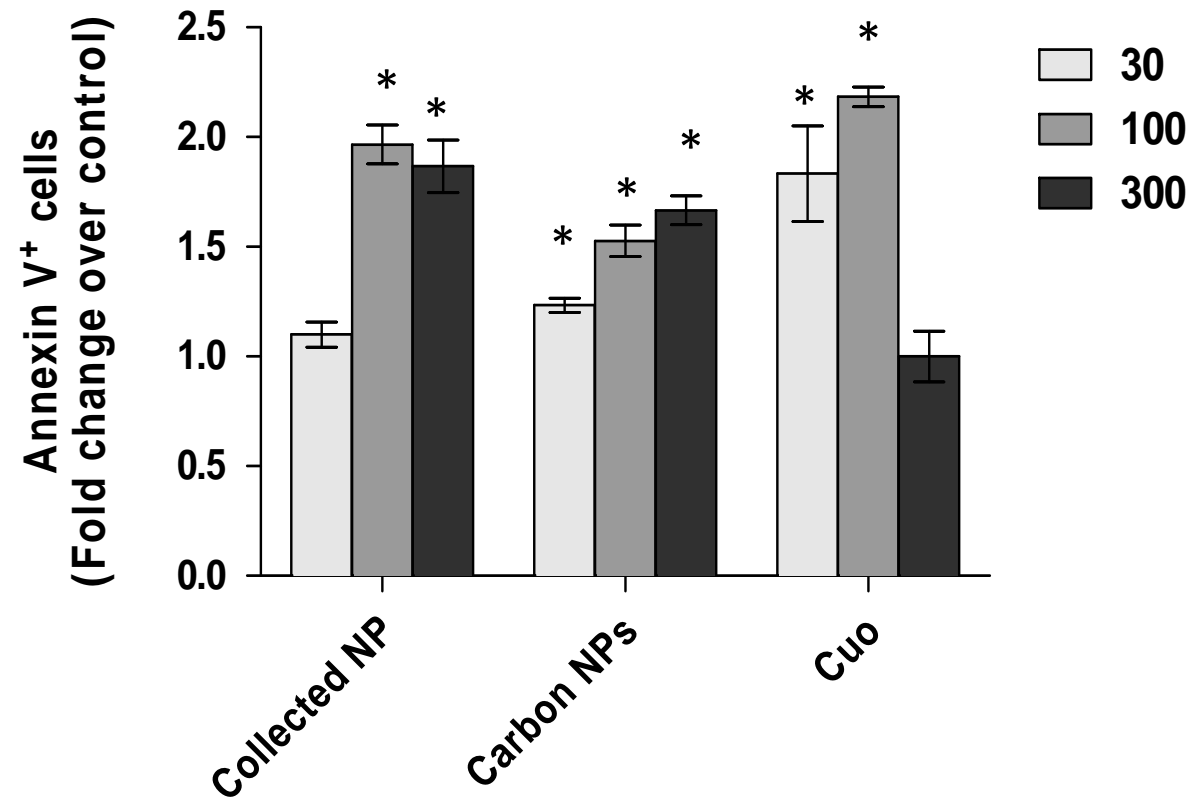


Delivered dose makes a difference

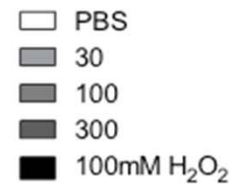
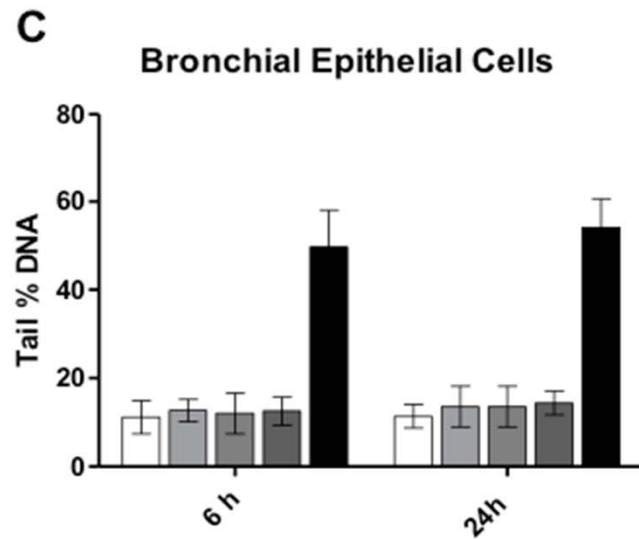
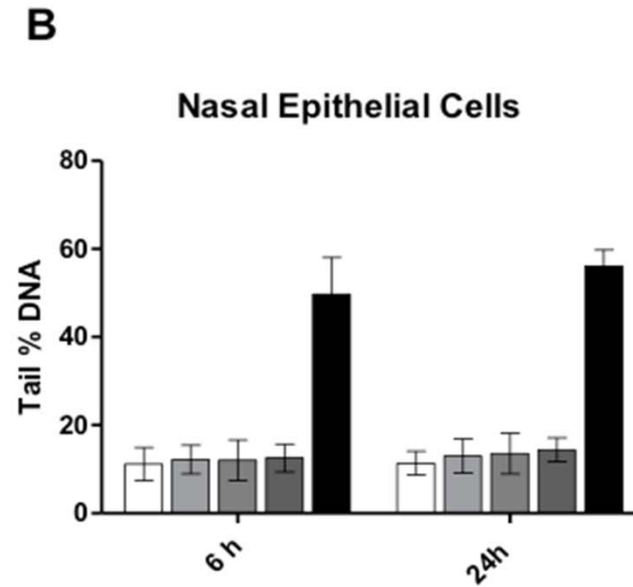
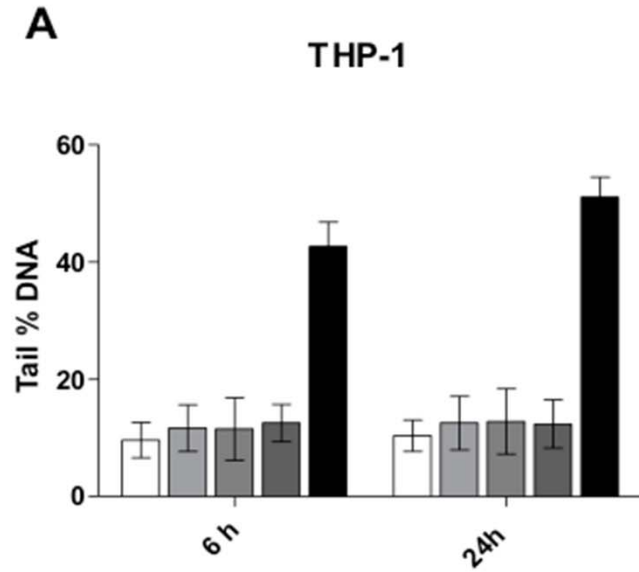


Administered dose (µg/mL)	Effective Dose at 6hr 0.1x	Effective dose at 24 hrs, 0.2x	Lung equivalency	CuO 6hrs	CuO 24 hrs
30	3	6	0.13 µg/mL estimated	15	30
100	10	20	1.3 µg/mL at 6 hr,	30	100
300	30	60	0.6 µg/mL @ 24 hrs	150	300

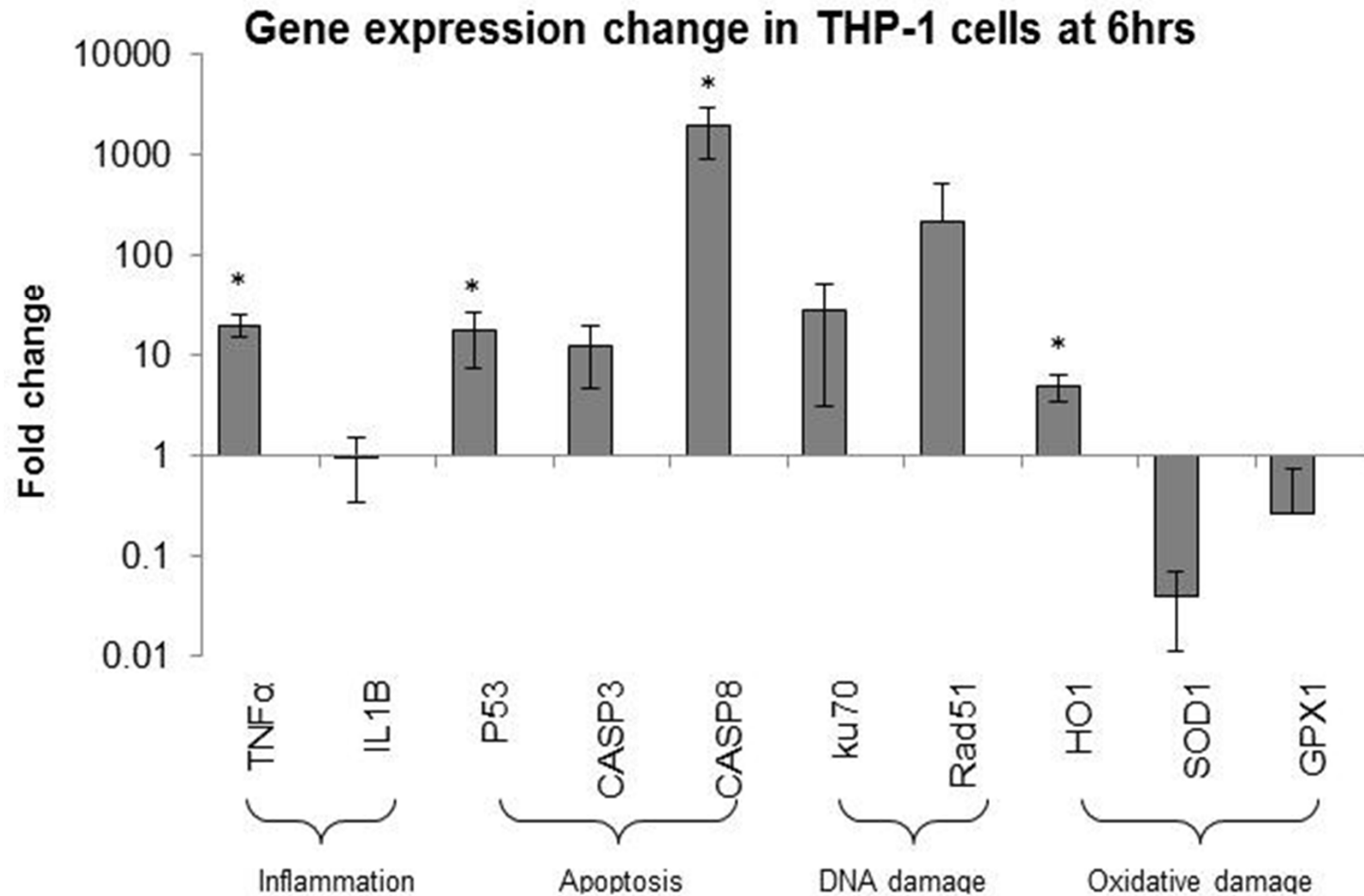
Apoptosis Results



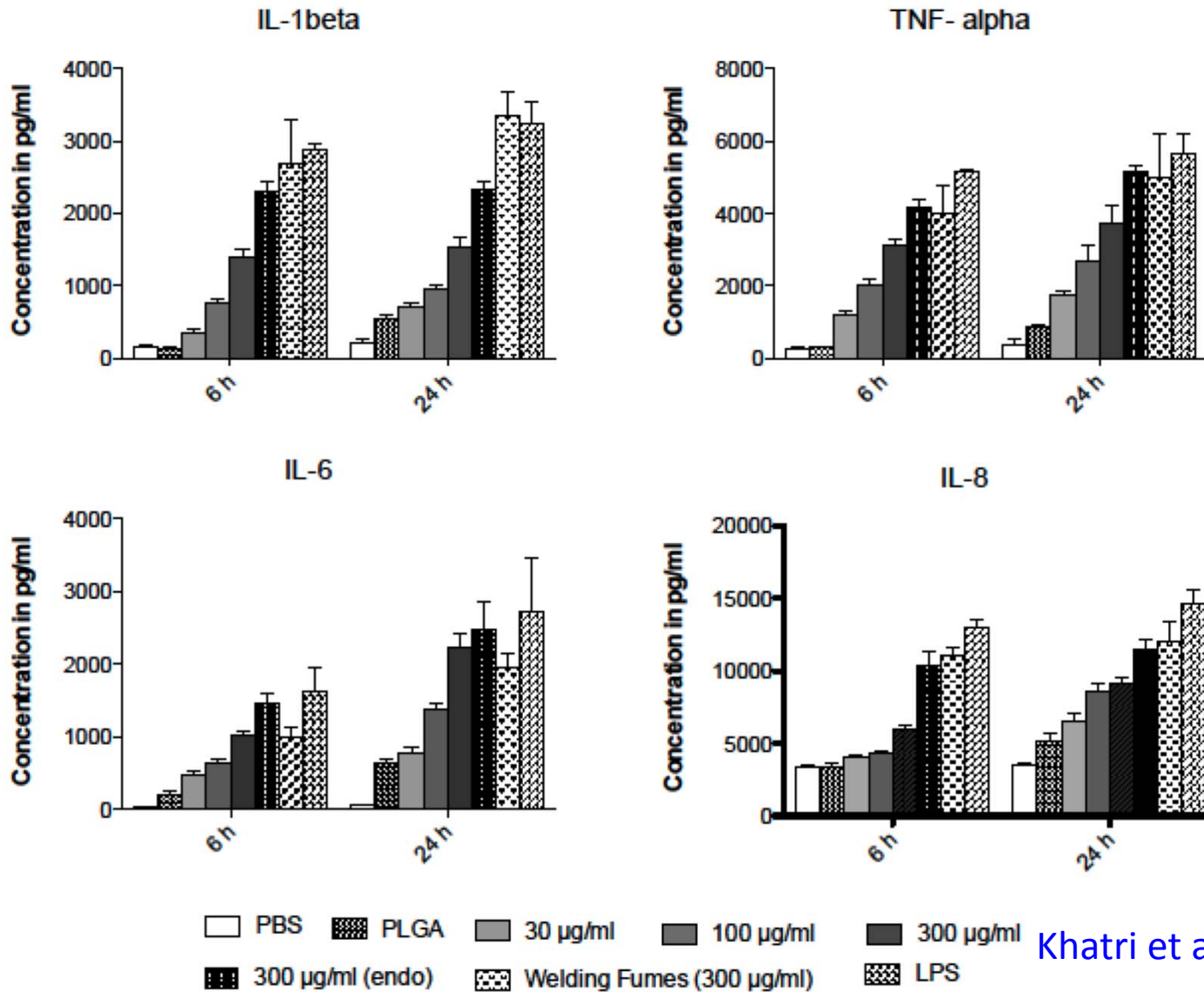
DNA damage- Comet assay



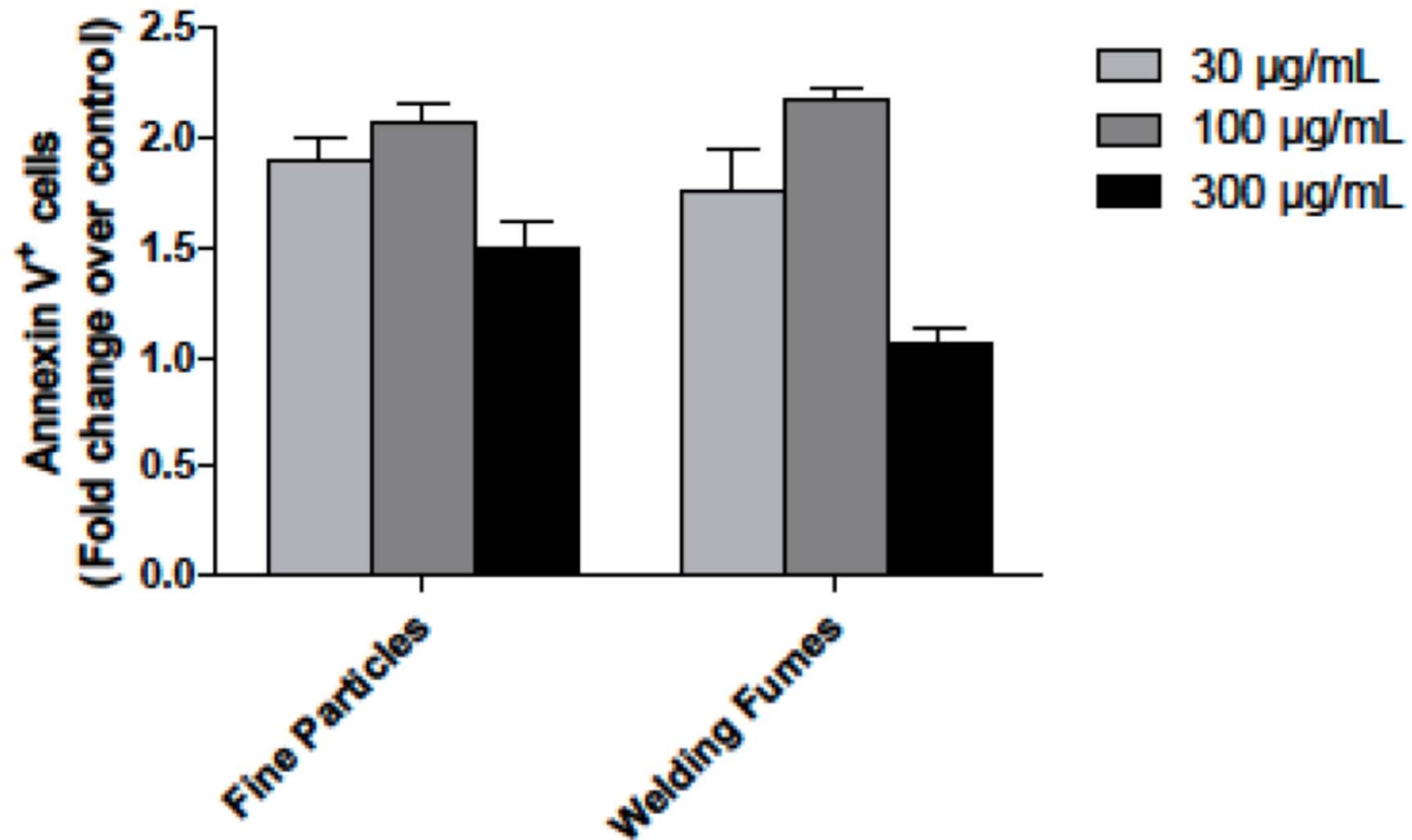
Gene Expression (RT-qPCR), 5ug/mL



PM_{0.1-2.5}, Cytokines in THP-1

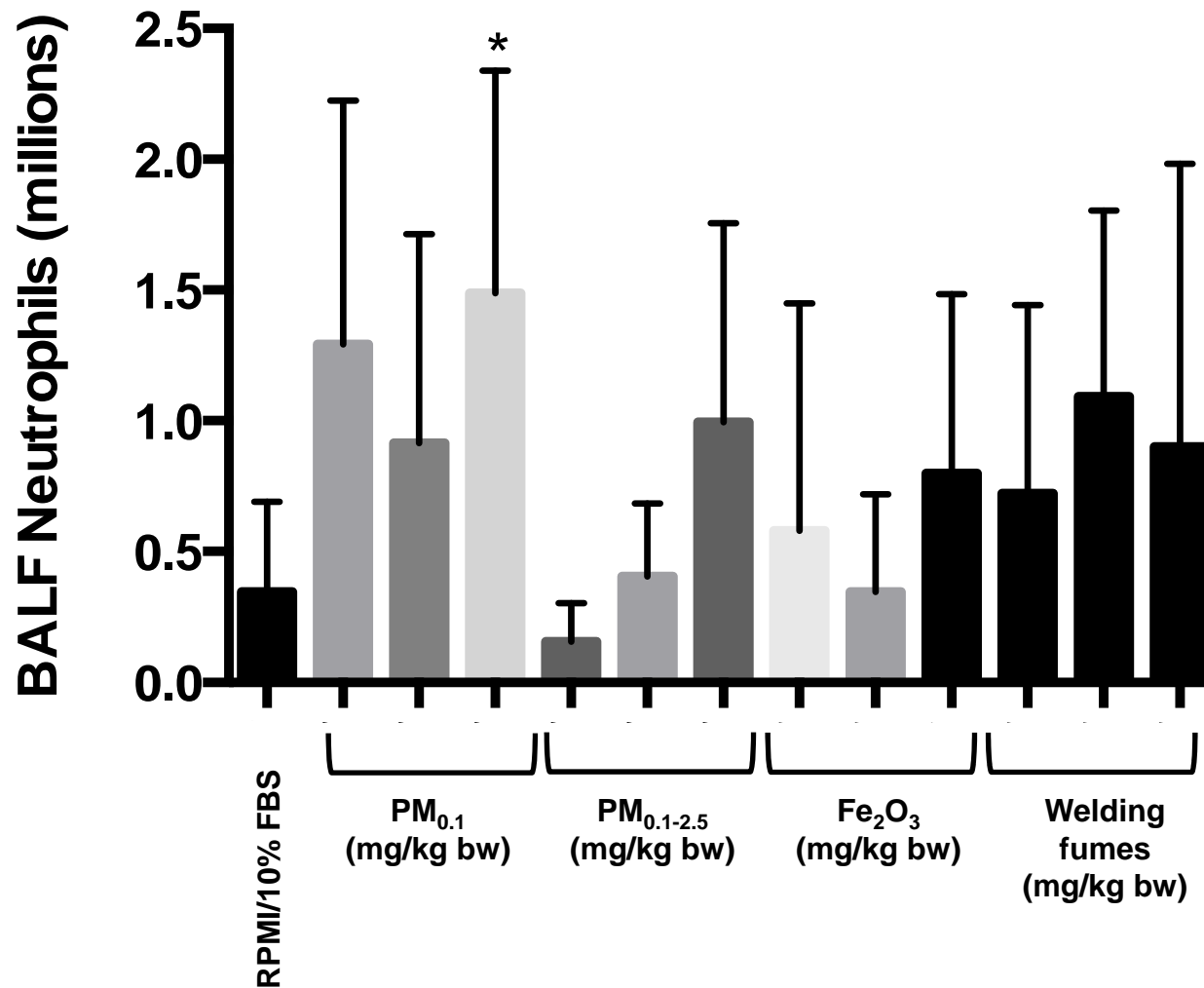


PM_{0.1-2.5}, Apoptosis in THP-1



In vivo instillation study in mice done at Harvard, Prof. Demokritou's group

Lavaged neutrophils 24 hours post-particle exposure



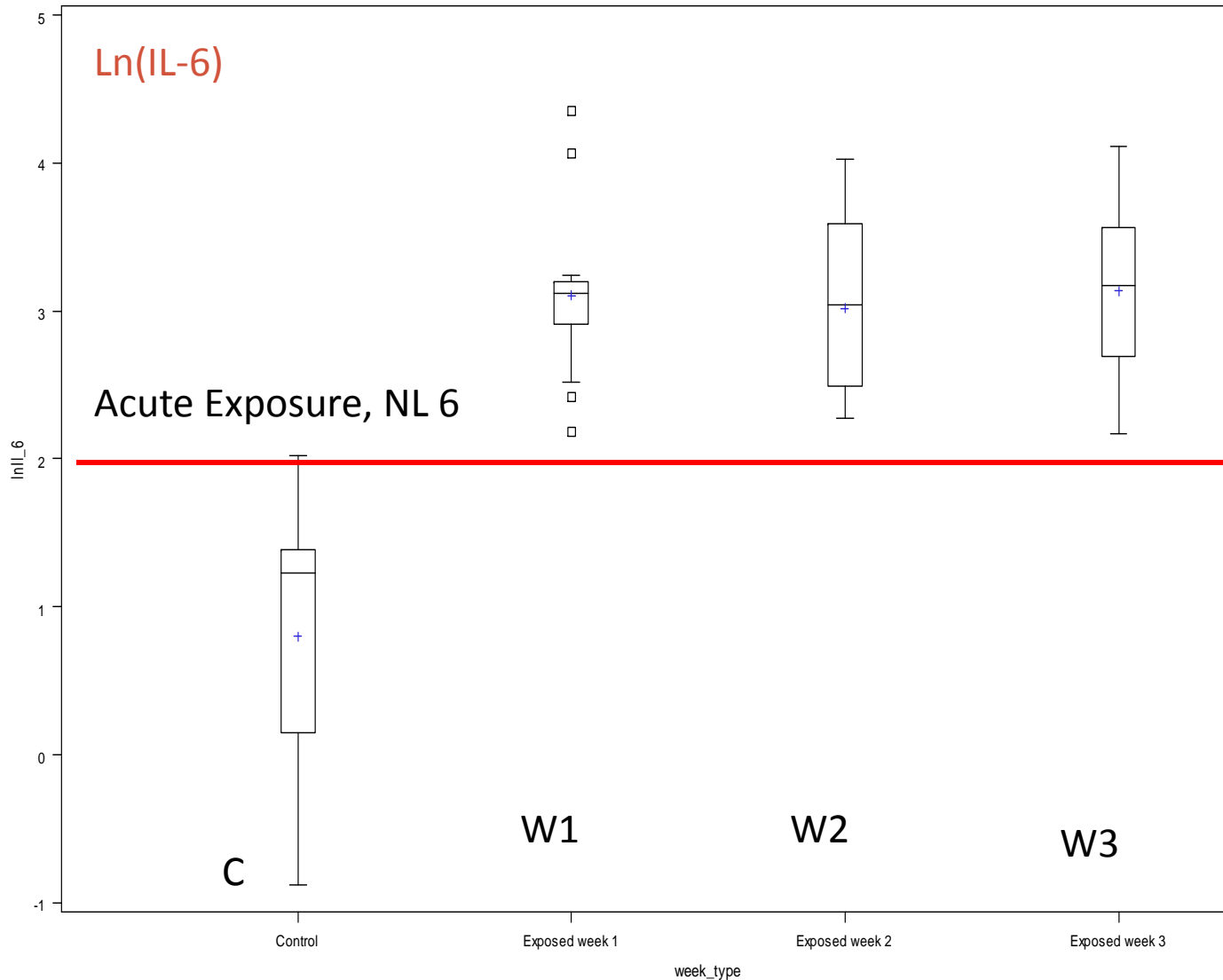
Summary of cytokine production

Khatri et al 2013, Inh Tox

Test System Cytokine	<i>In Vitro</i> (Fine particle dosing)			<i>In Vitro</i> (Nanoparticle dosing) Khatri et al 2013			<i>In Vivo</i>
	THP-1	Nasal Epithelial Cells	Small Airway Epithelial Cells	THP-1	Nasal Epithelial Cells	Small Airway Epithelial Cells	Nasal lavage of Healthy Volunteers (Khatri et al 2012)
	↑	↑	↑	↑	↑	↑	
IL-8	***	**	**	***	***	***	***
IL-6	**	-	-	***	-	-	***
IL-1β	*	-	-	**	-	*	***
GM-CSF	-	-	-	**	-	*	-
TNF-α	*	-		***	*	-	***
MCP-1	-	-	-	***	-	-	***
VEGF	-	-	-	*	**	**	***
IL-1α	-	-	-	-	***	*	-
G-CSF	-	-	-	-	-	-	***
Fractalkine	-	-	-	**	-	-	***
EGF	-	-	-	-	***	***	***
IFN-γ	-		-	-	*	-	-

Chronic Exposures, NL

5 exposed in 3 centers; 2-3 weeks & 8 controls



Same picture for:

- ↑ Total Protein
- ↑ IL-8
- ↑ TNF- α
- ↑ MCP-1
- ↑ 8-OH-dG

↓ G-CSF

Under-expressed

No change:

*VEGF,
IL-10,
Fractalkine,
EGF, IL-1 β*

Conclusions

- Approach is generalizable
- Suitable for a large number of life cycle scenarios of nano-enabled products
- It provides important context for risk and hazard assessment to Nanotech companies
- Important methodological insights for in vitro testing and comparative assessment
- Currently used to validate FRAS BOD as a screening assay



Questions?



Laser Printer Emissions & Human Health

“International foundation nano Control” database in Germany:

- 2,500 persons who claim to be sick from laser printer emissions
- 90% report respiratory tract complaints, glossalgia, chronic cough, rhinitis, and inflammation of the throat, tongue and paranasal sinuses
- ~ 30% developed asthma
- Inflammation of eyes, skin, diffuse pain and loss of hair



The hierarchical oxidative stress model

