Safe and Sustainable Nanotechnology: Using toxicology to benefit innovation and development

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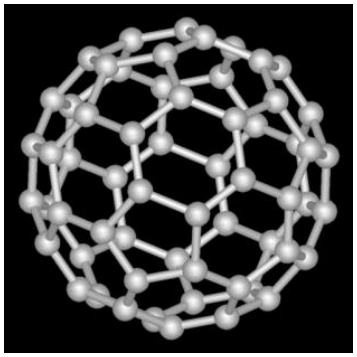
Toxicology Award Winner

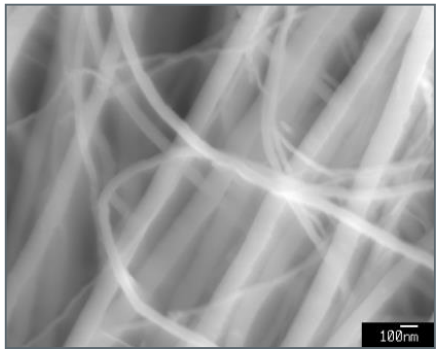
2015-16

LEADERS IN IDEAS AND SOLUTIONS

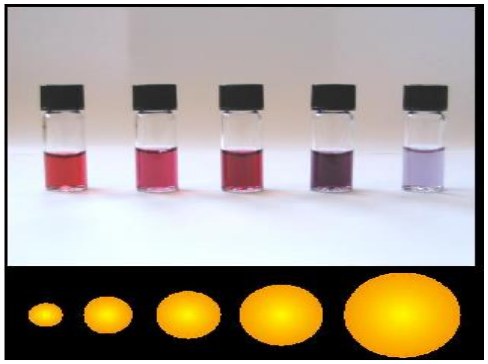
The diversity of nanomaterials



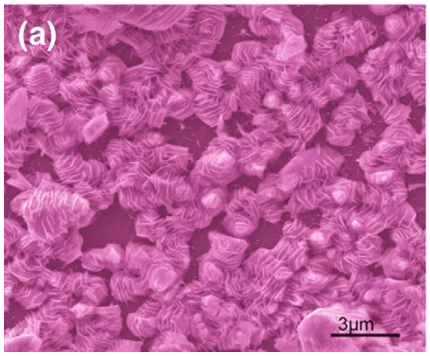
C60 Fullerenes



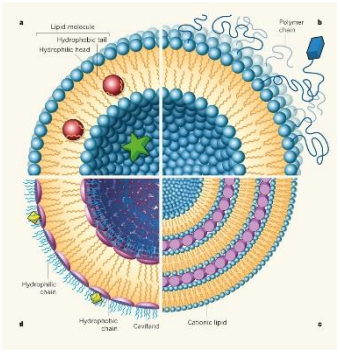
Carbon nanotubes



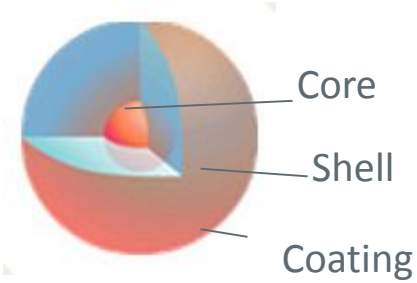
Nanoparticle gold



ZnO nano flowers



Liposomes



Quantum Dots

- Size, shape, charge…
- Matrices…
- Aged, weathered…
- Released…

Exposure to nanomaterials

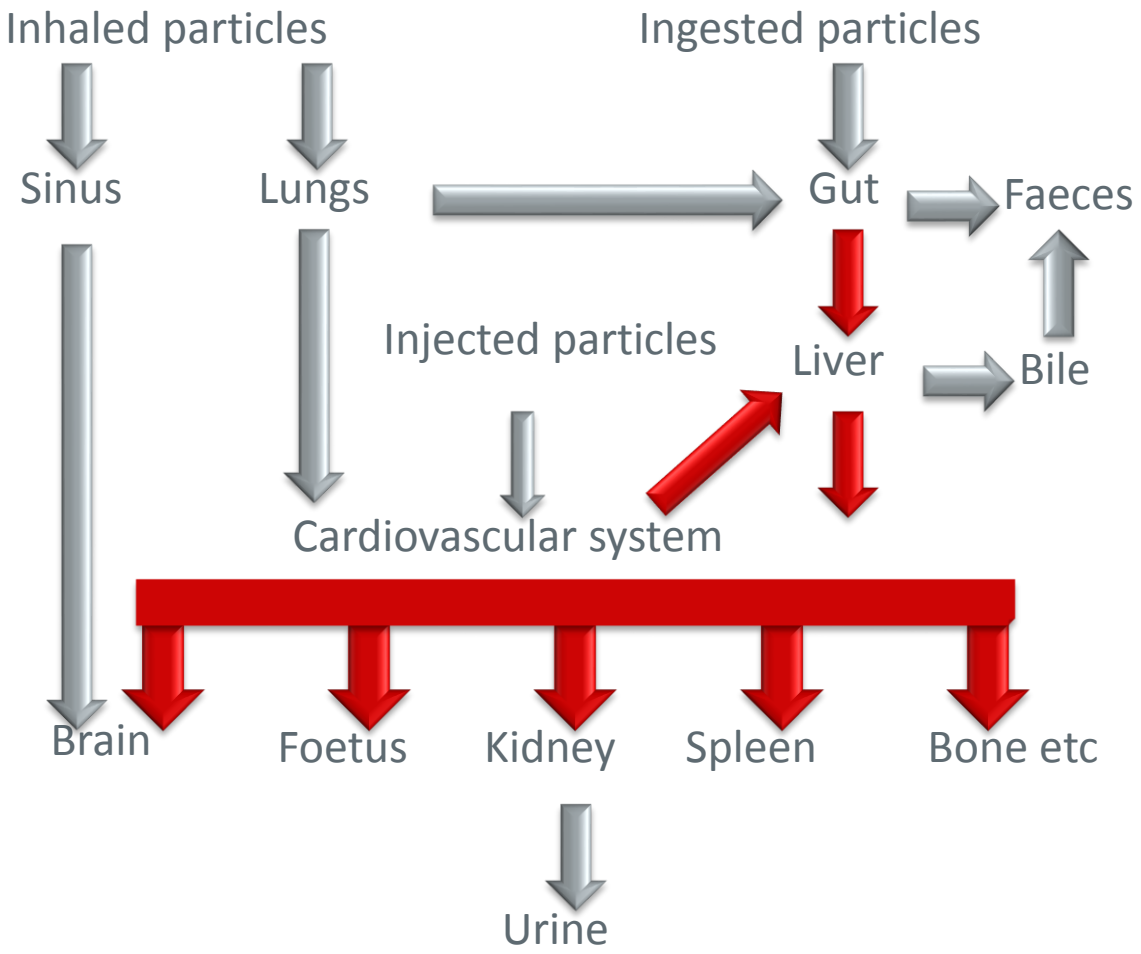
Human Exposure

Incidental ↑ Purposeful

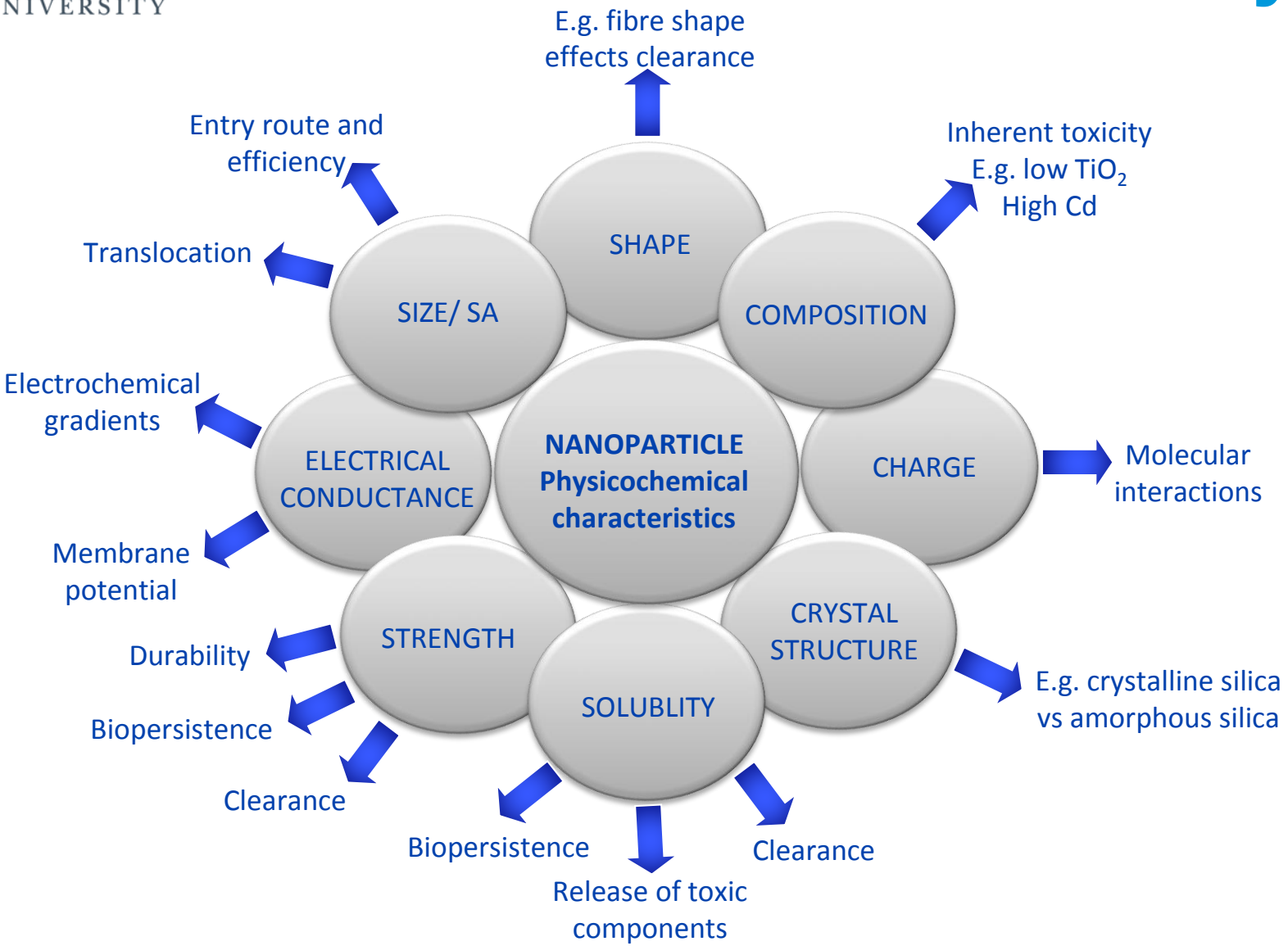
Incidental ↓ Purposeful

Environmental Exposure

Widening the routes and targets

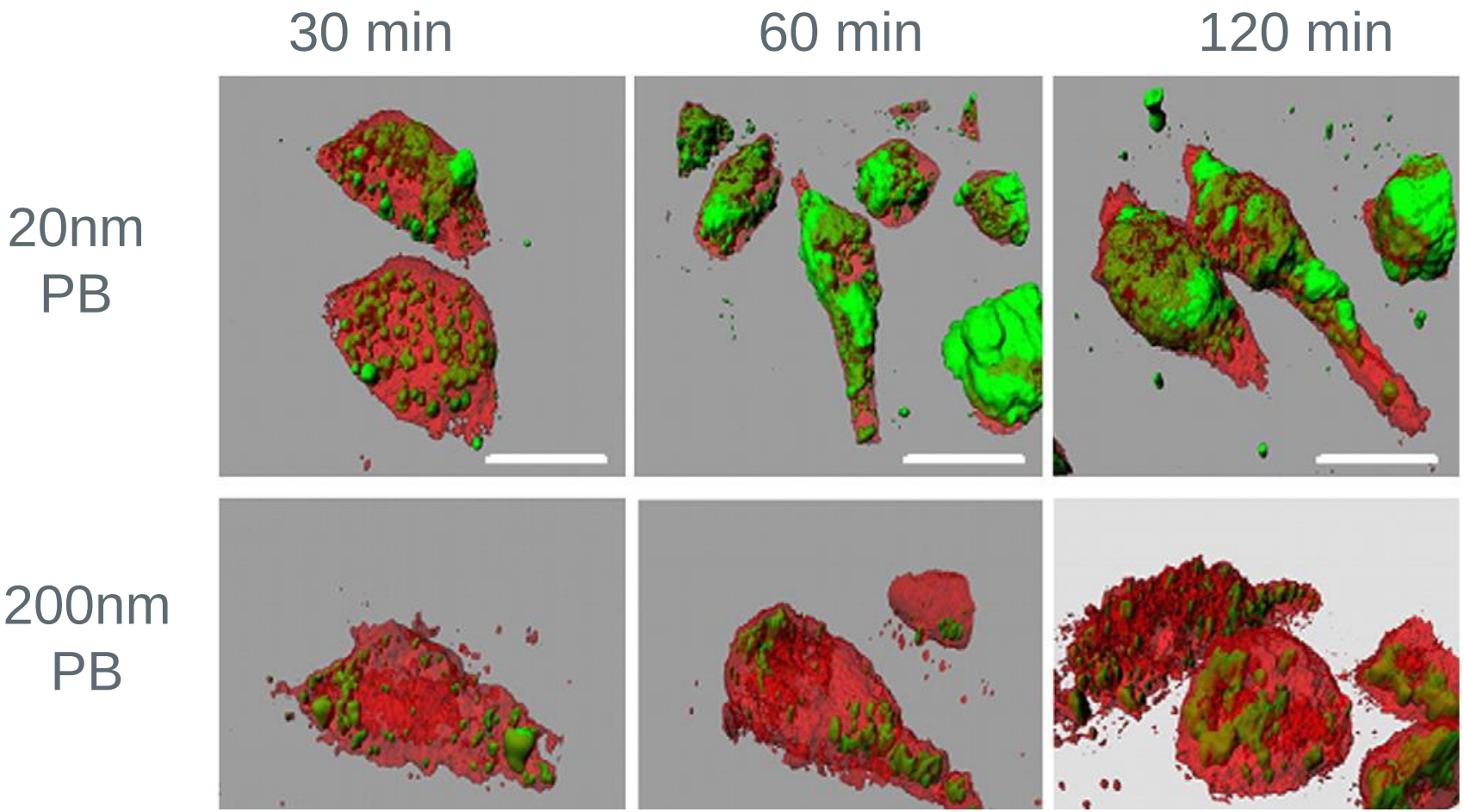


Characteristics vs Toxicity



Stone and Kinloch Nanotoxicology (book), 2007, Ed Monteiro and Tran.

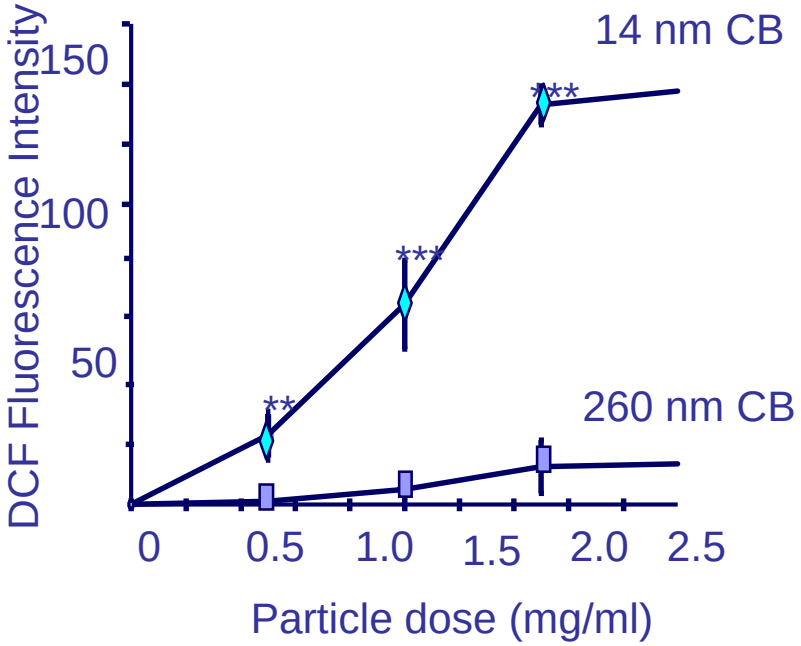
Size and uptake



Clift *et al*. 2008 TAAP 232; 418-427.

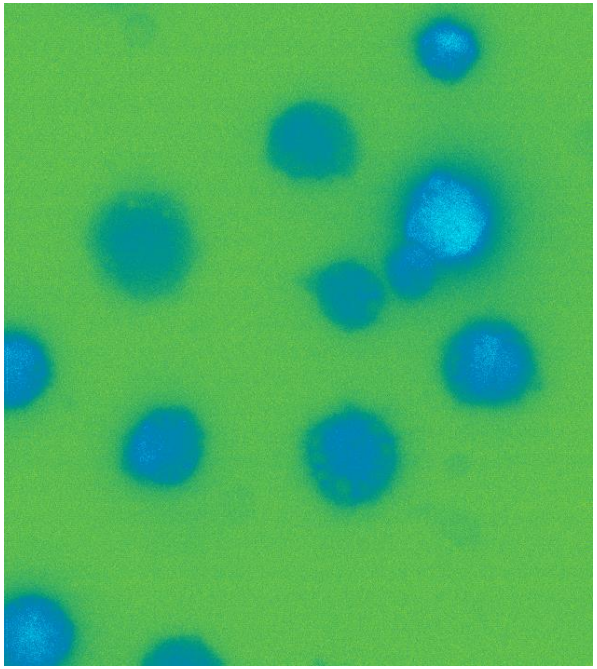
Surface and cell reactivity

Reactive oxygen species



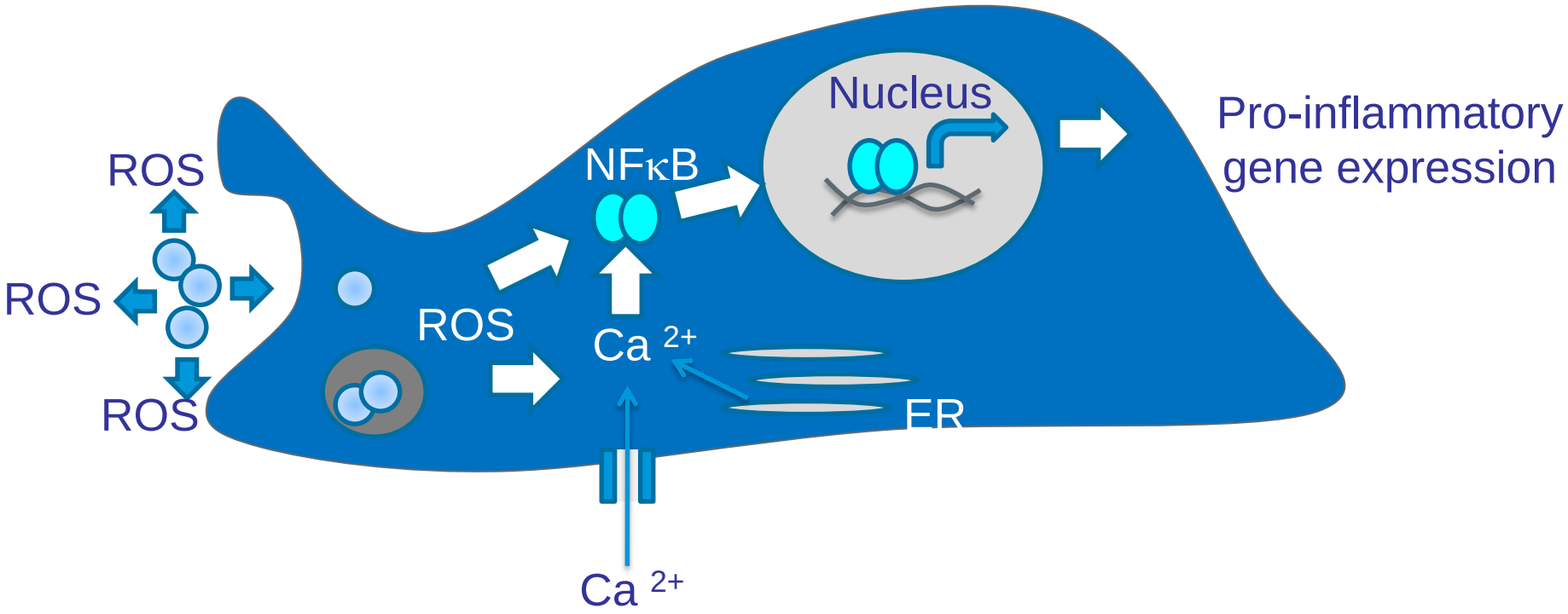
Wilson *et al.* 2002 TAP 184: 172-179.

Calcium signalling



Brown *et al.* 2004 AJP 286; L344-L353.

Understanding mechanism allows identification of ‘endpoints’ or biomarkers

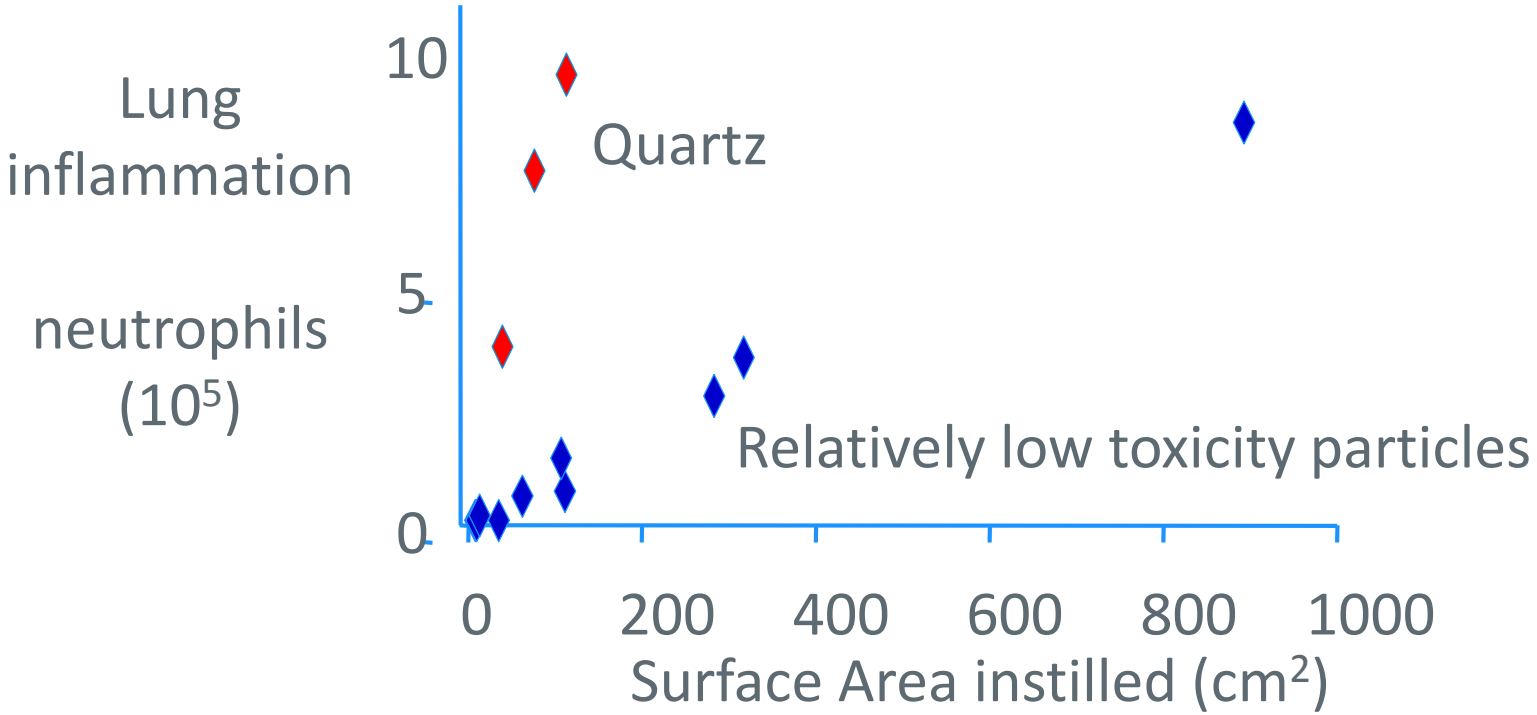


Surface area

Stone and Kinloch Nanotoxicology (book), 2007, Ed Monteiro and Tran.

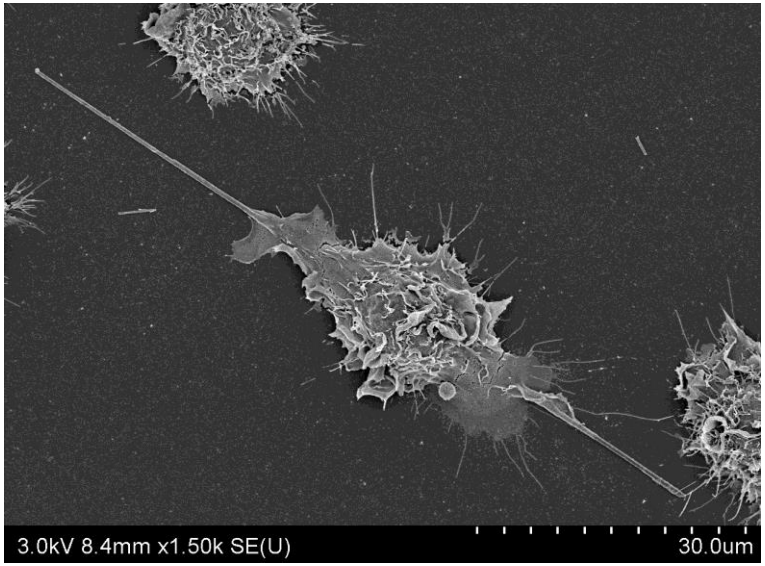
Diameter	100 μm	10 nm
Surface area	0.03 m^2/g	286 m^2/g
% atoms at surface	0.001 %	10.5 %

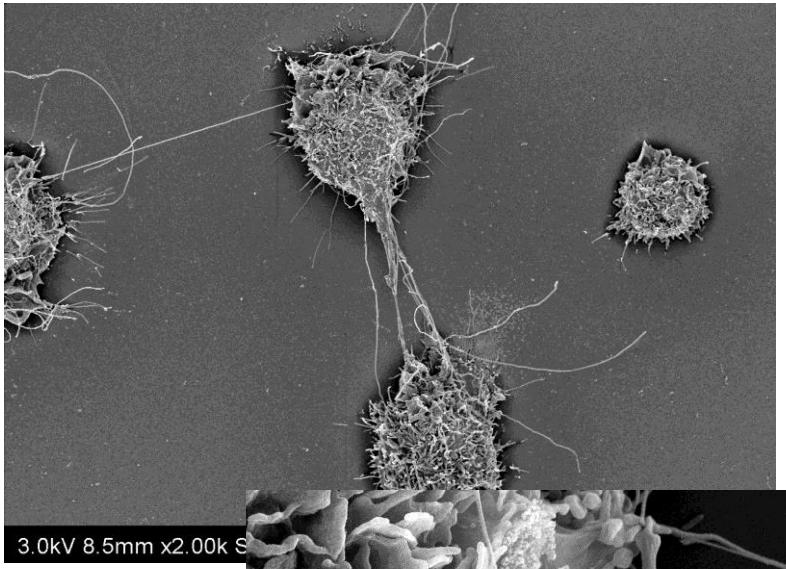
Surface area and reactivity

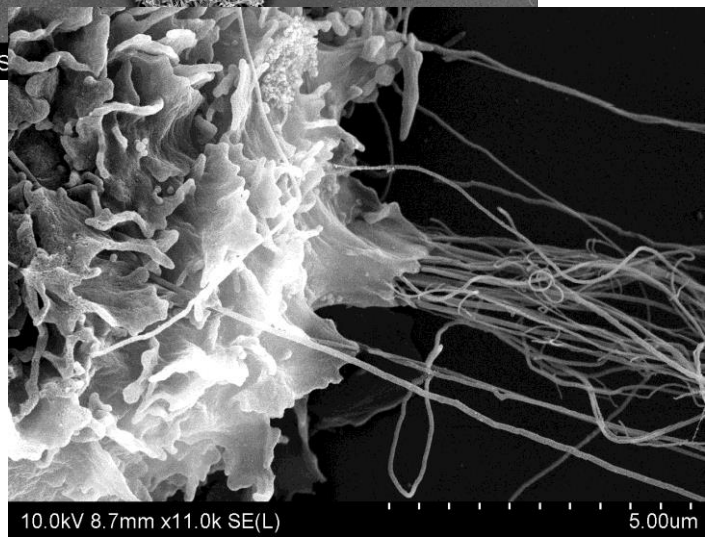


Duffin *et al*. 2002 Ann Occup Hyg 46 [suppl 1]; 242-245.

Carbon nanotube frustrated phagocytosis

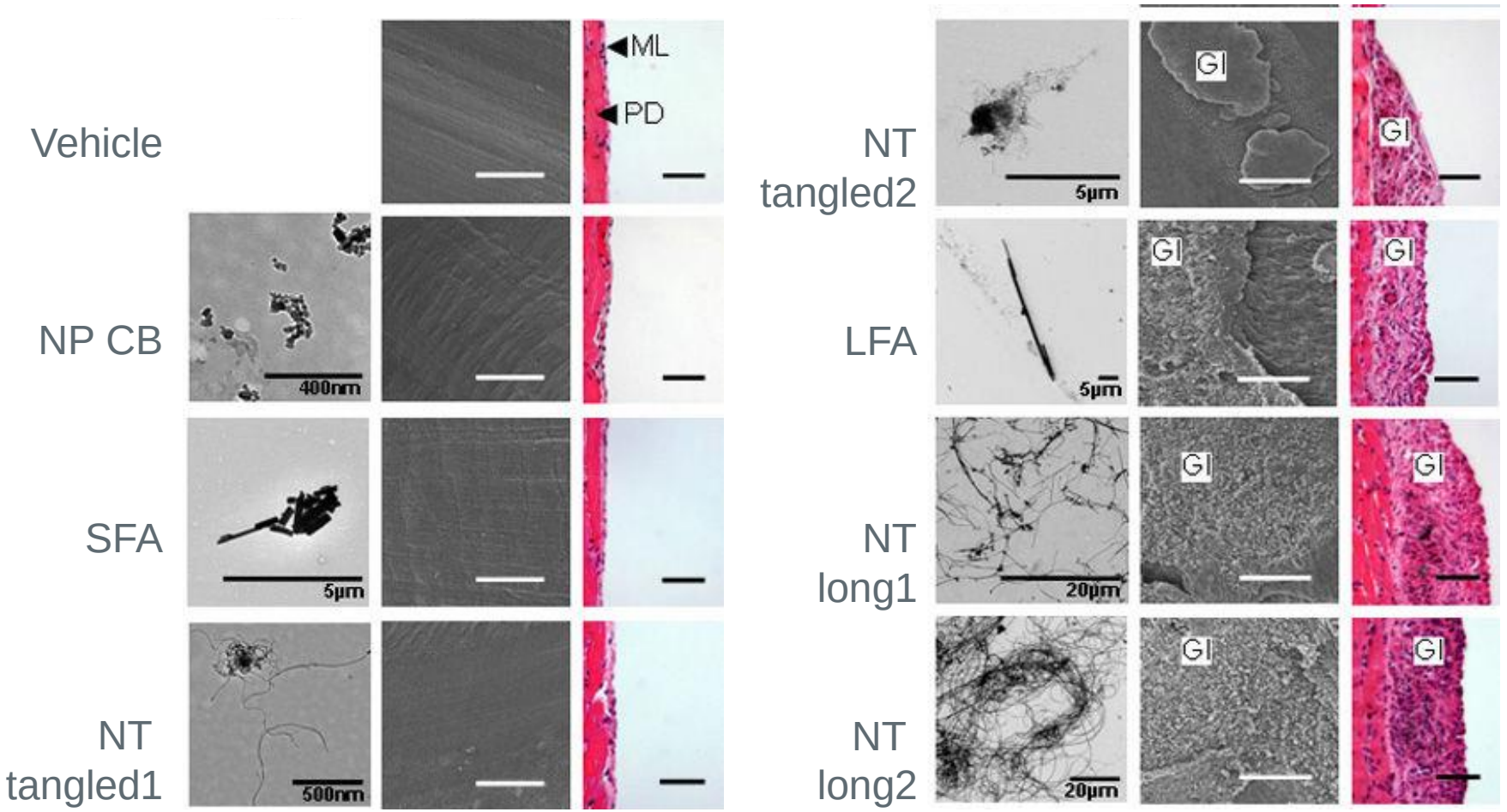






Boyles et al. 2015 Toxicology In Vitro 29

MWCNT induced pathology



Poland *et al*. 2008 Nature Nanotech 3, 423-428.

SUN Strategy to focus testing

- 8 NM to be assessed for exposure, hazard and RA
- All NM to be incorporated into a formulation (eg. Polypropylene, paint/coating or food)
- All formulations to be processed (e.g. cryomilled) to generate a fragmented product
- Safety-by-design approach to modulate NM properties and toxicity

SUN Strategy to focus testing

- Human and environmental hazard testing
  - Pristine NM and controls (e.g. soluble metals)
  - Fragmented NM/matrix
  - Fragmented matrix
  - $S_{by}D$ NMs
- Budget for hazard assessment was limited, therefore needed to be strategic regarding testing.
- Human hazard testing
  - *In vitro*
  - STIS
  - STOS

SUN Strategy to focus testing

1. What existing information is available (gap analysis)?
2. What are the likely uses of each NM/matrix case study?
3. What exposure scenarios (and therefore routes) are most likely for each case study?
4. Which hazard models best reflect the most likely exposure routes?
5. Identify priority NM for testing
   - Little info existing
   - Likely to be of high use
   - Exposure likely
   - Toxicity likely
   - *In vitro* toxicity observed

6. One material chosen to be tested in all hazard models
7. One *in vitro* model chosen to test all NMs

SUN Strategy to focus testing

SUN strategy combines:

- **Flexible Intelligent Testing Strategy**
- **Integrated Testing Strategy**
- **Alternative Testing Strategies**
- **Interpolation (data mining)**
- **Common sense approach**

Particle prioritisation

	Human Toxicity and Ecotoxicity testing	Inhalation and oral for FP?
CNT-PP car parts	CNT –PP car parts	Not a priority
Org.pig-PP car parts	STIS exists. Food grade. May not be respirable. Tiered approach starting with macrophage.	STOS?
F_2O_3-PE products	Too close to organic pigment, therefore not a priority	Not a priority
SiO_2 in food	Pancake feeding study with varied SiO_2 content. Link to nanodefine in terms of lowest detectable dose. Check gap analysis before deciding in vitro strategy.	STOS?
WCCo-lowfriction	Dominated by Co? Simple in vitro screen to compare WC, Co and WCCo. (papermill coating to drums, applied annually by spray)	In vitro only

Particle prioritisation

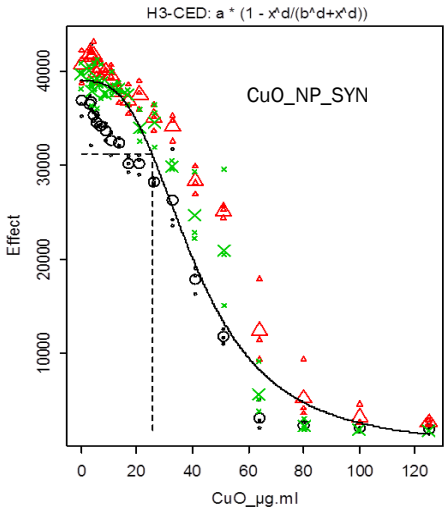
CASE STUDIES	Human Toxicity and Ecotoxicity testing	Inhalation and oral for FP?
TiO_2 photo-catylatic tiles	*Not priority*	*Not a priority*
CuO wood protection	CuO - Go ahead with STIS, STOS, macrophage, hepatocyte. Dermal information in literature.	STIS and STOS for pristine, and either FP or SbyD
CNT-epoxy marine coating	CNT epoxy marine coating – STIS and 90 day and feeding studies exist for CNT. Require epoxy control. Use a tiered approach with macrophages as first screen.	STIS of FP. STOS of FP.

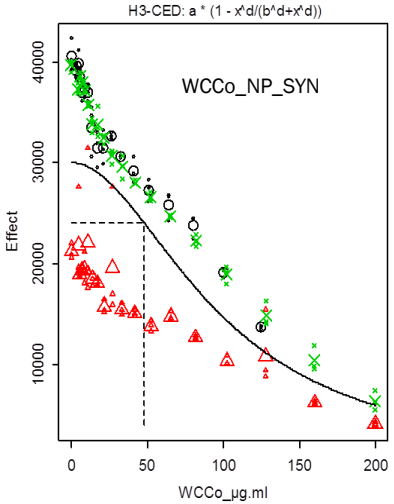
SUN
In vitro screening Macrophage and Hepatocyte models

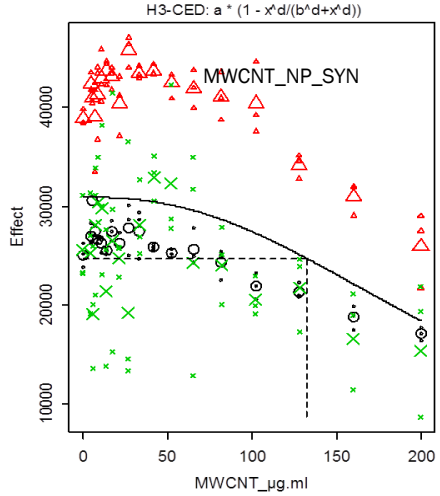
Heriot-Watt University
Karolinska Institute

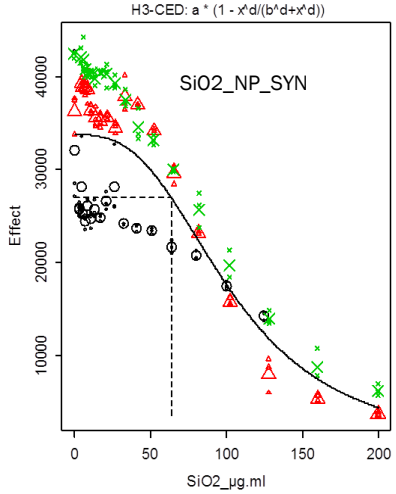
In vitro macrophage responses

- **RAW264.7 macrophages**
  - Alamar blue assay
  - BMD20 and EC50 by PROAST 38.9
    - Tested concentrations: 0-125 µg/ml
  - Cytokine release at concentrations of 0.5xBMD20, 1xBMD20 and 2xBMD20
    - TNF-α, IL-6, IL-1β, IL-10, IL-12, MCP-1 (MCAF; CCL2), MIP-1β (CCL3), MIP-1α (CCL4), RANTES (CCL5), KC (IL-8, CXCL8).



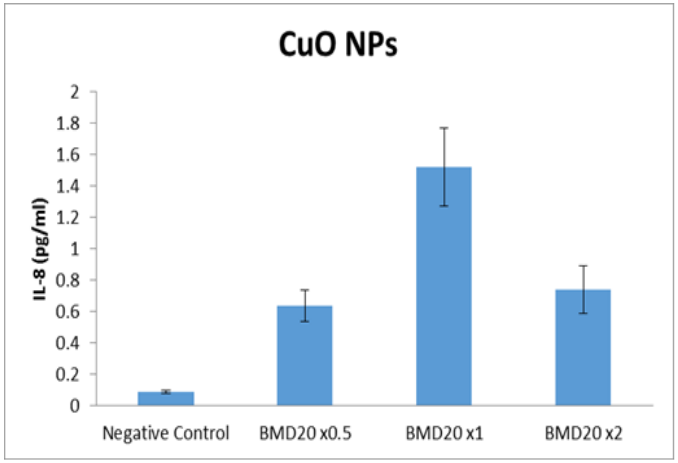


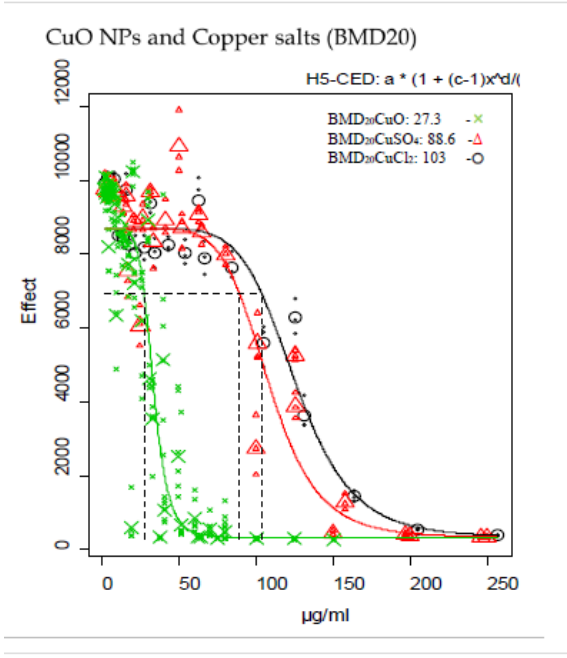


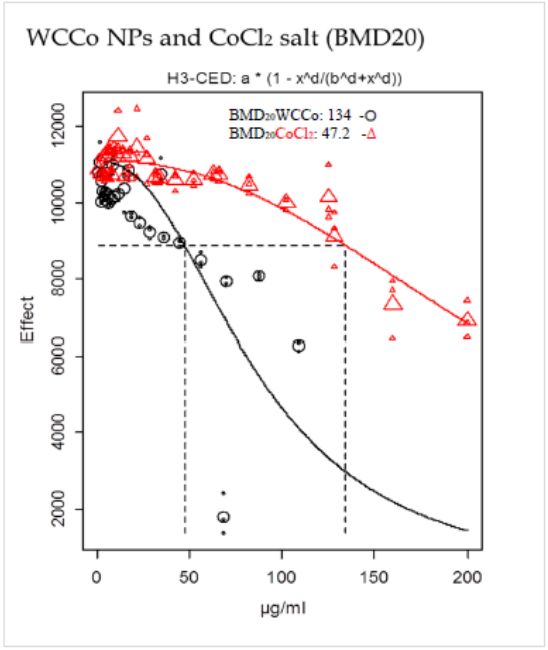


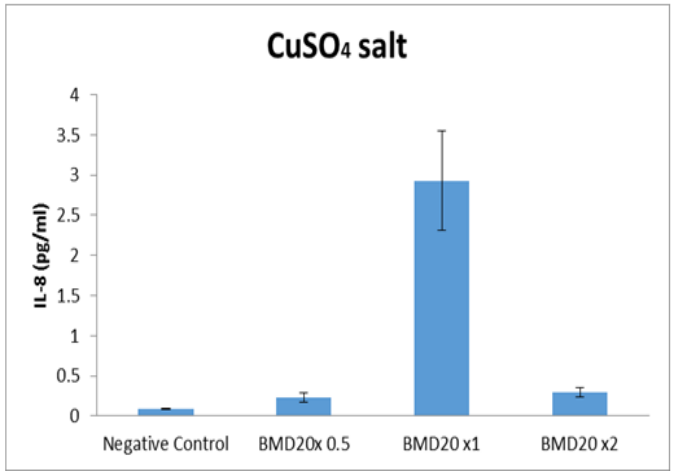
In vitro hepatocyte responses

- C3A hepatocytes
  - Alamar blue assay
  - BMD20 and EC50 by PROAST 38.9
    - Tested concentrations: 0-125 µg/ml
  - Cytokine release at concentrations of 0.5xBMD20, 1xBMD20 and 2xBMD20
    - TNF-α, IL-6, IL-1β, IL-10, IL-12, MCP-1 (MCAF; CCL2), MIP-1β (CCL3), MIP-1α (CCL4), RANTES (CCL5), KC (IL-8, CXCL8).









In vitro macrophage responses

NOAA	Time point	BMD_{20} (µg/ml)	EC_{50} (µg/ml)
CuO_1_NP_SYN	24h	25.50	40.97
$CuCl_2$	24h	55.60	63.91
WCCo_1_NP_SYN	24h	48.10	98.08
$CoCl_2$	24h	32.70	58.54
MWCNT_1_NP_SYN	24h	132	>200
MWCNT_1_NP_SYN	48h	>200	>200
Pigment_1_NP_SYN	24h	123	>125
Fe2O3_1_NP_SYN	24h	>125	>125
SiO2_1_NP_SYN	24h	63.70	103.3
TiO2_1_SOL_MPG_SYN	24h	>200	>200
TiO2_2_SOL_HCl_SYN	24h	>200	>200

In vitro hepatocyte responses

NOAA	Time point	BMD_{20} (µg/ml)	EC_{50} (µg/ml)
CuO_1_NP_SYN	24h	25.8	32.54
$CuSO_4$	24h	88.6	112
WCCo_1_NP_SYN	24h	157	235
$CoCl_2$	24h	47.2	98
Pigment_1_NP_SYN	24h	240	276
SiO2_1_NP_SYN	24h	229	273

CuO nanoparticle – Industrial collaboration

Tiered strategy

Stage 1: Identify existing data

Stage 2: Prioritise NM
- lacking data,
- likely to be toxicity due to chemistry,
- potential for exposure and
- high volume of use

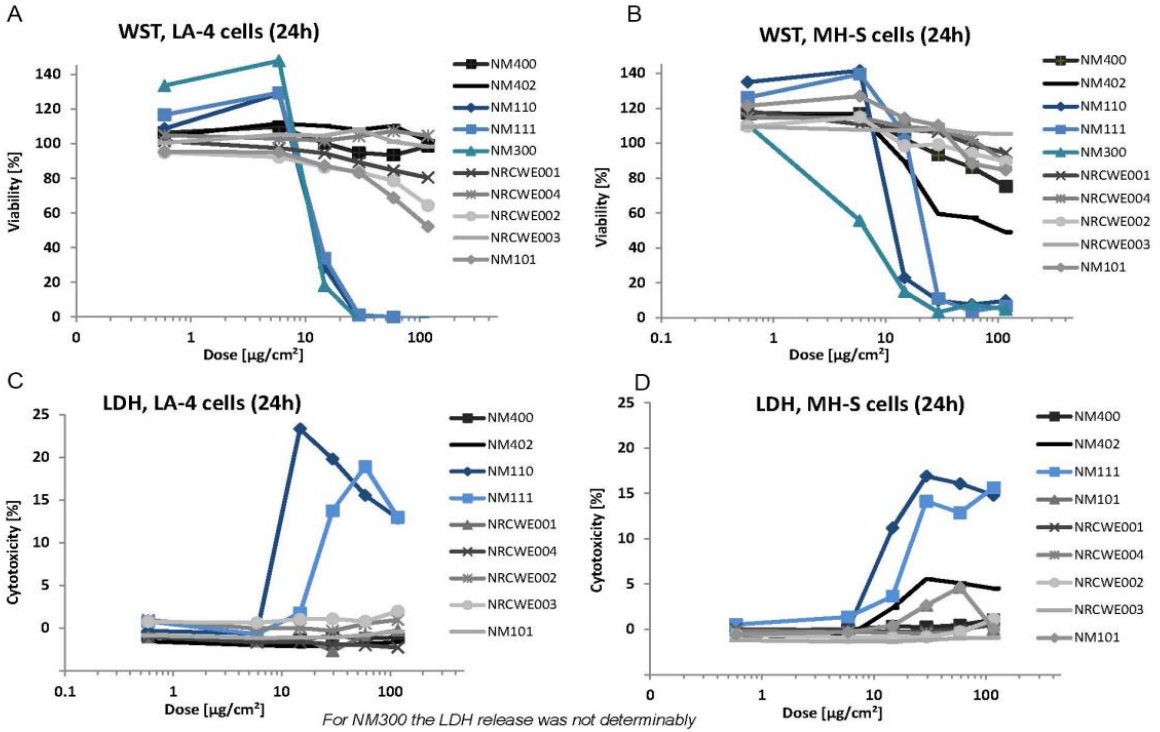
Stage 3: Assess toxicity *in vitro* with macrophages and hepatocytes, compared to other nanomaterials and relevant salts.

Stage 4. Use *in vitro* data to design dose range for rodent inhalation study

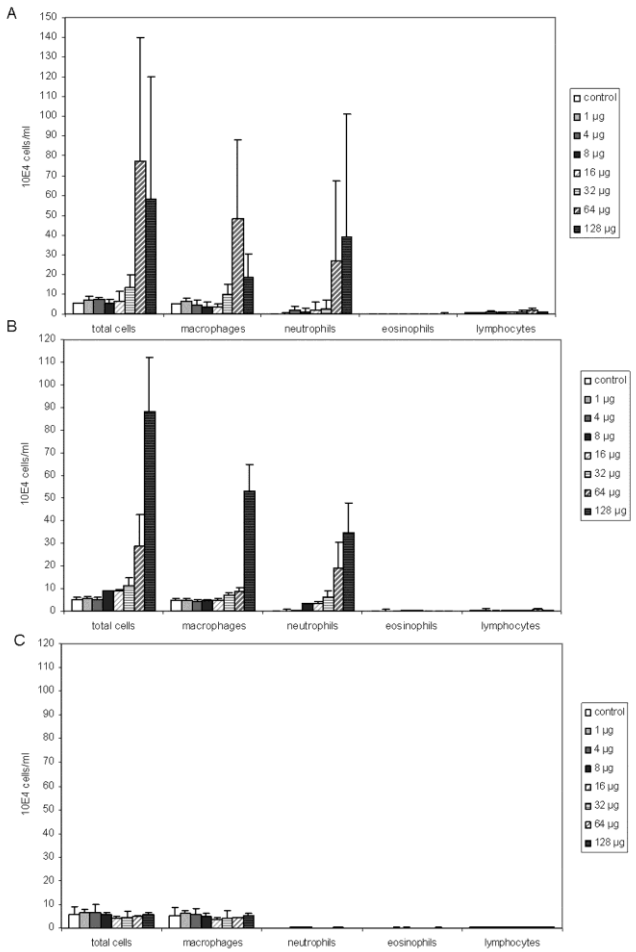
CuO

ENPRA data

In vitro



In vivo



Used *in vitro* and *in vivo* data from ENPRA combined with in vitro data from SUN to decide upon *in vivo* dose range

SUN
Short Term Inhalation Studies Nano CuO pristine and $S_{by}D$ candidates

RIVM

Study protocol STIS

Klein et al., Arch Toxicol (2012) 86: 1137-1151

DAY	-3	-2	-1	1	2	3	4	5	6	7	8-27	28	29
				EXP	EXP	EXP	EXP	EXP	EXP	REC	REC	REC	
TOX	train	train	train	x	x	x	x	x	B+H			B+H	
KIN		train	train	train	x	x	x	x	x	O			O

TOX - toxicological assesssment

KIN - bio-kinetics assessment

Train - training of rats in nose-only tubes

EXP - exposure to CuO

REC - recovery period

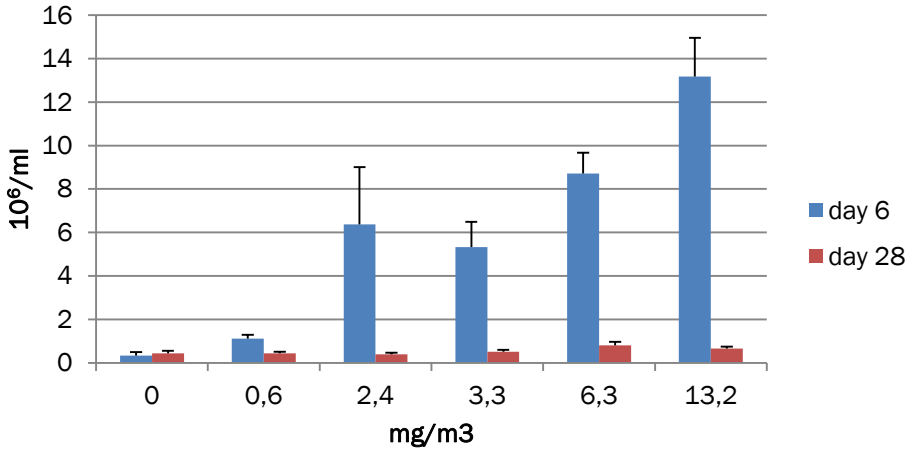
B - BALF

H - Histology

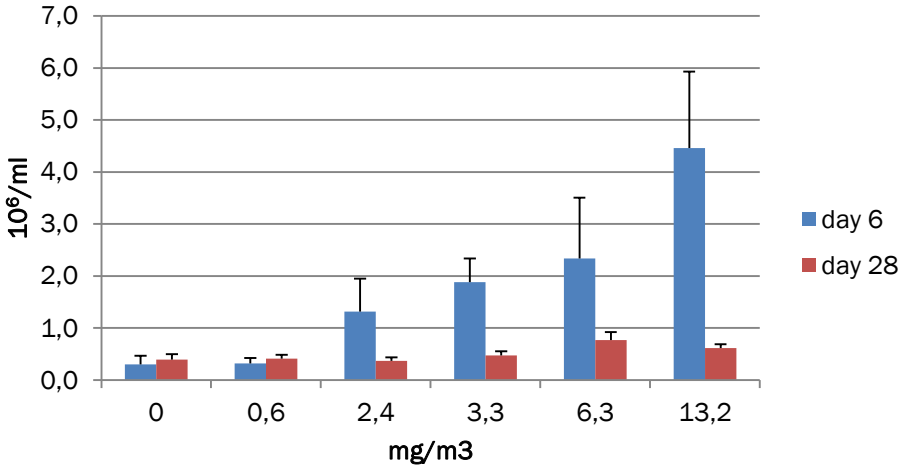
O - Organs harvested for Cu content

Inhalation hazard of Pristine CuO NM

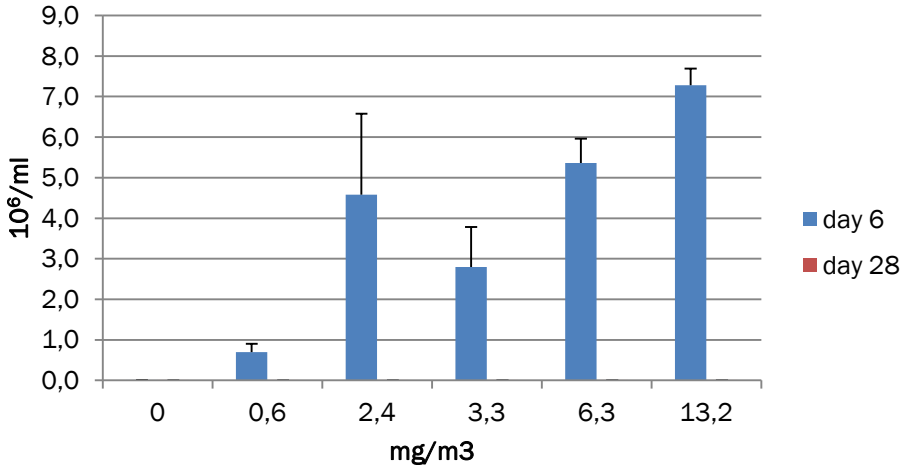
Total cell number BALF



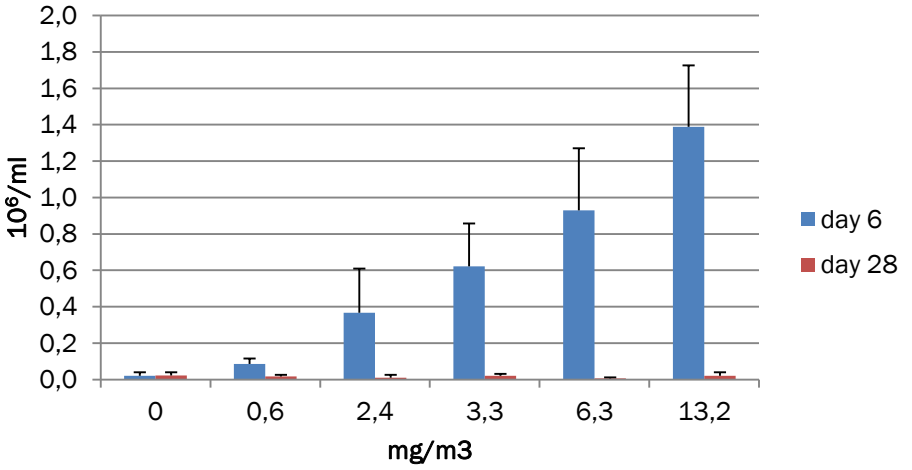
macrophages BALF



neutrophils BALF



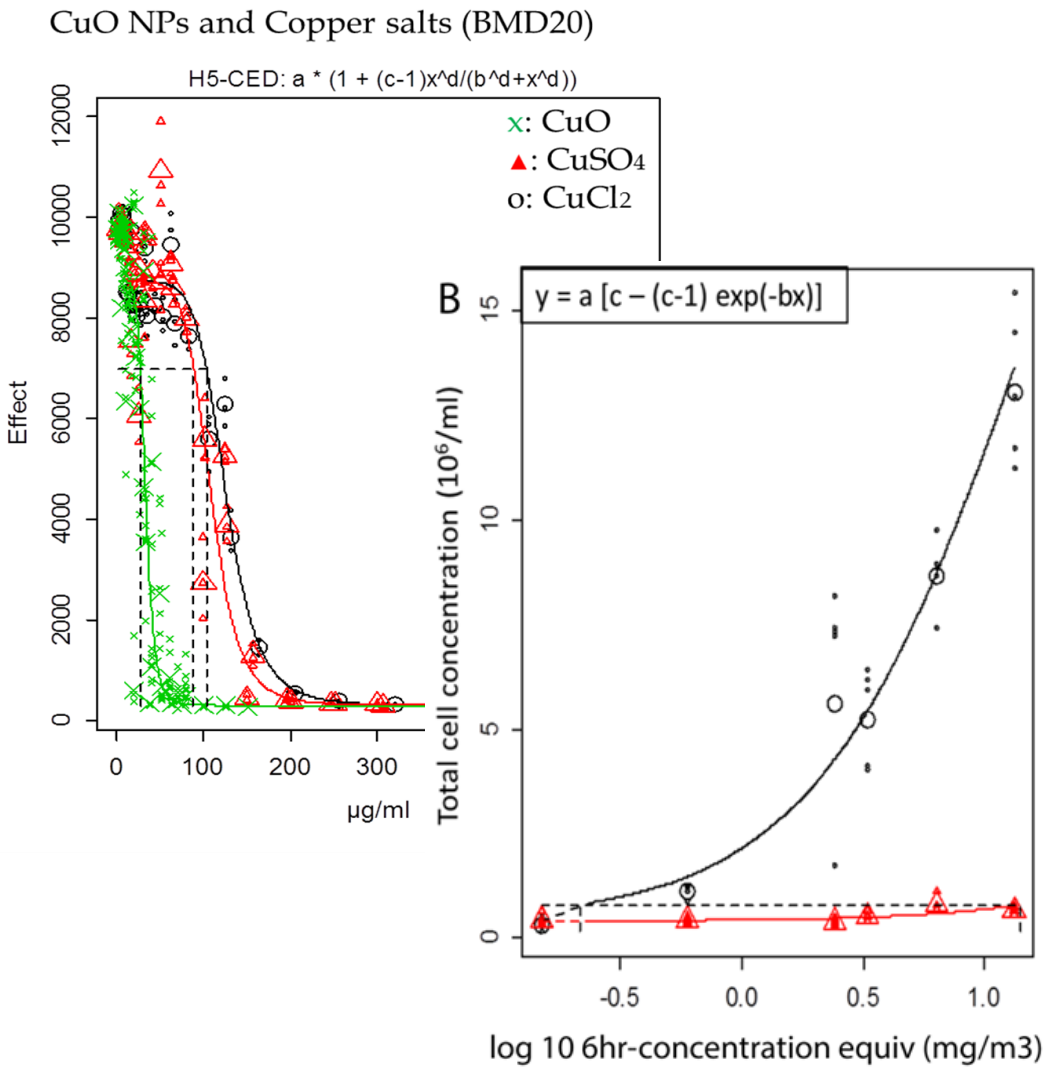
lymphocytes BALF



CuO Pristine summary

Tiered strategy results pristine CuO

- Relatively toxic *in vitro* compared to other NM and Cu salts
- Produced inflammation in lung at day 6
- Inflammation largely resolved by day 28, but small elevation of macrophages remains



CuO nanoparticle – in polymer matrix

- Within product, CuO NPs are embedded in a polymer matrix
- Impossible to generate fragmented particles at room temperature.
- Only possible to generate fragmented particles in very cold conditions while wet.
- This suggests exposure to fragmented particles containing CuO NPs is likely to be low or unlikely
- Therefore risk to consumers/workers once incorporated should be low
- Eventually generated FP which are awaiting testing.
  - Requires inverted culture due to low density of polymer

STIS modified CuO NP

Surface coatings

- Ascorbate (ASC, negative) coating suggested by WP6
- Also provided citrate (CIT),
- polyethyleneimine (PEI)
- polyvinylpyrrolidone (PVP)

Safety by Design

Details	Label	BMD_{20}
Pristine	CuO	33.2
Buffer	101	18.7
Citrate	102	25.7
PVP	103	16.6
PEI	104	20.4
Ascorbate	105	64.8

Phosphate buffer

Surface modification

In vitro screening identified

PEI-capped > uncapped NPs > PVP-capped > CIT-capped > ASC-capped

PEI and ASC tested *in vivo* via STIS

STIS modified CuO NP

Day 6

- Both induced dose dependent inflammation at day 6
- Tissue cytokine protein analysis at day 6
  - PEI CuO NP increased IL1α, IL1β and MIP2
  - ASC CuO NP did not increase IL1α or IL1β, but did increase MIP2

Day 28

- PEI – residue of inflammatory cells at day 28 (similar to pristine)
- ASC – no inflammatory cells at day 28

Conclusion

- ASC coating appears to dampen CuO NP induced inflammation in lung

Summary

- A range of characteristics influence toxic potential
- Gap analysis combined with consideration of:
  - NM uses
  - Exposure scenarios and incidence
  - *In vitro* toxicology (using existing protocols)
- Data mining, ITS, ATS, common sense
- In vitro models (macrophage and hepatocytes) generated ranking data and refinement data to inform *in vivo* studies
- STIS
  - CuO pristine, dose dependent inflammation at 6 days, largely resolved at day 28.
  - CuO ascorbate modification decreased inflammation

Conclusions

- Toxicity information can be used to prioritise decision making for product use and for design
- Intelligent testing strategies including Alternative testing strategies are useful to improve efficiency and ethics of NM safety assessments

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