SmartNanoTox

SmartNanoTox Project Online Conference, 24 June 2020

Mechanisms of toxicity related to surface activity, and shape-induced cell-particle interaction

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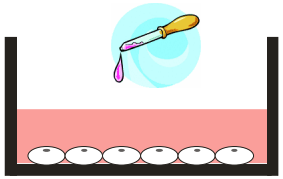
SmartNanoTox
Smart Tools for Gauging Nano Hazards

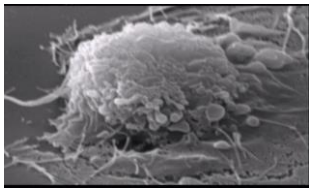
Horizon 2020
European Union funding
for Research & Innovation

Cells, cytotoxicity



In vitro, submerged

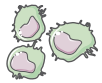
NR8383, rats

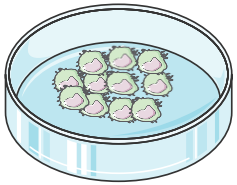


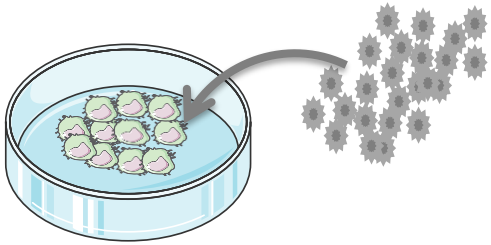
THP-1, human

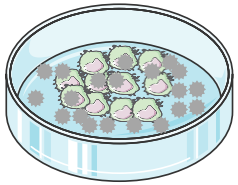
PMA

Macrophages M0
THP-1









T-24

T0

Exposition to Nanomaterial

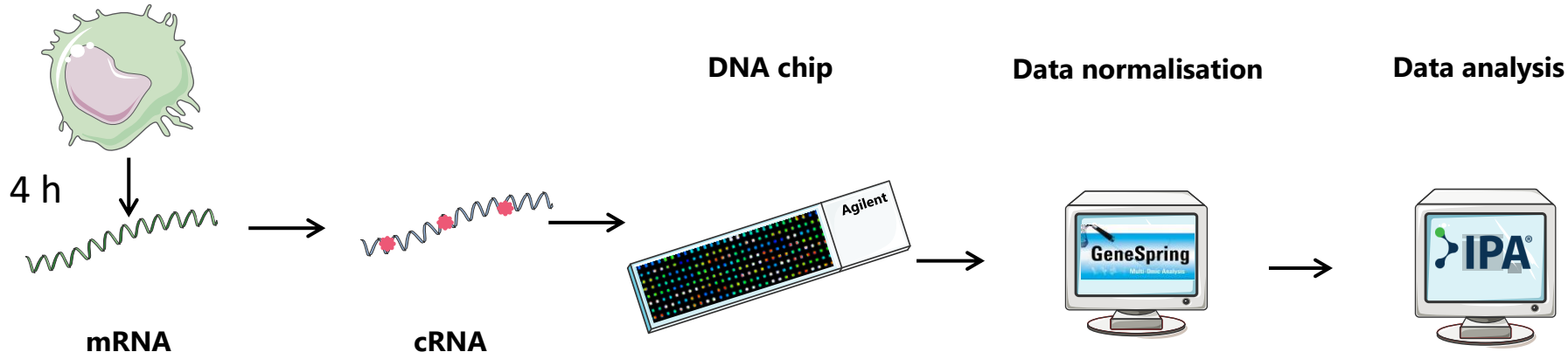
T+24

Viability/cytotoxicity assays
LDH, WST-1, Alamar blue

IC_{50}

Transcriptomic protocol, simplified

¼ IC_{50} Nanomaterial



Binding to cyanin-3

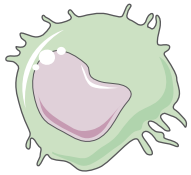
Hybridization

Benjamini Hochberg test
p-value < 0,001
FC > I1,5I

Regulated pathways
p-value < 0,05
or
P-value < 0,001

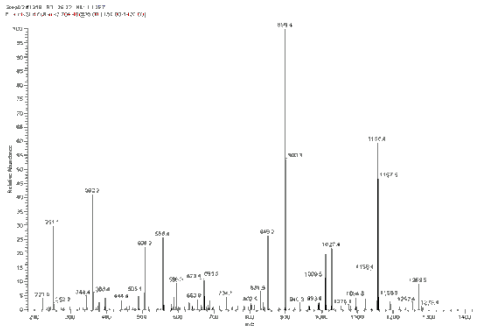
Proteomic protocol, simplified

¼ IC_{50} Nanomaterial



24 h

Proteins



Mass Spectrometry

Data analysis

Ingenuity Pathway Analysis

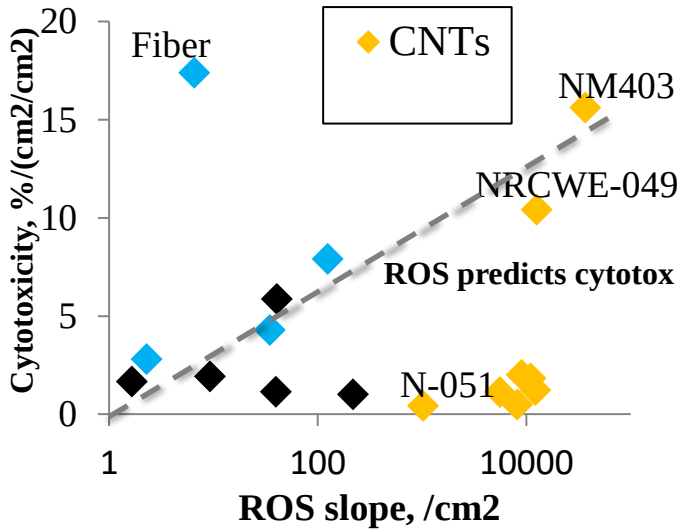
IPA®

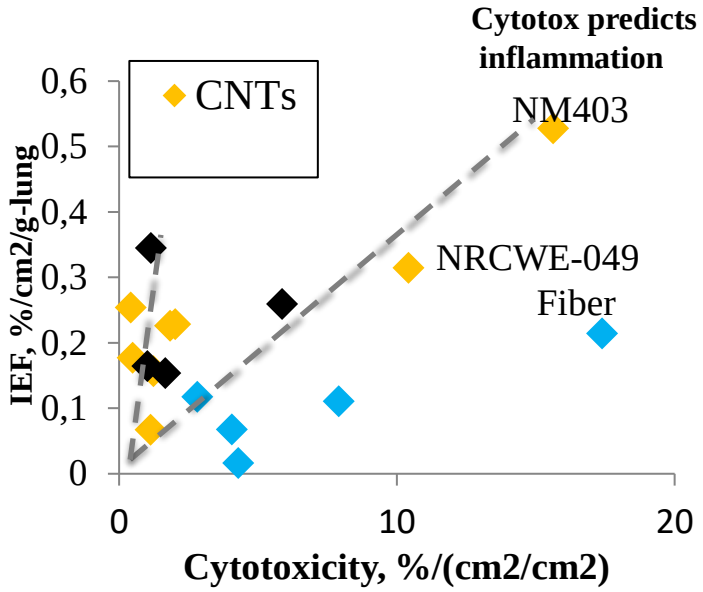
STRING

Functional protein association networks

Biomolecular and biomedical Research (Ireland)
H. Cassidy et D. Gomez

ROS–Cytotoxicity/Cytotoxicity-Inflammatory Correlations





- Not all of the observed cytotoxicity (reduction in viability) is due to nanomaterial-induced ROS
- Cytotoxicity (viability) predicts acute lung inflammation (mouse), but only for some of the nanomaterials

- 3 Enrichment score 6

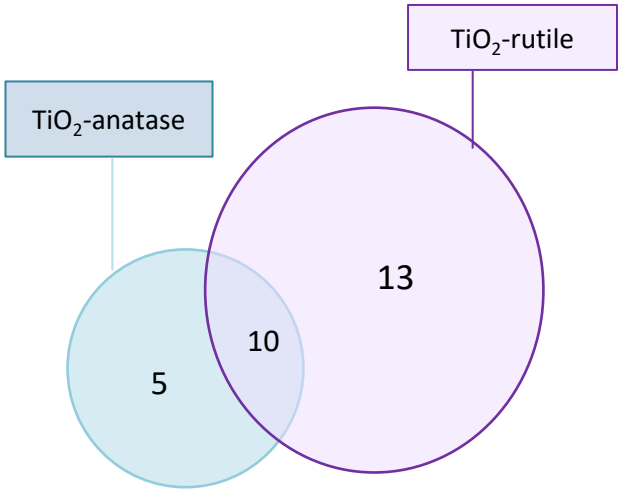
No activity pattern available

Normal regulation

Response		Enrichment score	
Functional categories	Canonical pathways	ZnO NM110	$ZnFe_2O_4$ NRCWE-021
Stress response	• Mitochondrial dysfunction		
	• Oxidative phosphorylation		
	• mTOR signaling		
	• NRF2-mediated oxidative stress response		
	• Sirtuin signaling		
Cell cycle/ proliferation	• PI3K/AKT signalling		
	• VEGF signaling		
Protein synthesis/ modification	• EIF2 signaling		
	• Regulation of eIF4 and P70S6K signaling		
	• Protein ubiquitination pathway		
	• Unfolded protein response		
Cell mobility	• Paxillin signaling		
	• Integrin signaling		
Lipid homeostasis	• Superpathway of cholesterol biosynthesis		
Cell modelling	• Actin cytoskeleton signaling		
Cancer	• Cancer drug resistance by drug efflux		
Metal exposure response	• Iron homeostasis signaling pathway		

Doumandji et a., Cell Biol Toxicol, 2020

TiO2, anatase vs rutile, dysregulated genes in NR8383



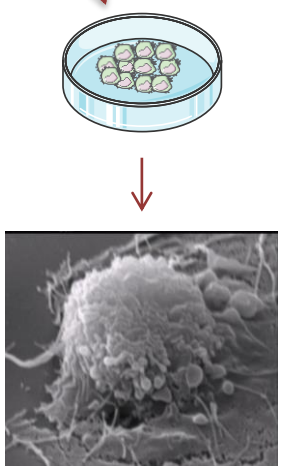
p-valeur < 0,001, FC > 20

Genes	Genes name	FC (exposure to anatase)	FC (exposure to rutile)
Cxcl2	Chemokine (C-X-C motif) ligand 2	63.36	71.32
Slpi	Secretory Leukocyte Peptidase Inhibitor	51.27	31.19
Slpil3	Secretory Leukocyte Peptidase Inhibitor 3	49.57	28.27
Tac4	Tachykinin 4	42.53	56.20
Tbkbp1	TBK1 Binding Protein 1	23.24	24.43
Phf19	PHD Finger Protein 19	20.03	20.33
Pla2g2d	Phospholipase A2 Group IID	-31.56	-56.16
Dynlt3	Dynein Light Chain Tctex-Type 3	-31.57	-83.03
Cybb	Cytochrome B-245 Beta Chain	-36.61	-85.31
Calcr	Calcitonin Receptor	-36.77	-100.62

TiO_2 :3 cm^2/cm^2

Submerged

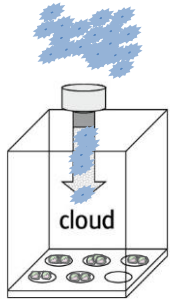
TiO_2 anatase



4h

Air-Liquid Interface

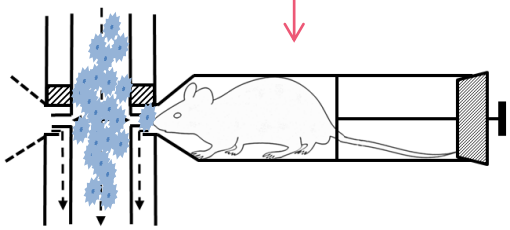
TiO_2 anatase



4h

Nose-only

TiO_2 anatase



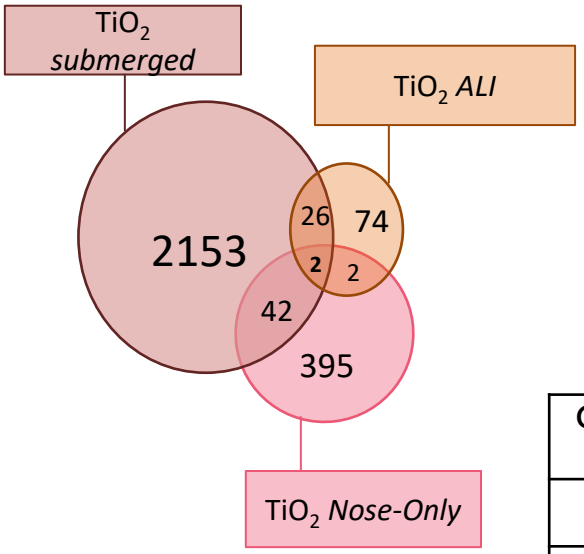
Exhaust

Exposition for 28 j, 6h/j, 5j/s

J 0 - 6 h post-exposition

Transcriptomic

L Gaté
C Seidel
L Chezeau



p-value < 0,05, FC > 1,5

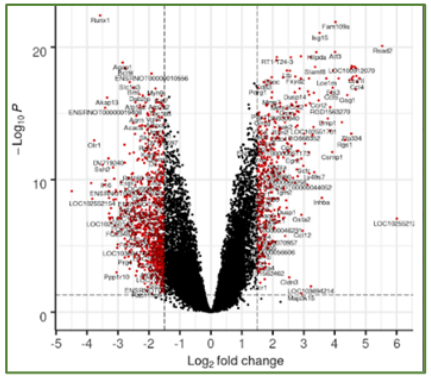
Gene ID	FC *sub*	FC *ALI*	FC *nose*	Protein	Biological fonction
Ccl4	3,92	2,12	1,76	C-C Motif Chemokine Ligand, 4	inflammatory response
Cxcl2	63.36	3,16	3.72	C-X-C Motif Chemokine Ligand 2	chimiotaxis, inflammatory response
Ccl3	3.56	__	1.94	C-C Motif Chemokine Ligand, 3	inflammation
Mmp7	2.72	__	2.66	Metalloproteinase 7	cell division, inflammation
Ccl7	__	1,53	4,8	C-C Motif Chemokine Ligand, 7	inflammatory response

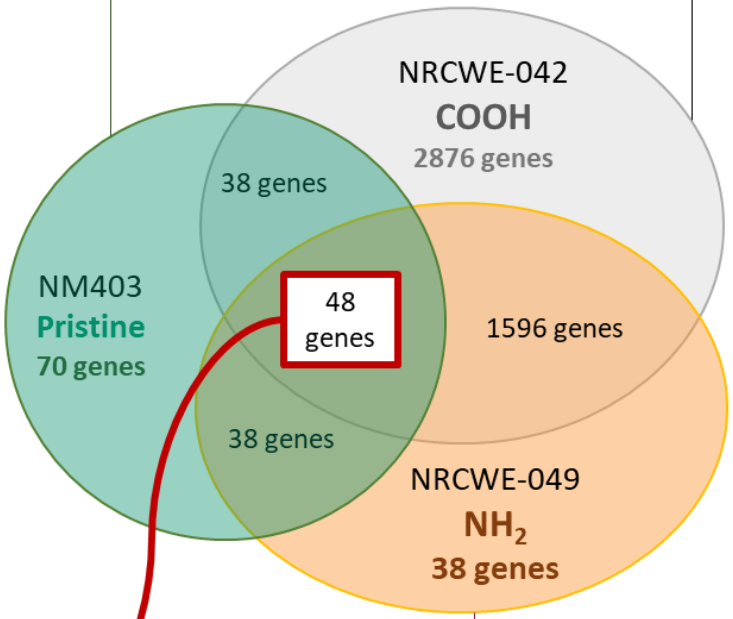
Functionalisation effects on the regulated answer to CNTs, in NR8383

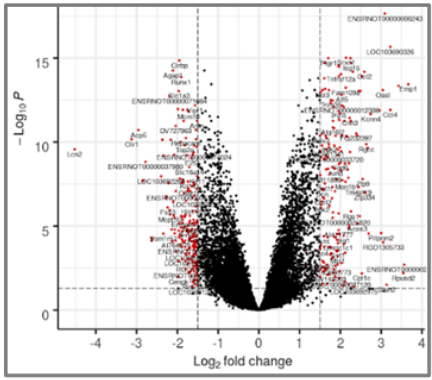
NR8383
Time: 4 h
Doses: ¼ IC_{50}

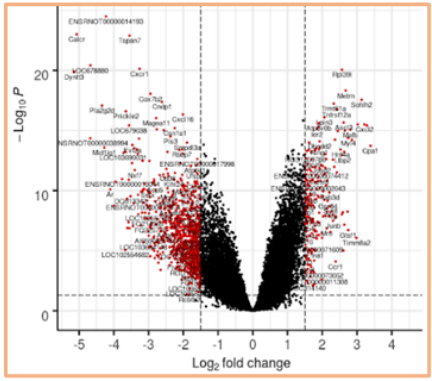
Oxidative stress

Traduction









Commons

Cytoskeleton; Ribosomes

Inflammation

Cytokines production

Passed both cut-offs and up-regulated

Failed to pass both cut-offs

Adapted from Nahle et al., J Nanobiotech, 2020

Functionalisation effects on the regulated response to NMs, in NR8383

<table>
<tr><th colspan="3">Dysregulated pathways after NMs exposure of NR8383</th></tr>
<tr><th>NM403</th><th>NRCWE-042</th><th>NRCWE-049</th></tr>
<tr><td rowspan="4">• Role of Braca1 in DNA damage
• Atm signaling
• Vitamin-C Transport
• Cell cycle: G2/M DNA Damage Checkpoint Regulation
• NrF2 mediated oxidative stress response</td><td colspan="2">Common</td></tr>
<tr><td colspan="2">• Mitochondrial dysfunction
• EIF2 signaling
• Sirtuin signaling
• Oxidative phosphorylation</td></tr>
<tr><td colspan="2">Different</td></tr>
<tr><td>Regulation of eIF4 and p70S6K signaling</td><td>mTOR signaling</td></tr>
</table>

NM403
Pristine
DNA damage
Oxidative stress

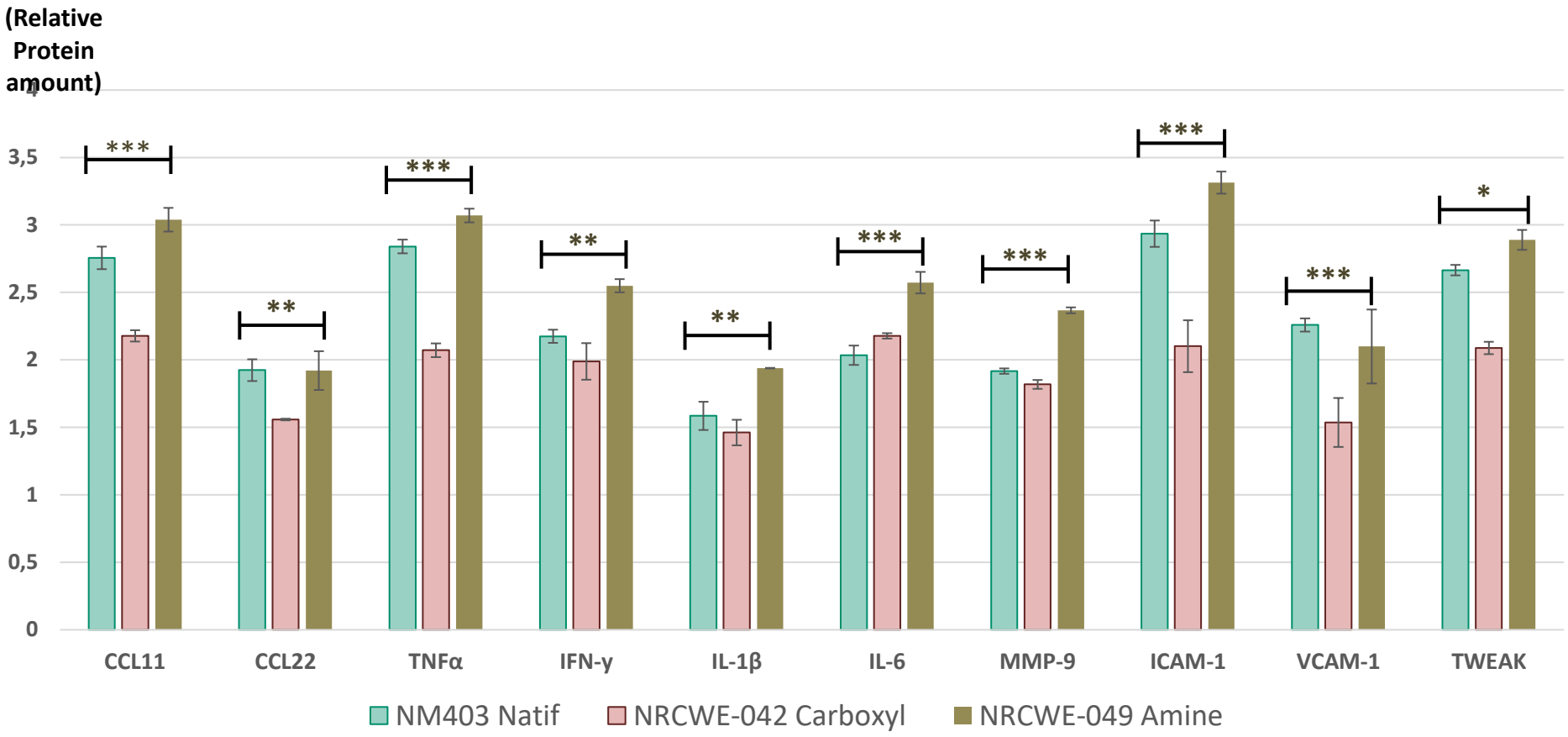
NRCWE-042
Fonction : COOH
ER Stress
Protein synthesis

NRCWE-049
Fonction : NH_2
ER Stress
Protein synthesis

Adapted from Nahle et al., J Nanobiotech, 2020

SmartNanoTox
Smart Tools for Gauging Nano Hazards

Cytokines expression in NR8383 after exposure to NM

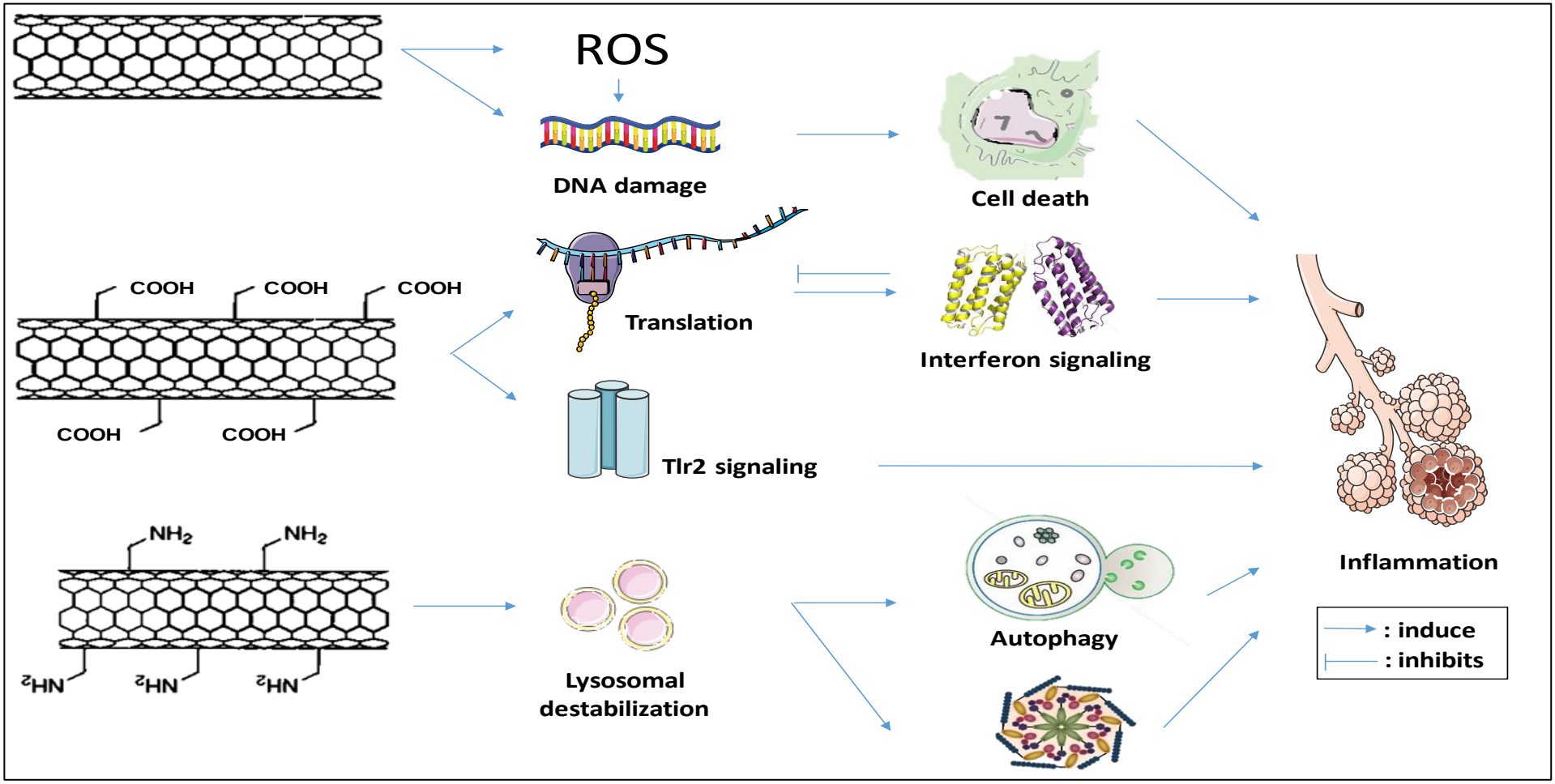


NR8383
Time: 24 h
Doses: ¼ IC_{50}

*** p<0,10 ** p<0,05 *** p<0,01**

Nahle et al., J Nanobiotech, 2020

Functionalisation effects on the regulated response to NMs, in NR8383



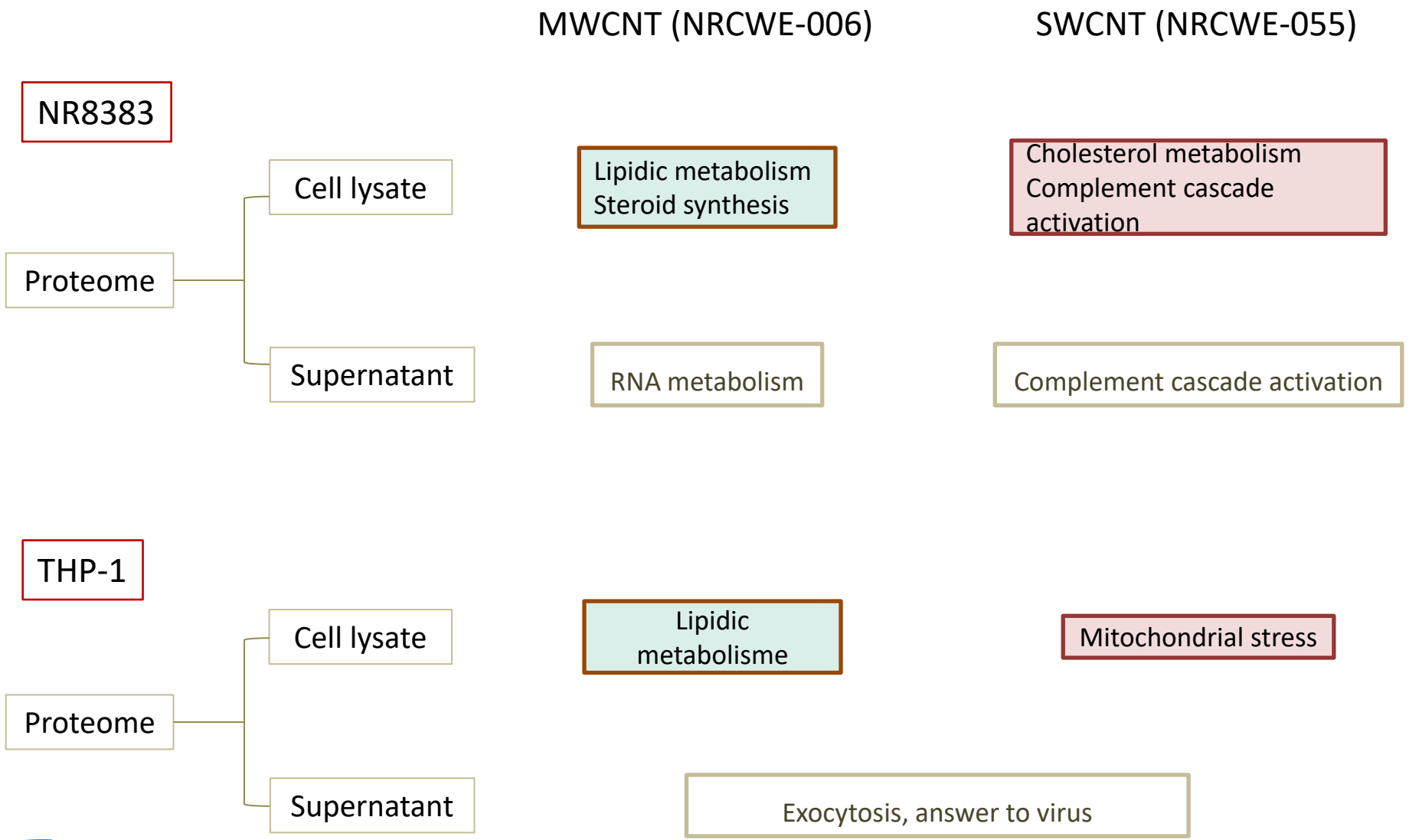
SW *vs* MW effects on the regulated answer to NMs, in NR8383

- 3 **Enrichment score** 6

Time = 4h
Doses: SWCNT = 11 cm^2/cm^2
MWCNT = 1 cm^2/cm^2

Cells	Canonical pathways	
	SWCNT (NRCWE-055)	MWCNT (NRCWE-006)
THP-1 Human	• Mitochondrial Dysfunction • Oxidative Phosphorylation • **Sirtuin Signaling Pathway** • **EIF2 Signaling** • mTOR Signaling	• **Sirtuin Signaling Pathway** • mTOR Signaling • Regulation of eIF4 and p70S6K Signaling • **EIF2 Signaling** • Sumoylation Pathway
NR8383 Rat	• **EIF2 Signaling** • Mitochondrial Dysfunction • Oxidative Phosphorylation • **Sirtuin Signaling Pathway** • mTOR Signaling	• **EIF2 Signaling** • Protein Ubiquitination Pathway • **Sirtuin Signaling Pathway** • Oxidative Phosphorylation • Mitochondrial Dysfunction

Proteomic primary analysis of SW *vs* MW effects in NR8383



Biomolecular and biomedical Research (Ireland)
H. Cassidy et D. Gomez

S Nahle, PhD defense, 2019

KEs and AOs for some MWCNT

Printex-90

KEs
- ROS formation
- Inflammation

AO
- Cancer
- Cardiovascular effects

NRCWE-006

KEs
- ROS formation
- Genotoxicity

AO
- Cancer
- Cardiovascular effects

NM403

KEs
- ROS formation
- Inflammation
- Genotoxicity

NM401

KEs
- ROS formation
- Inflammation

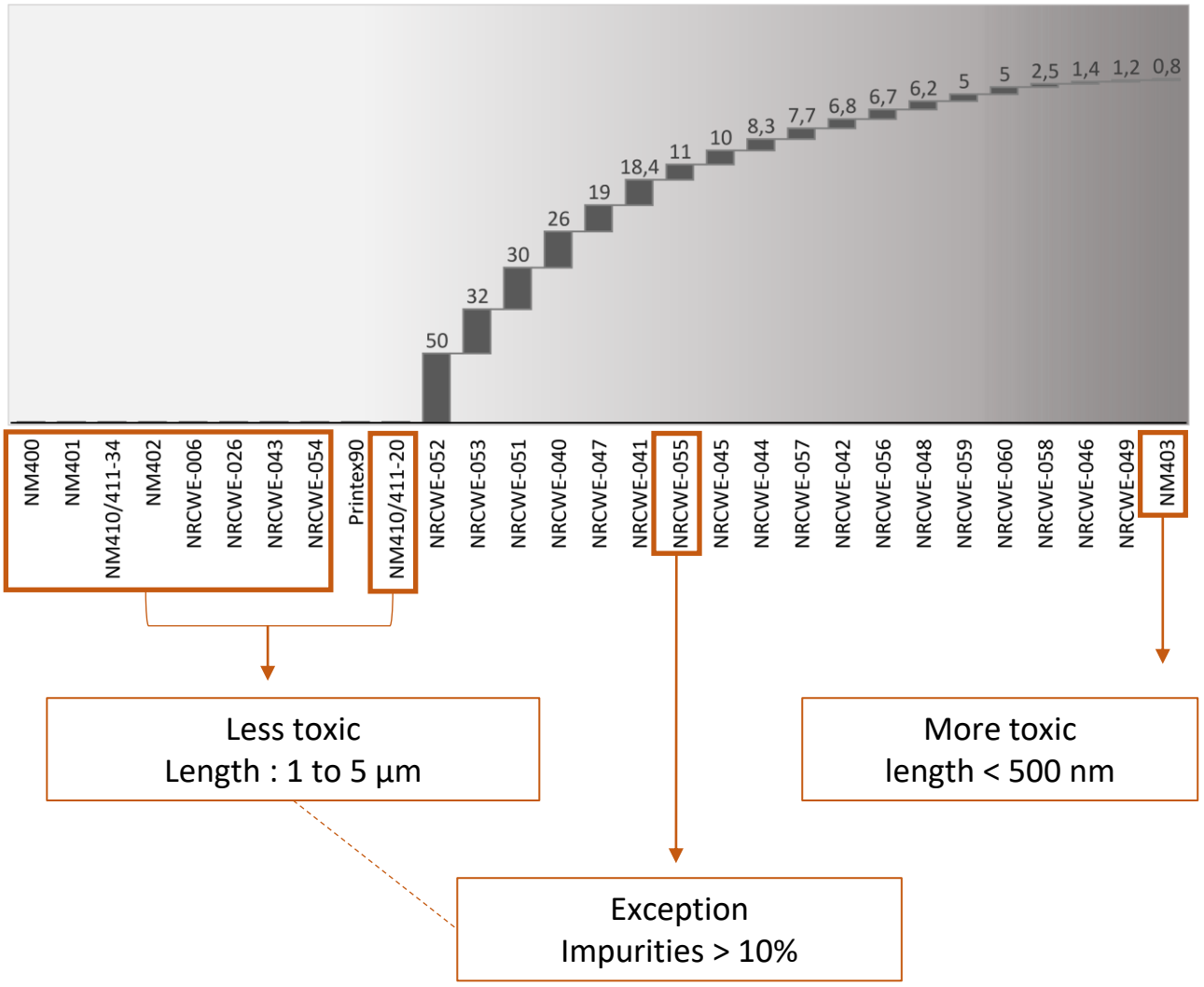
AO
- Cardiovascular effects

NRCWE-042

KEs
- ROS formation

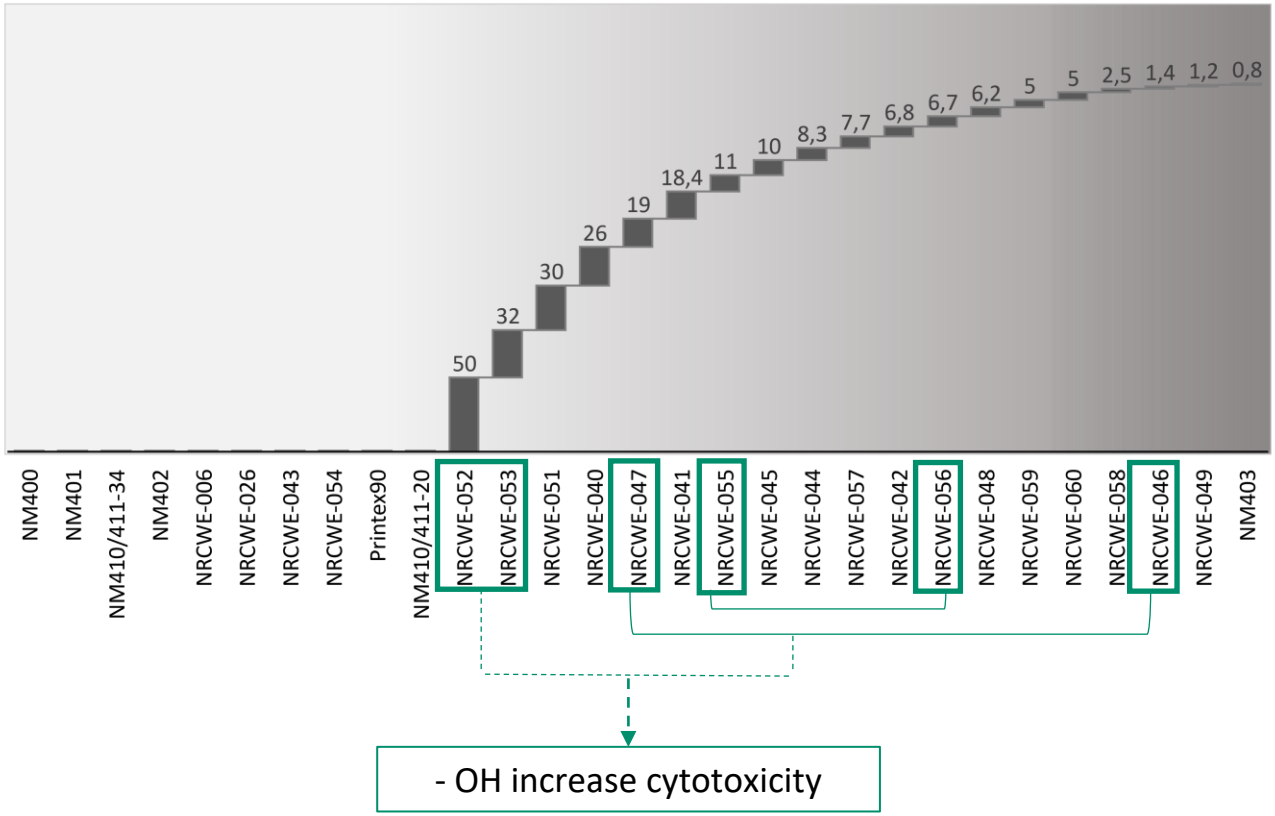
AO
- Cardiovascular effects

CNT length effect on IC_{50}, NR8383, WST-1

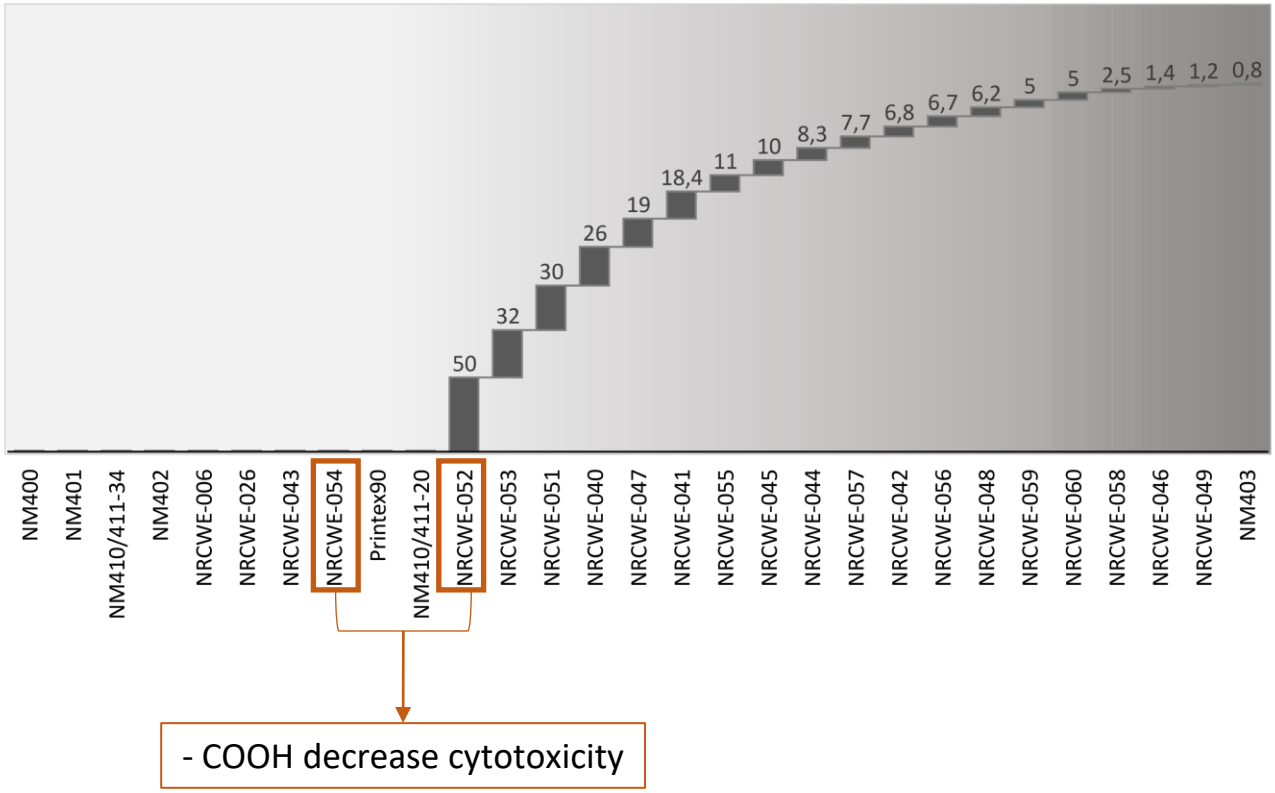


S Nahle, PhD defense, 2019

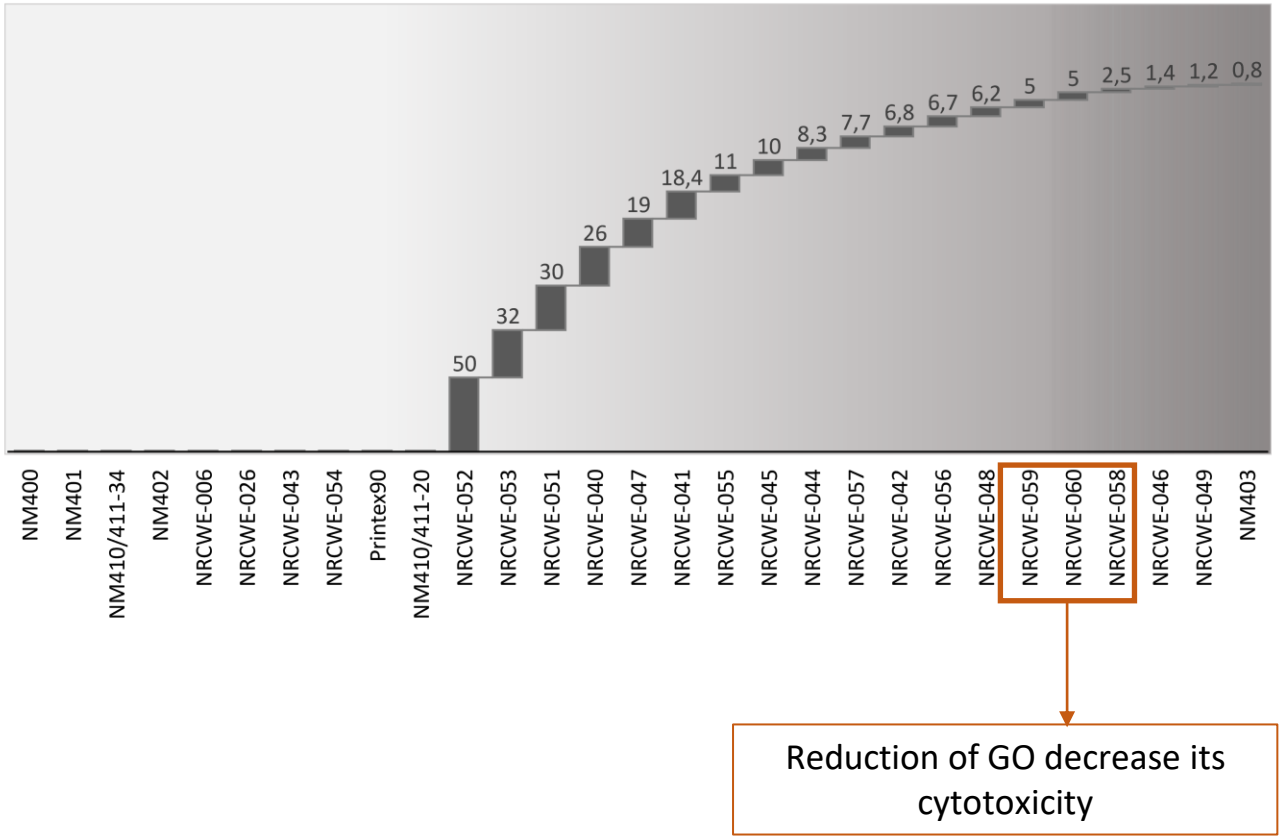
CNT hydroxylation effect on IC_{50}, NR8383, WST-1



CNT carboxylation effect on IC_{50}, NR8383, WST-1



Reduction of graphene oxide effect on IC_{50}, NR8383, WST-1



Reduction status of GO:
NRCWE-058<NRCWE-060<NRCWE-059

S Nahle, PhD defense, 2019

Thank you for your attention!