Mechanisms of response to NMs in soil invertebrates

integrating from gene expression to organism effect and AOPs

Mónica JB Amorim

Susana IL Gomes, Janeck J Scott-Fordsmand

Outline

1. Motivation
2. Approach & Aims
3. Test (nano)materials
4. Test (nano)biology
5. Results and discussion
    i. Copper; ii. Silver
6. Mains

Motivation

Cost-effective methods are urgent

Prediction of long term effects using short term effect kw

Standard methods (e.g. OECD) may underestimate ENMs effects and lack specificity

Understanding the mechanisms of ENMs can ultimately :

- **provide better inside to potential effects**
- **substantiate knowledge for grouping / ranking**
- **enable safer-by-design and sustainability**
- **support a knowledge based RA and ITS**
- **…**

Approach & Aims

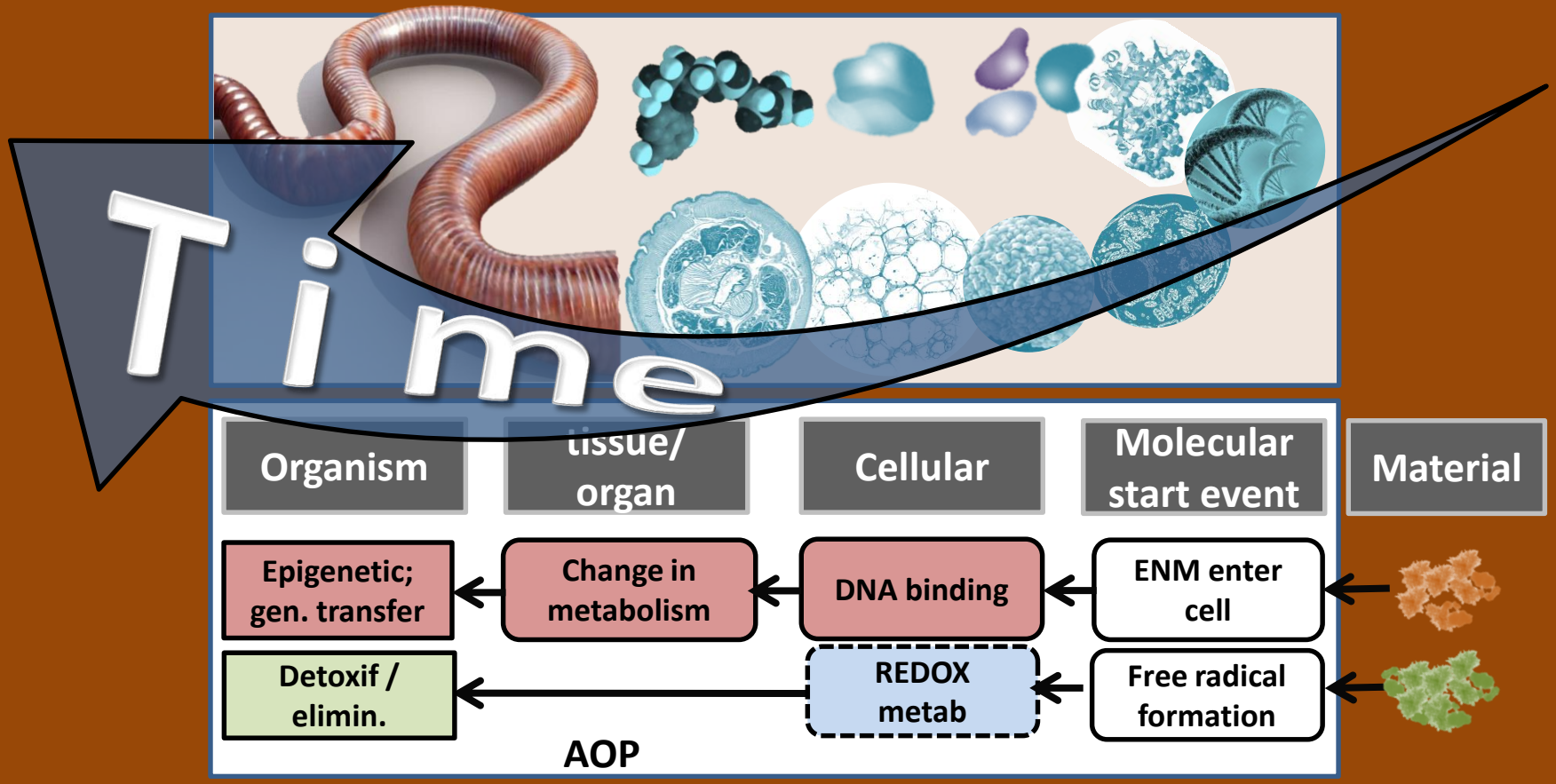
- ❑ **Integration: SYSTEMS TOXICOLOGY**

- ❑ **Effects at various levels:**
  - **Population**
  - **Organism**
  - **Cell**
  - **Sub-cellular**

- ❑ **Anchored experiments**

- ❑ **AOP – Adverse Outcome Pathways**

Approach & Aim



Adverse Outcome Pathways

Test (nano)materials

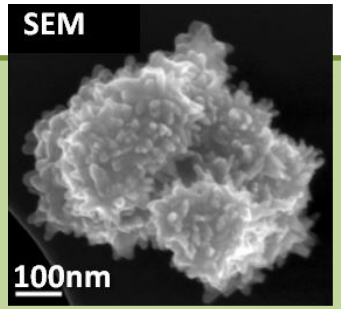
Copper

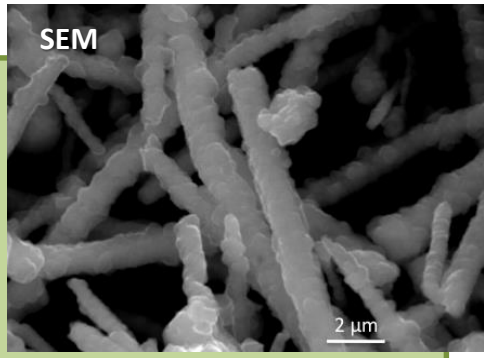
i. Nanoparticles (Cu-NPs)

ii. Nanowires (Cu-Nwires)

iii. salt ($CuCl_2$, $CuNO_3$)

iv. aged salt ($CuSO_4$) [historical field contamination]





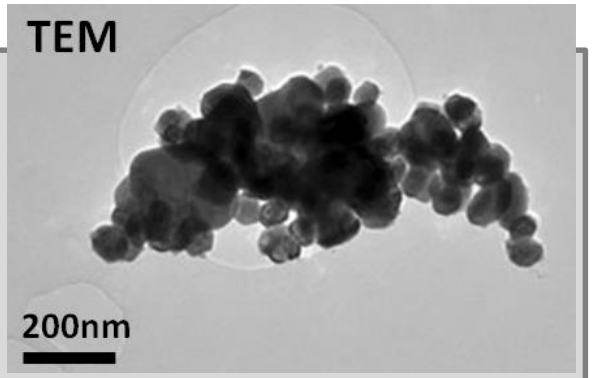
Silver

i. Non-coated Ag-NPs (NC)

ii. PVP-coated Ag-NPs (Coated)

iii. Dispersed Ag-NPs (300K)

iv. salt ($AgNO_3$)



Test (nano)biology

1. Survival and reproduction: OECD/ISO guidelines
2. Stress enzymes (ROS), damage (LPO), energy reserves
3. Gene expression profile: high-throughput (HTP) tool

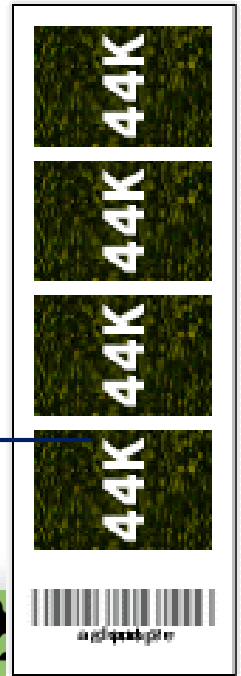
organism

tissue / cell

Sub- cell

mRNA → cDNA → + Cy3-dCTP → cRNA

Hibridise



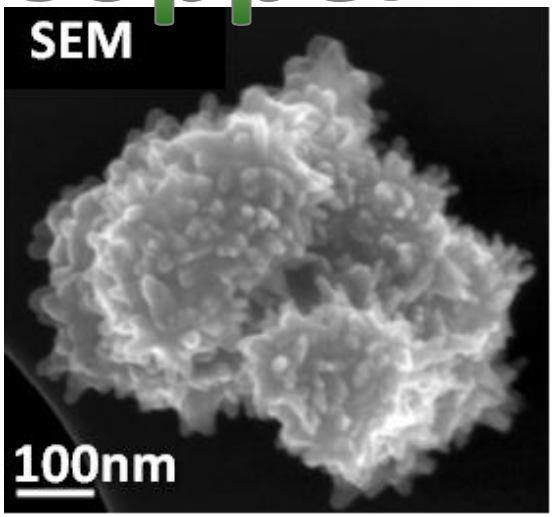
*4x44K Agilent HD Microarray

Data analysis

*BMC Genomics (2014) 15: 302

Results materials – Copper

(DLS, TEM, SEM, ISE, AAS, Seq. extraction, XANES)



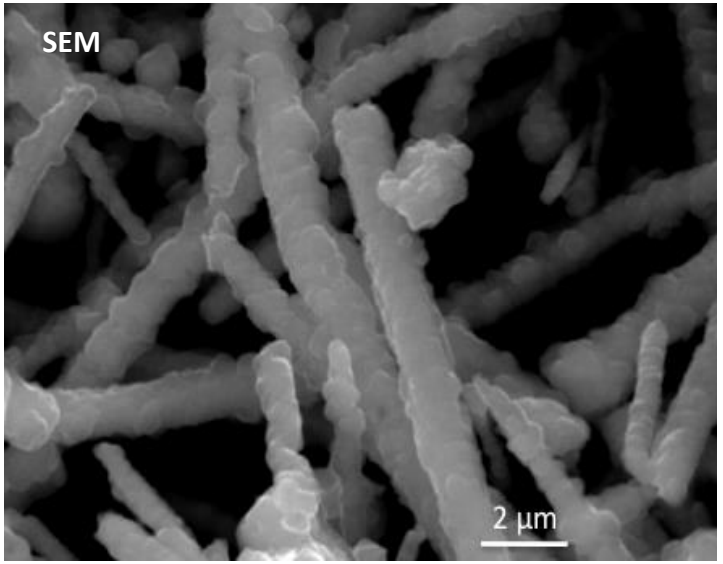
i. nanoparticles (Cu-NPs)

25 nm, surface oxidation (≈ 40% in soil),

“flower-like” morphology

ii. (nano)wires (Cu-Nwires)

500 nm diameter, >10 µm length



iii. salt ($CuNO_3$)

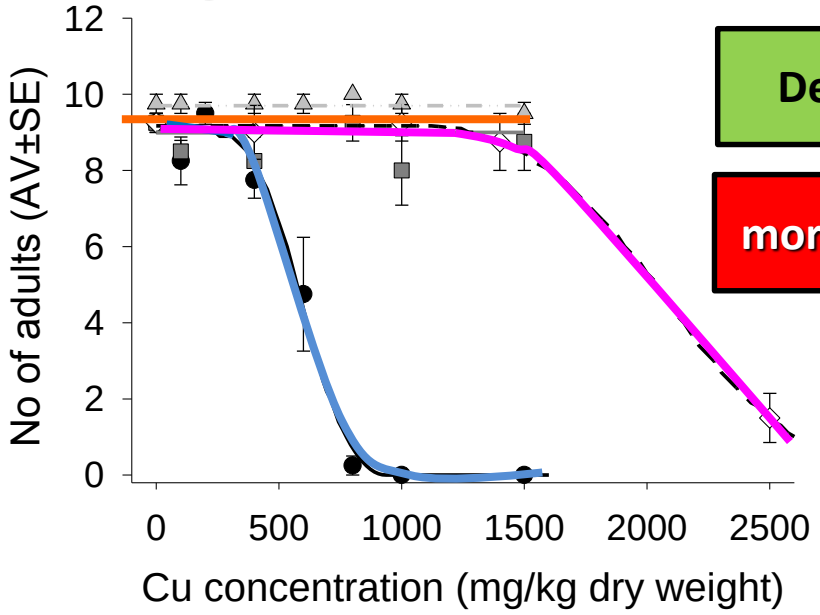
Soluble

iv. salt (Cu-Field) - aged

80 years old field contamination

Results organism- Copper

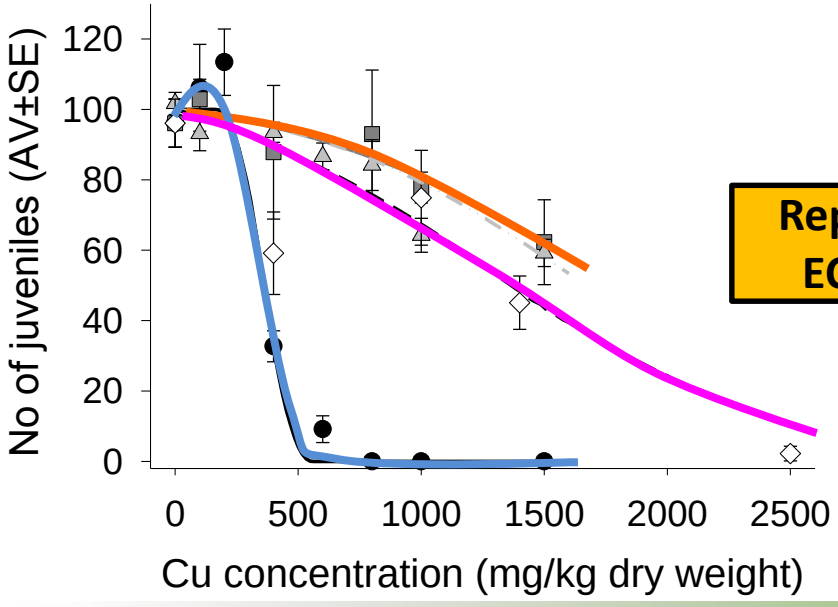
Survival



Detox

mortality

Reproduction



Reprod EC50

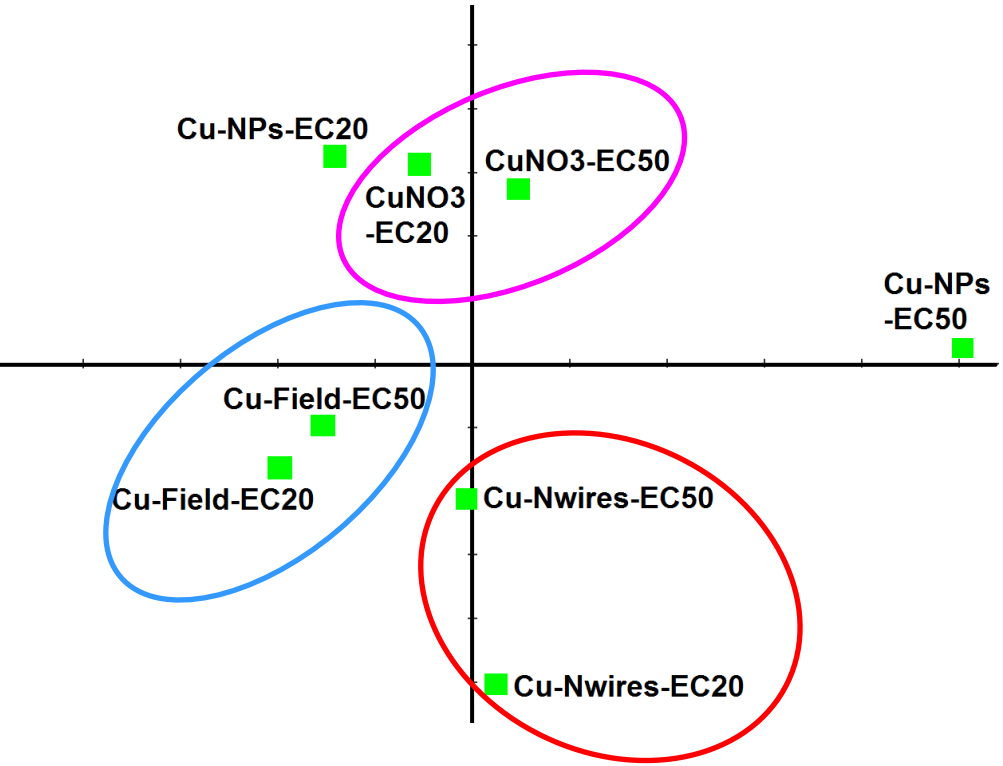
Cu-salt
Cu-NPs
Cu-Nwires
Cu-Field

Results genes - Copper

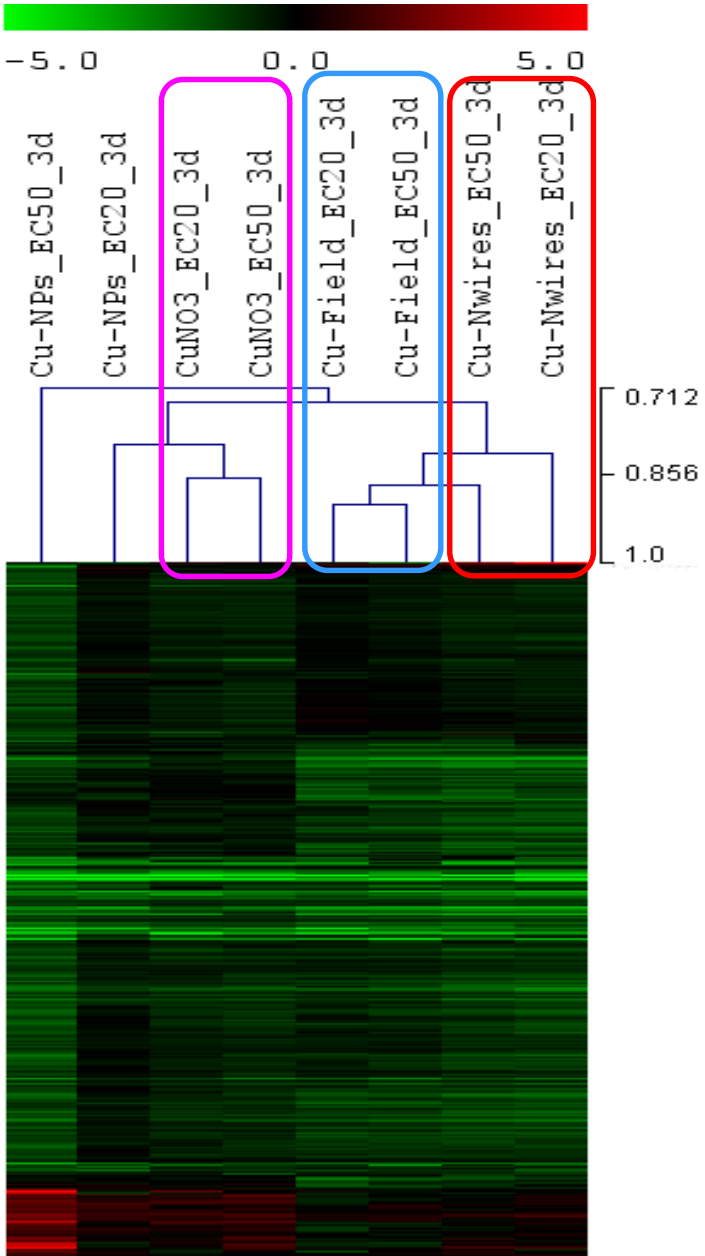
Clustering by Cu form

Less differentiation between EC_{20} & EC_{50}

PCA:3 days



GENE EXPRESSION



Results genes- Copper

Cu-salt ($CuNO_3$ + Cu-field):

Calcium regulation — Calcium regulation

Cilium assembly → chemoreceptor system — Regulation: chemosensory system

Cu-salt (Cu-field) + Cu-NMs (NPs + Nwires)

Translation

Energy metabolism — Affected differently between Cu-salt and Cu-NMs — Alteration in energetic metabolism

Cu-NMs (NPs + Nwires)

Histone modifications → impairment of DNA repair; DNA damage

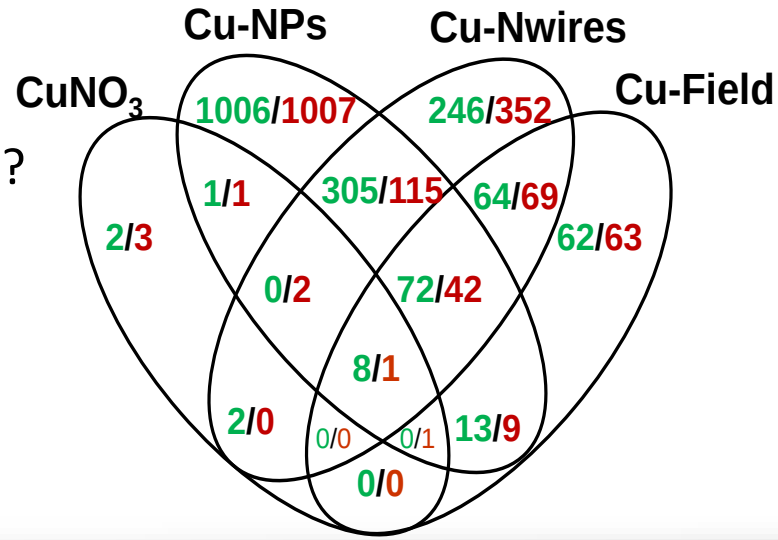
Cell cycle control — ↓histone modifications ↓ DNA repair — Interaction with DNA

Cu-NPs

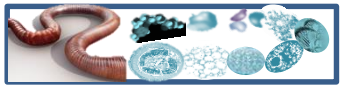
Ubiquitin related processes → apoptosis activation? — Pro-apoptotic stimuli

Cu-Nwires

Locomotion behaviour — Locomotion behaviour



AOP - Copper



Toxicant	Molecular start event	Cellular	tissue/ organ	organism

Cu-Nwires

Cu-NPs

Cu-salt

Interaction with DNA

Pro-apoptotic stimuli

Free radical formation

Locomotion behaviour

↓histone modifications
↓ DNA repair

↑Cyt c

Mitochondrial dysfunction

Alteration in energetic metabolism

regulation:↑SOD ↑GPx ↓GSH ↑GSSG

Oxidative stress

regulation: ↑GPx

↓Cyt c

Regulation: chemosensory system

Calcium regulation

?

Reproduction / Epigenetic

Detoxification

Reproduction EC50

↑ LPO

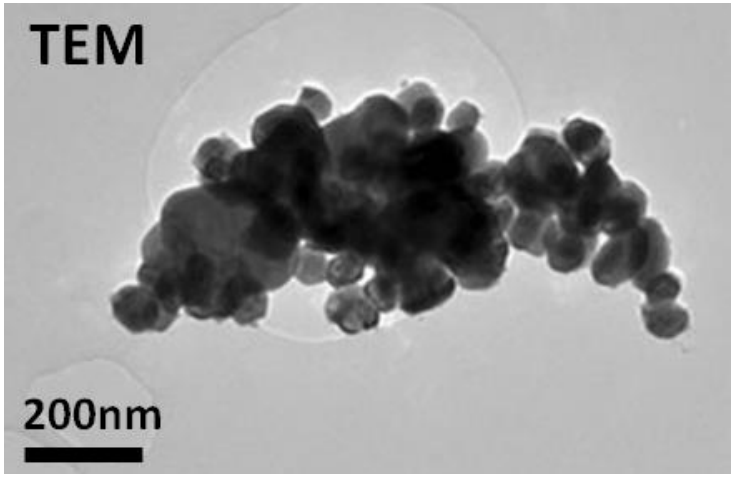
mortality

Start event
Biol event based on DEG

Adverse event
Semi-adverse event
Improved event

Results materials – Silver

(DLS, TEM, SEM, AAS, …)



i. Non-coated Ag-NPs (NC)

20-30 nm, spherical, 99% Ag

ii. PVP-coated Ag-NPs (Coated)

20-30 nm, spherical, 99% Ag, 0.2% w/w PVP

iii. Dispersed Ag-NPs (300K)

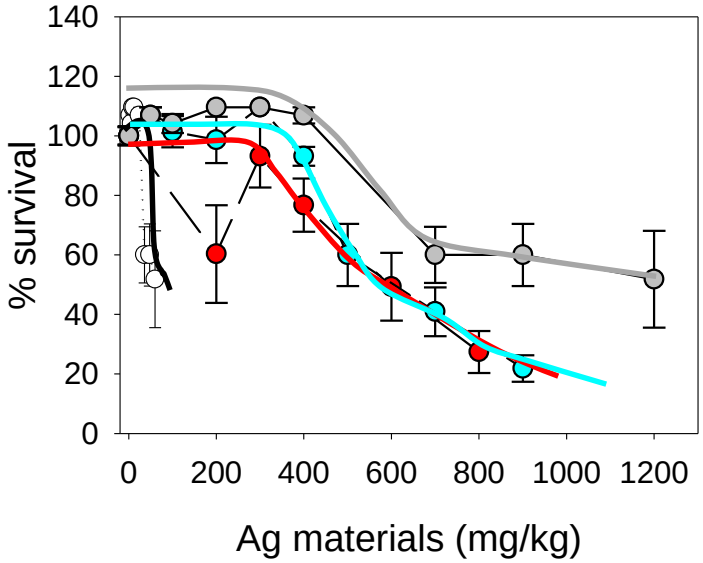
15 nm, spherical, dispersed in Tween 20, 10.2% w/w Ag

iv. salt ($AgNO_3$)

Soluble

Results organisms - Silver

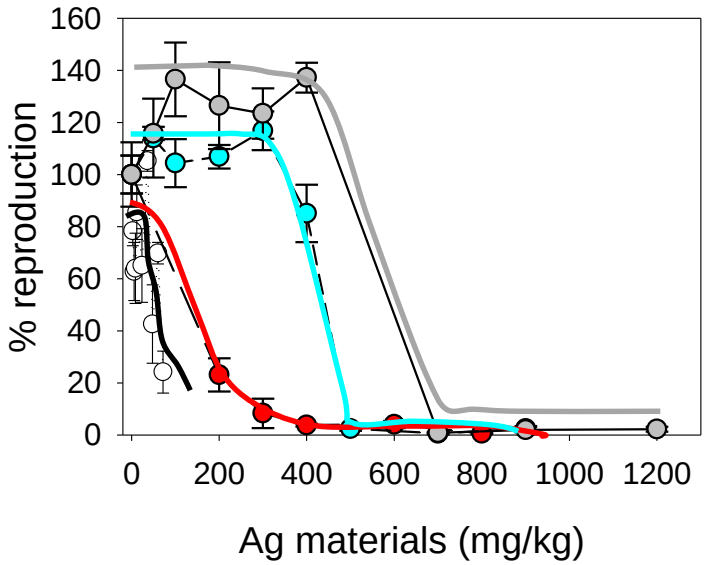
Survival

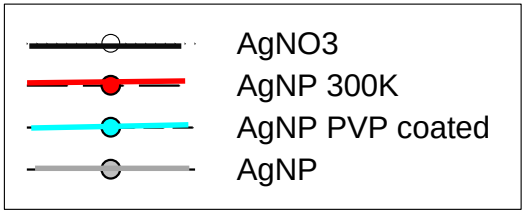


Detox

mortality

Reproduction



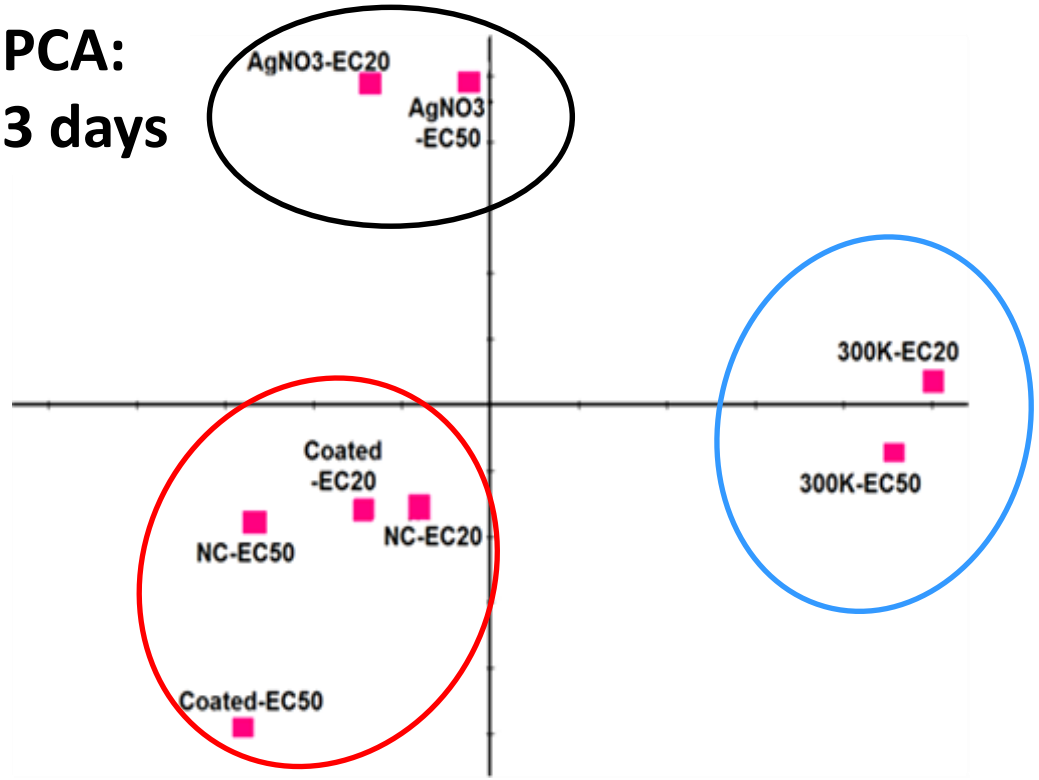


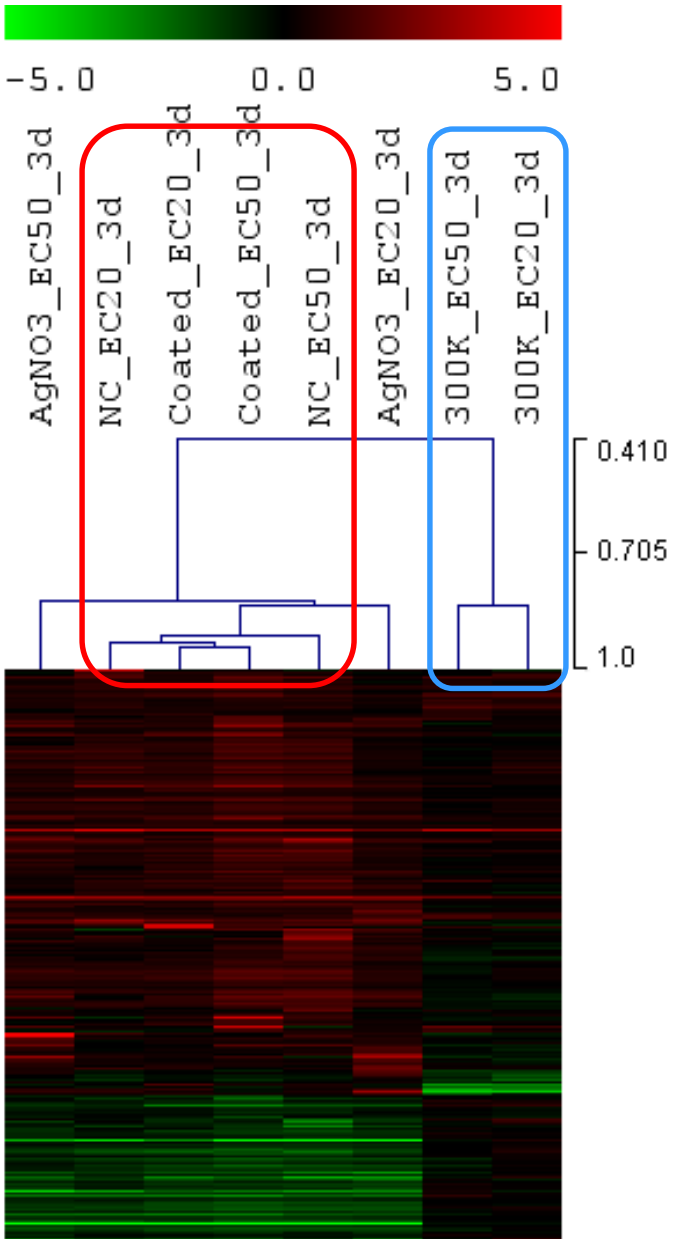
Reprod EC50

Results genes- Silver

Clustering by Ag form

PCA:
3 days





Results genes - Silver

Ag

Cell cycle control → impairment of DNA repair

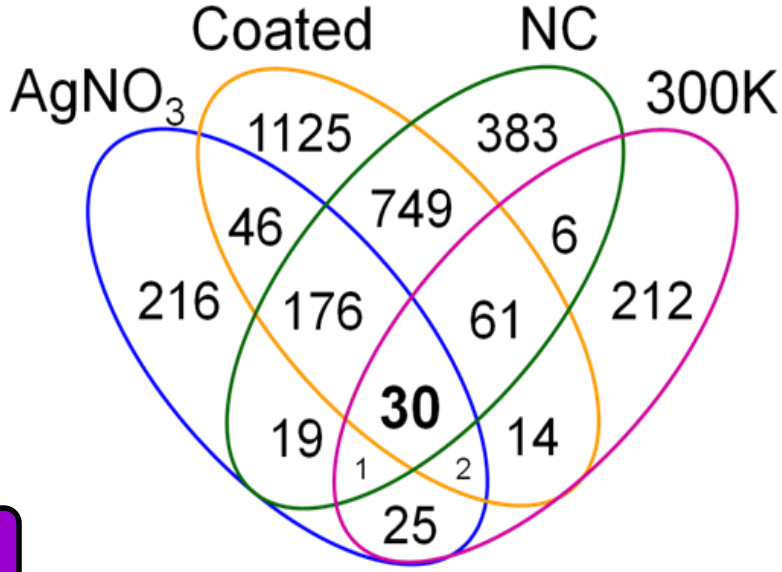
DNA damage

Impairment of cell cycle control

Ag-salt + Ag-NPs Coated and NC:

Impairment of neurotransmission (↓ sodium, potassium and calcium channels and transporters)

Impairment of neurotransmission



Ag-NPs 300K:

↑GST (indication of oxidative stress response)

Detection of bacterium

Oxidative stress regulation:↑GST

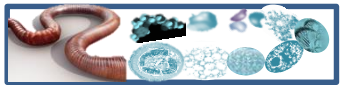
Free radical formation

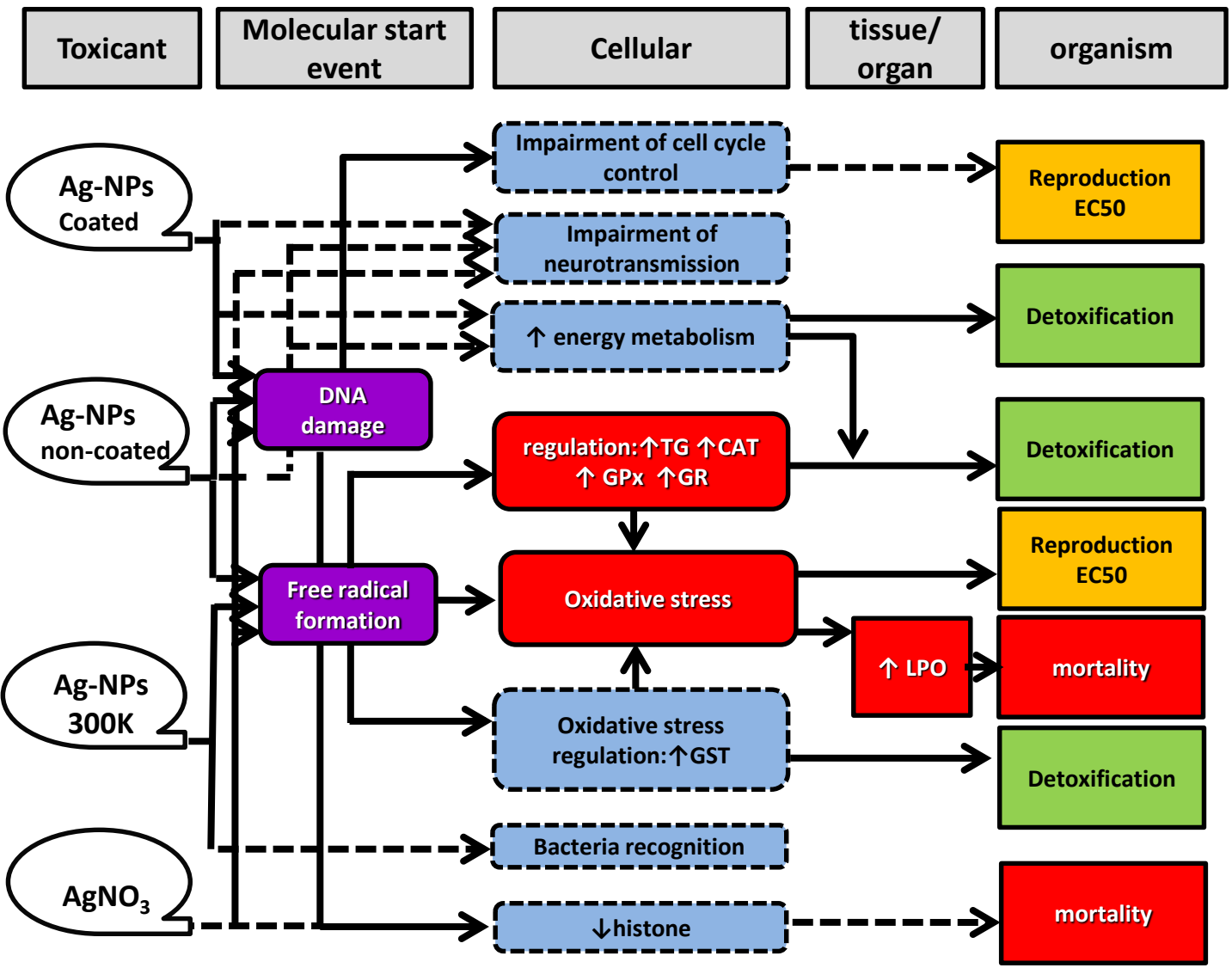
Ag-NPs Coated and NC

Energy metabolism (activation of carbohydrate metabolism → high energy use/demand)

↑ energy metabolism

AOP - Silver



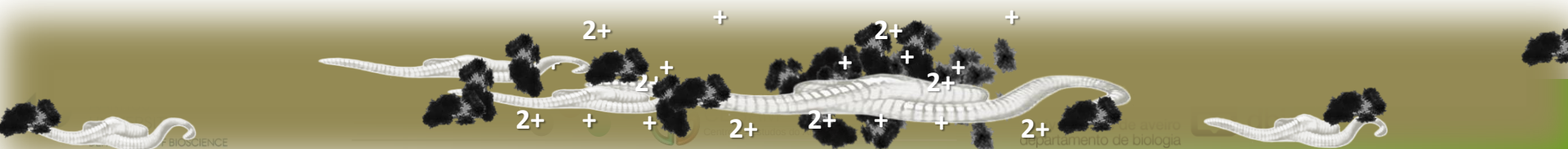

Toxicant
Molecular start event
Cellular
tissue/ organ
organism
Ag-NPs Coated
Ag-NPs non-coated
Ag-NPs 300K
AgNO3
DNA damage
Free radical formation
Impairment of cell cycle control
Impairment of neurotransmission
↑ energy metabolism
regulation:↑TG ↑CAT ↑ GPx ↑GR
Oxidative stress
Oxidative stress regulation:↑GST
Bacteria recognition
↓histone
↑ LPO
Reproduction EC50
Detoxification
Detoxification
Reproduction EC50
mortality
Detoxification
mortality
Start event
Biol event based on DEG
Adverse event
Semi-adverse event
Improved event

Mains

→ **Differentiation between materials was possible at the lower levels of biological organization -** “material fingerprints” based on transcriptomic analysis.

→ **Cellular and molecular effects following <u>short-term</u> exposure could be linked to or act as a <u>proxy of</u> reproduction effects following <u>longer-term</u> exposure.**

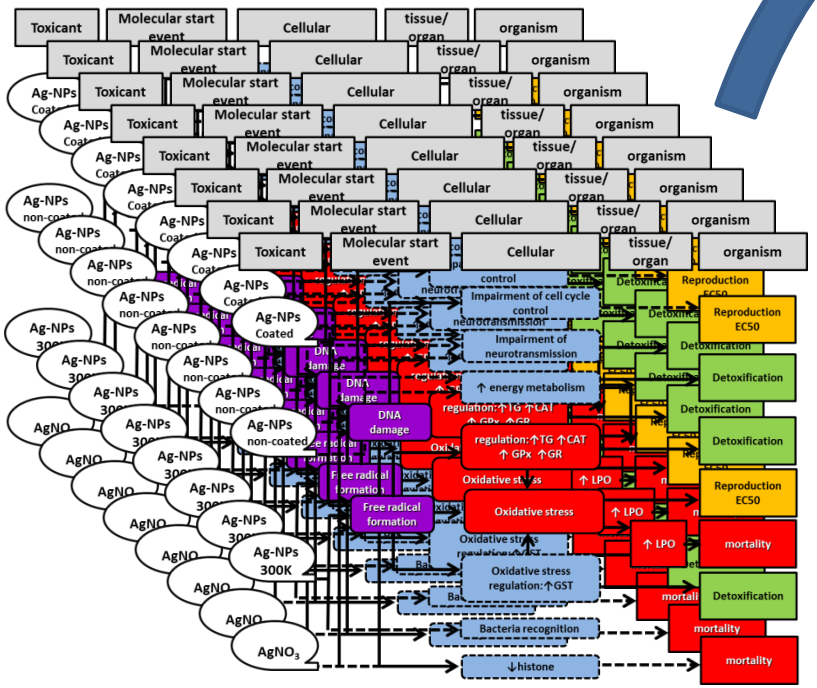
→ **Identification of material related AOPs.**



Aims *vs* Output

Aims	--->	Output
Effects <u>various levels</u>	- Standard tests - sub-cellular - anchored expts.	**Correlation between <u>short and long term</u>**
Mechanisms	- HTP gene expression	**Excellent tool for hypothesis formulating → - Intelligent Testing Strategy -**
Cu and Ag NMs <u>mechanisms of toxicity</u>	- HTP gene expression - Enzymes - Energy metabolites	**- <u>Affected pathways</u> - <u>Similar</u> and <u>dissimilar</u> pathways per material - Key <u>discriminating</u> points**
AOP	- Collection of outputs - various methods	- Partial AOP **- Knowledge Based RA**
Integration: <u>Systems Toxicology</u>	- Integration analysis	**- Progress towards ST - Advance in understanding - …**

Finally..



AOPs

- inside to potential effects
- knowledge for grouping / ranking
- enable safer-by-design
- support a KBRA and ITS

Effects

LONG TERM ← SHORT TERM

Acknowledgements

The XANES studies were perform at DESY, Hamburg, Germany.

Study funded by FEDER through COMPETE-Programa Operacional Factores de Competitividade, and by National funding through FCT-Fundação para a Ciência e Tecnologia, within the research project **nanokA** FCOMP-01-0124- FEDER-008944 (Refª. FCT PTDC/BIA-BEC/103716/2008), a PhD grant (SFRH/BD/63261/2009) and the EU-FP7 **MARINA** (Ref. 263215).