How well can standard soil tests provide the needed evidence for risk assessment of nanomaterials?

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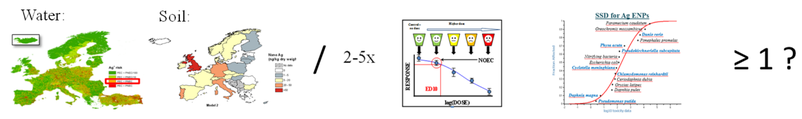
Introduction: What is so difficult about Nano RA? – The fact that the testable exposures do not reflect real world exposures

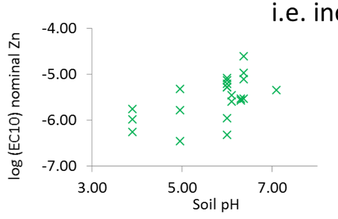
Nano RA - What would we like to do?

A) Rank toxicities: e.g. Ag NP > ZnO NP >TiO2 NP
or 5nm Ag NM > / < 50nm Ag NM ?
or Ag Citrate NP ? Ag PEG NP ? Ag PVP NP

B) Do basic Environmental Risk Assessment: (PEC/PNEC ≥ 1 ?)



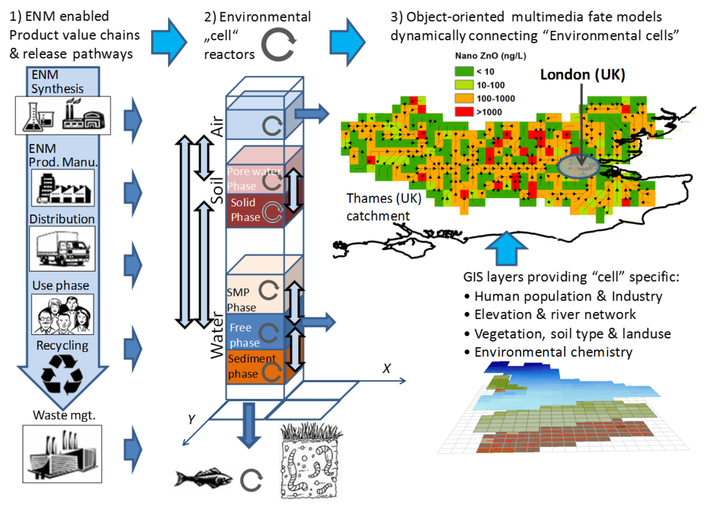
C) Apply "basic Environmental Risk Assessment" to "Real world" :
i.e. include: - real world exposure form
- real world media
- bioavailability effects



Analyse combined Literature data (x)

Conclude : Soil pH ↑ -> Toxicity of Zn ↓

Nano RA – Why so difficult?



Conceptual workflow for a framework to deliver dynamic multimedia fate prediction both in a generalised model environment and GIS enabled mode.

Are we trying to *fly without getting good at crawling first"?*

E.g. Science behind current EU Zinc Soil RA:
- took 20-30 year to develop, putting data from 100's of papers together
- Years of addressing safety factors
- Much is based on empirical based models, transfer (pH/OM) and aging factors (x3)

To reach this standard for nano will require:
- Standard tests for ranking of NM forms
- Ability to test exposure relevant NM forms
- Diverse data allowing comparison of:
  - NM forms, media, test species, exposure times

The main problem for environmental NM risk assessment in soils is that the NM forms manufactured (and thus testable) are not those that will arrive in the environment.

Long term exposure, ageing & transformations: Standard tests with "pristine" NMs may both under & overestimate toxicity

Long term aging of soils pre-exposure – effects on toxicities:

SPRINGTAIL TEST WITH ZnO:
Soils spiked and aged before standard OECD Springtail test

- Zn^{2+} "ages" as expected with EC_{50}s increasing with soil age
- Uncoated ZnO forms ages as expected becoming less toxic
- Coated ZnO is more toxic and aging is delayed 6+ months

EC50 values in mg Zn/kg dry soil

Time (months)	coated ZnO NP	uncoated ZnO NP	non-nano ZnO	$ZnCl_2$
T=0	873[a] (659-1087)	1964 (1635-2293)	1591 (-)	299[a] (181-415)
T=3	749[a] (463-1035)	2847 (-)	3628<EC_{50}<8359	912[b] (-)
T=6	576[a] (263-888)	-	-	-
T=12	1817[b] (1344-2291)	>5855*	>8359*	707[b] (419-996)

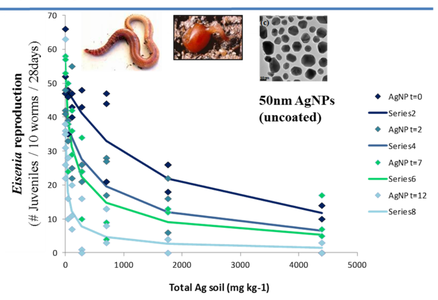
Waalewijn-Kool et. al. (2013) *Environmental pollution: 178, 59-64*

EARTHWORM TEST WITH SILVER:
Soils spiked and aged before standard OECD Earthworm test

- Ag^{+} "ages" as expected with EC_{50}s increasing with soil age
- AgNP becomes 10 - 40 times more toxic with aging
- AgNP reaches Ag+ tox, but does not exceed it.

Soil EC50 (mg/kgdry soil)	T=0	T=2mth	T=7mth	T=12mth
		AgNP 10x more toxic @ 7mth		
AgNP	1420 (407-2432)	588 (65-1110)	142 (5-278)	34 (-117)
$AgNO_3$	49 (46-51)	30 (16-43)	90 (29-151)	104 (63-144)

Diez-Ortiz, M *et. al.* (2015) Environ. Poll. 203:191-198



Effect of transformations on particle toxicity

- **Particles artificially aged to mimic post WWTP speciation**

Standard C elegans test:
- In moderately hard reconstituted water
- 24h Mortality test without food

Treatments:
- Control
- Ionic control Ag^{+} Pristine PVP Ag NP
- Artificially "Aged" sulfidised Ag NP

Conclusions:
- Tox: Ag^{+} > Ag NM > sulfidised AgNM
- < 20% of mortality in NM treatments attributed to free Ag^{+} in experiments without feeding

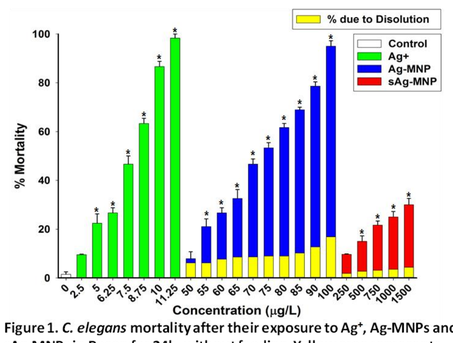


Figure 1. *C. elegans* mortality after their exposure to Ag^{+}, Ag-MNPs and sAg-MNPs in Recon for 24h without feeding. Yellow area represents amount of mortality due to dissolution of Ag^{+}. * indicates significantly different than control ($p < 0.001$)

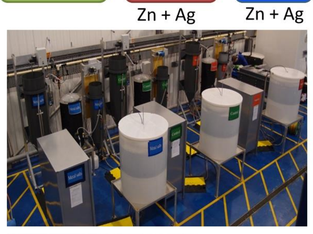
Starnes et. al. (2015) Environ Poll. 196:239-246.

"Real world" transformations: These and especially their consequences cannot be predicted just from standard tests

Sludge production in pilot WWTP

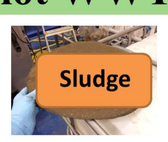
Three sewage sludge streams

Control | Ionic (Zn + Ag) | NP (Zn + Ag)



Zn limit: 2800 mg/kg
Equivalent Ag: 250 mg/kg

US EPA Guideline (CFR 40 part 503)



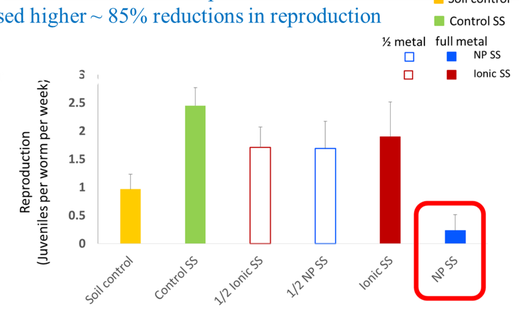
Control SS | Ionic SS | NP SS

- Mixed with soil to Max. Zn loading from sewage sludge in US soils = **1400 mg Zn/kg**
- **Aged 6months in outdoor mesocosms**

Effects on earthworm reproduction

Standard OECD Earthworm test of 6-month aged SS

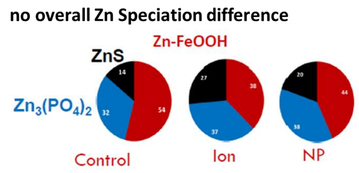
The highest **"Full Metal"** exposure matched 10 years of yearly SS application
The **"½ metal"** treatment was made diluting with control sludge (~ same OM%)
- Adding sludge (OM) even moderately polluted improves EW reproduction
- "½ metal" sludge whether NMs or ions reduced reproduction 20%
- "Full metal ionic" had no further effects on reproduction
- "Full metal NM" caused higher ~ 85% reductions in reproduction



What is different about the NP metals?

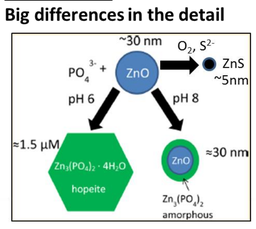
Question: What "difference" caused the SS metals to be more toxic?
Synchrotron speciation work by Greg Lowry and Jason Unrine's groups:

Pilot WWTP:
No ZnO left and
no overall Zn Speciation difference



Characterisation in Pilot plant solids:
Zn: no overall difference / Ag: <LOD

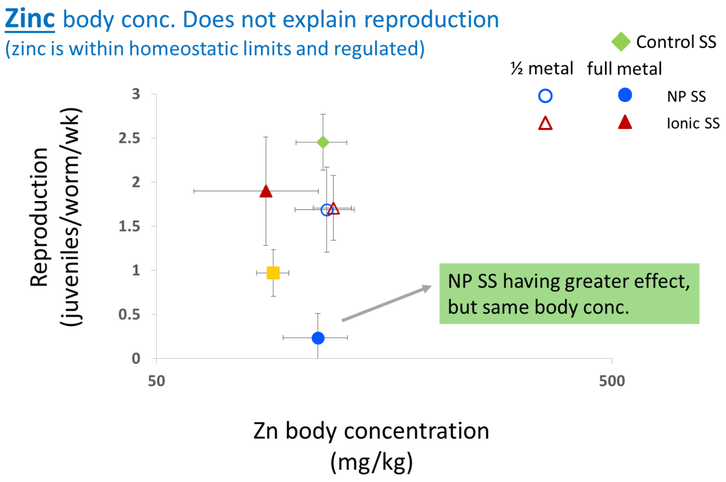
Lab reactions:
Big differences in the detail



Mechanistic Lab experiments:
Understand reactions and rates
-> effects on fate and tox

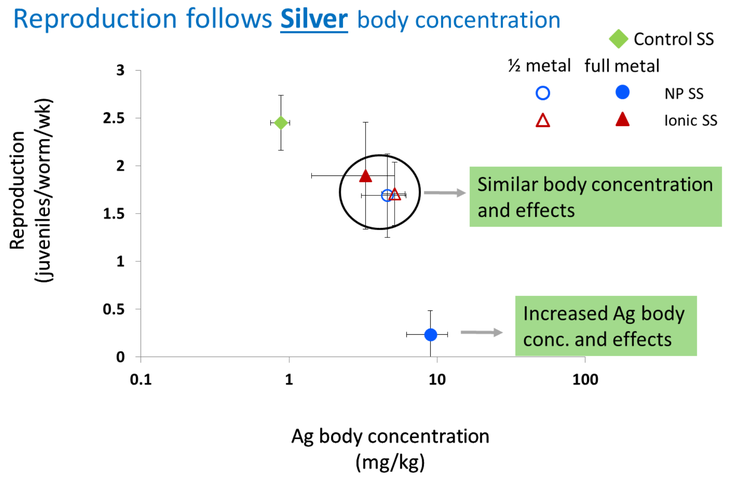
Reproduction + Earthworm Zn body concentration

Zinc body conc. Does not explain reproduction
(zinc is within homeostatic limits and regulated)



Earthworm Silver body concentration

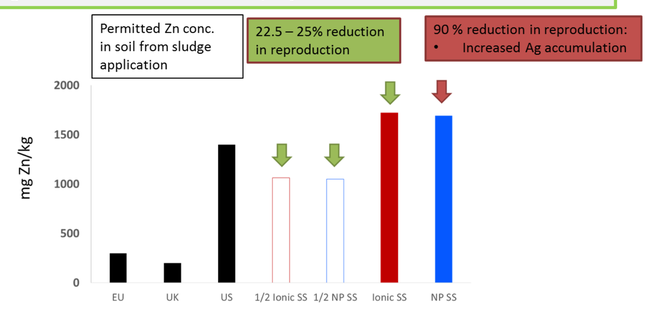
Reproduction follows **Silver** body concentration



Consequences for NM sludge application to land?

Can we use ionic metal RA & limits in sludge for NM metals?

Possibly for ZnO, but probably not for Ag, However……
- Doses were max expected US sludge Zn & Ag levels based on Zn limit
- There is no Regl. limit for Ag so this gave 125mg Ag/kg exposure in the soil
- This is several orders of mag. above the worst case EU soil Ag loading of 1.3µg/kg modelled in the NanoFATE project.



CONCLUSION: We need to test exposure relevant NM forms, at levels and under durations relevant to NM fate processes

www.NanoFATE.eu (EU CP-FP7 247739)

www.guideNANO.eu (EU CP-FP7 604387)

UK (NERC) / US (EPA)

www.NanoFASE.eu (EU H2020 646002)