The impact of engineered nanomaterials on aquatic species: ISA-TAB compatible dataset mined from literature (2007-2016)

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Project Scope & Aims

- Primary scope of the project was to model the toxicity of engineered nanomaterials (ENMs) at multiple levels
- Design and create a database (DB) containing extracted peer-reviewed published toxicity data
- Analyse the extracted data and identify the most significant ENM characterisation parameters and potential correlations with observed toxicity
- Identify potential gaps in ENP characterisation and toxicity endpoints and resulting gaps in cross-study comparability and continuity

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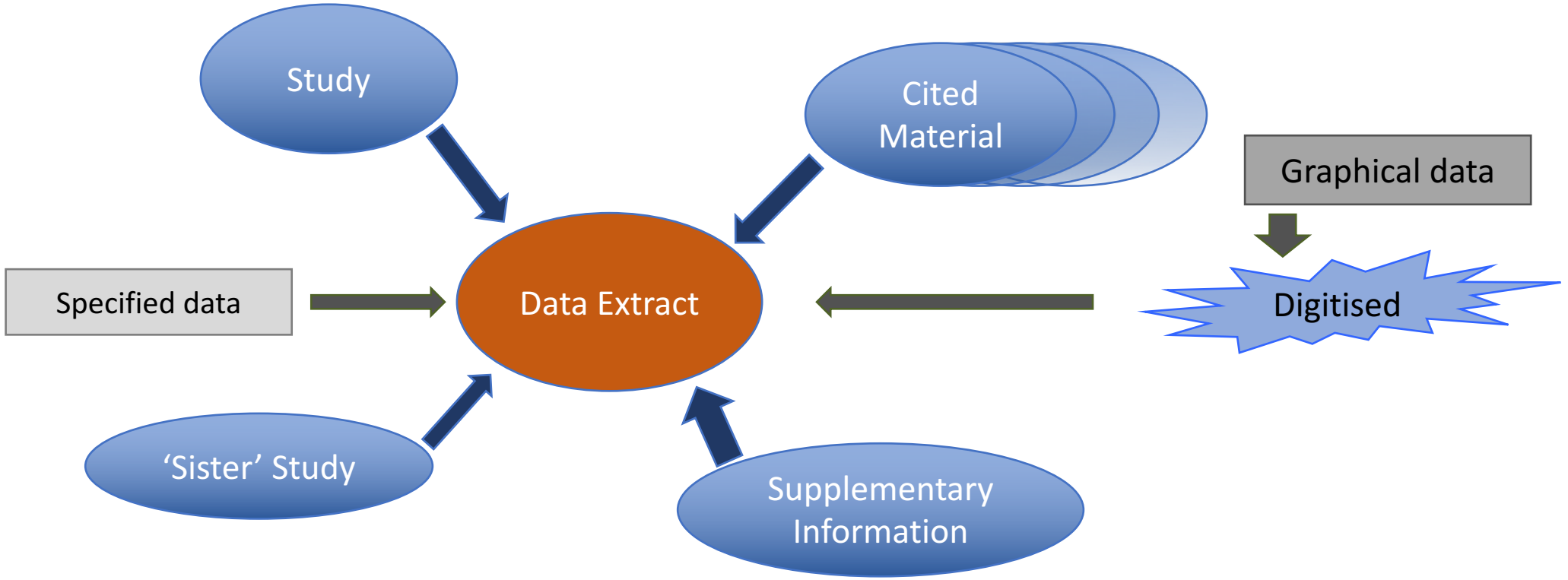
DB Structure - 1



- **Requirements:**
  - **Main focus on Ag ENMs**
  - **Gross endpoints**
  - **Aquatic organisms**
- **Current DB contains 108 studies**

Inclusion criteria & evaluation

- 1st QC check for candidate (published) studies:
  - ENMs origin (commercial (0) / in house (1))
  - ENMs characterisation (no in house (0) / in house characterisation (1))
  - Any study with a combined score of 0 was excluded from the DB
  - Low threshold to maximise study inclusion

ENP Descriptors & Assay Endpoints

Particle Characteristics

Size
Size distribution
Hydrodynamic diameter
Concentration
Surface area
ζ-potential
Morphology
Dissolution
Elemental composition
Crystallite size
Crystal structure
Aggregation
Chemical speciation
Energy band gap

Assay Details

Too variable to standardise

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Study rating and QC

- Each study was evaluated for the completeness of ENP characterisation using a custom grading system
- Characterisation score is included in the DB as a separate entry
- QC of the bio-assay was not possible. Studies were to variable to standardise

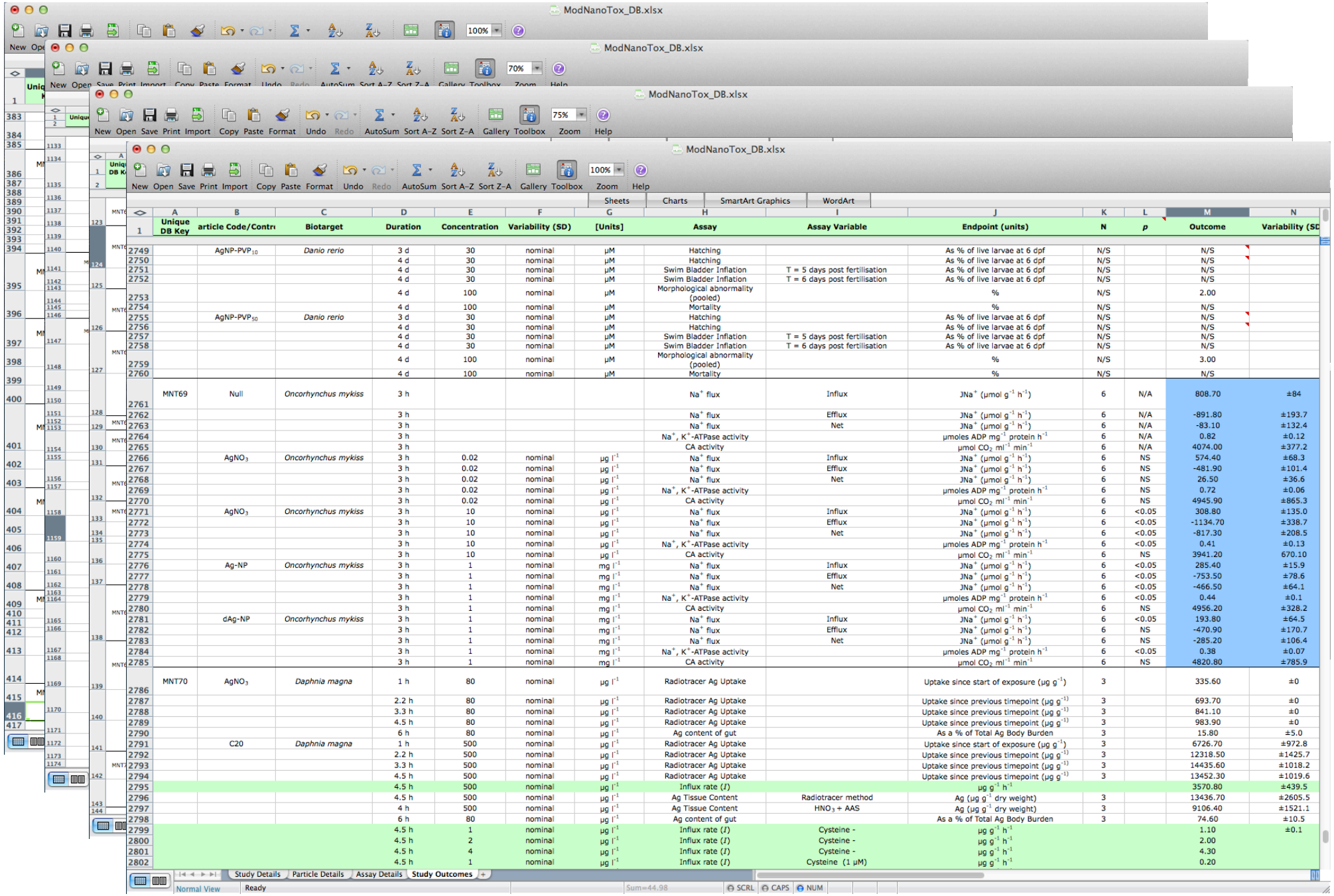
Parameter	Appropriate Techniques	Pristine Particles	In situ	Over exposure duration
Size	TEM/AFM/NTA	0-2	0-2	0-2
Morphology	TEM	0-2	0-2	0-2
Solubility	Dialysis / Ultrafiltration	0-2	0-2	0-2
Surface Properties	XPS / SEM-EDX	0-2	0-2	0-2
Aggregation	DLS/SEM /TEM	0-2	0-2	0-2

0 = absent; 1 = qualitative; 2 = quantitative, sufficient for statistical analysis

DB Structure - 1

Bibliographic Info	Particle Characteristics	Assay Details	Study Outcomes
Unique Database Key	Unique Database Key	Unique Database Key	Unique Database Key
Characterisation Score	Source	Binomial	Particle Code / Control
Status	Batch number	Common Name	Biotarget
Author Surname	Core Chemistry	Source	Duration
Author Forename	Shell Chemistry	Gender / Life-stage	Concentration
Affiliation	Surface Modification	Maintenance and Preparation	Variability
e-mail for correspondence author	Size	Media	Concentration Units
Title/Description	Shape	Illumination (photoperiod)	Assay
Year	Particle Code	Illumination (flux)	Assay Variable
Journal	Nanoparticle Descriptor	Temperature	Endpoint (units)
DOI	Method	Agitation	N
Volume	Particle Preparation	pH	p
Issue	Instrument	Exposure Route	Outcome
Start Page	Medium	Exposure Duration	Variability
End Page	Occasion	Depuration Duration	Calculation Details
PDF	Measured Outcome	Endpoints Measured	Author Derived Conclusions
Availability of Supp. Info	Outcome	Endpoint Method	
Supplementary PDF	Measured Variability	Controls Included	
Précis of Study	Variability		

DB Structure - 2



ModNanoTox_DB.xlsx

Sheets | Charts | SmartArt Graphics | WordArt

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Unique DB Key	article Code/Contr	Biotarget	Duration	Concentration	Variability (SD)	[Units]	Assay	Assay Variable	Endpoint (units)	N	p	Outcome	Variability (SD
2749		$AgNP\text{-}PVP_{10}$	*Danio rerio*	3 d	30	nominal	µM	Hatching		As % of live larvae at 6 dpf	N/S		N/S	
2750				4 d	30	nominal	µM	Hatching		As % of live larvae at 6 dpf	N/S		N/S	
2751				4 d	30	nominal	µM	Swim Bladder Inflation	T = 5 days post fertilisation	As % of live larvae at 6 dpf	N/S		N/S	
2752				4 d	30	nominal	µM	Swim Bladder Inflation	T = 6 days post fertilisation	As % of live larvae at 6 dpf	N/S		N/S	
2753				4 d	100	nominal	µM	Morphological abnormality (pooled)		%	N/S		2.00	
2754				4 d	100	nominal	µM	Mortality		%	N/S		N/S	
2755		$AgNP\text{-}PVP_{50}$	*Danio rerio*	3 d	30	nominal	µM	Hatching		As % of live larvae at 6 dpf	N/S		N/S	
2756				4 d	30	nominal	µM	Hatching		As % of live larvae at 6 dpf	N/S		N/S	
2757				4 d	30	nominal	µM	Swim Bladder Inflation	T = 5 days post fertilisation	As % of live larvae at 6 dpf	N/S		N/S	
2758				4 d	30	nominal	µM	Swim Bladder Inflation	T = 6 days post fertilisation	As % of live larvae at 6 dpf	N/S		N/S	
2759				4 d	100	nominal	µM	Morphological abnormality (pooled)		%	N/S		3.00	
2760				4 d	100	nominal	µM	Mortality		%	N/S		N/S	
2761	MNT69	Null	*Oncorhynchus mykiss*	3 h				Na^+ flux	Influx	JNa^+ (µmol g^{-1} h^{-1})	6	N/A	808.70	±84
2762				3 h				Na^+ flux	Efflux	JNa^+ (µmol g^{-1} h^{-1})	6	N/A	-891.80	±193.7
2763				3 h				Na^+ flux	Net	JNa^+ (µmol g^{-1} h^{-1})	6	N/A	-83.10	±132.4
2764				3 h				Na^+, K^+-ATPase activity		µmoles ADP mg^{-1} protein h^{-1}	6	N/A	0.82	±0.12
2765				3 h				CA activity		µmol CO_2 ml^{-1} min^{-1}	6	N/A	4074.00	±377.2
2766		$AgNO_3$	*Oncorhynchus mykiss*	3 h	0.02	nominal	µg l^{-1}	Na^+ flux	Influx	JNa^+ (µmol g^{-1} h^{-1})	6	NS	574.40	±68.3
2767				3 h	0.02	nominal	µg l^{-1}	Na^+ flux	Efflux	JNa^+ (µmol g^{-1} h^{-1})	6	NS	-481.90	±101.4
2768				3 h	0.02	nominal	µg l^{-1}	Na^+ flux	Net	JNa^+ (µmol g^{-1} h^{-1})	6	NS	26.50	±36.6
2769				3 h	0.02	nominal	µg l^{-1}	Na^+, K^+-ATPase activity		µmoles ADP mg^{-1} protein h^{-1}	6	NS	0.72	±0.06
2770				3 h	0.02	nominal	µg l^{-1}	CA activity		µmol CO_2 ml^{-1} min^{-1}	6	NS	4945.90	±865.3
2771		$AgNO_3$	*Oncorhynchus mykiss*	3 h	10	nominal	µg l^{-1}	Na^+ flux	Influx	JNa^+ (µmol g^{-1} h^{-1})	6	<0.05	308.80	±135.0
2772				3 h	10	nominal	µg l^{-1}	Na^+ flux	Efflux	JNa^+ (µmol g^{-1} h^{-1})	6	<0.05	-1134.70	±338.7
2773				3 h	10	nominal	µg l^{-1}	Na^+ flux	Net	JNa^+ (µmol g^{-1} h^{-1})	6	<0.05	-817.30	±208.5
2774				3 h	10	nominal	µg l^{-1}	Na^+, K^+-ATPase activity		µmoles ADP mg^{-1} protein h^{-1}	6	<0.05	0.41	±0.13
2775				3 h	10	nominal	µg l^{-1}	CA activity		µmol CO_2 ml^{-1} min^{-1}	6	NS	3941.20	670.10
2776		Ag-NP	*Oncorhynchus mykiss*	3 h	1	nominal	mg l^{-1}	Na^+ flux	Influx	JNa^+ (µmol g^{-1} h^{-1})	6	<0.05	285.40	±15.9
2777				3 h	1	nominal	mg l^{-1}	Na^+ flux	Efflux	JNa^+ (µmol g^{-1} h^{-1})	6	<0.05	-753.50	±78.6
2778				3 h	1	nominal	mg l^{-1}	Na^+ flux	Net	JNa^+ (µmol g^{-1} h^{-1})	6	<0.05	-466.50	±64.1
2779				3 h	1	nominal	mg l^{-1}	Na^+, K^+-ATPase activity		µmoles ADP mg^{-1} protein h^{-1}	6	<0.05	0.44	±0.1
2780				3 h	1	nominal	mg l^{-1}	CA activity		µmol CO_2 ml^{-1} min^{-1}	6	NS	4956.20	±328.2
2781		dAg-NP	*Oncorhynchus mykiss*	3 h	1	nominal	mg l^{-1}	Na^+ flux	Influx	JNa^+ (µmol g^{-1} h^{-1})	6	<0.05	193.80	±64.5
2782				3 h	1	nominal	mg l^{-1}	Na^+ flux	Efflux	JNa^+ (µmol g^{-1} h^{-1})	6	NS	-470.90	±170.7
2783				3 h	1	nominal	mg l^{-1}	Na^+ flux	Net	JNa^+ (µmol g^{-1} h^{-1})	6	NS	-285.20	±106.4
2784				3 h	1	nominal	mg l^{-1}	Na^+, K^+-ATPase activity		µmoles ADP mg^{-1} protein h^{-1}	6	<0.05	0.38	±0.07
2785				3 h	1	nominal	mg l^{-1}	CA activity		µmol CO_2 ml^{-1} min^{-1}	6	NS	4820.80	±785.9
2786	MNT70	$AgNO_3$	*Daphnia magna*	1 h	80	nominal	µg l^{-1}	Radiotracer Ag Uptake		Uptake since start of exposure (µg g^{-1})	3		335.60	±0
2787				2.2 h	80	nominal	µg l^{-1}	Radiotracer Ag Uptake		Uptake since previous timepoint (µg g^{-1})	3		693.70	±0
2788				3.3 h	80	nominal	µg l^{-1}	Radiotracer Ag Uptake		Uptake since previous timepoint (µg g^{-1})	3		841.10	±0
2789				4.5 h	80	nominal	µg l^{-1}	Radiotracer Ag Uptake		Uptake since previous timepoint (µg g^{-1})	3		983.90	±0
2790				6 h	80	nominal	µg l^{-1}	Ag content of gut		As a % of Total Ag Body Burden	3		15.80	±5.0
2791		C20	*Daphnia magna*	1 h	500	nominal	µg l^{-1}	Radiotracer Ag Uptake		Uptake since start of exposure (µg g^{-1})	3		6726.70	±972.8
2792				2.2 h	500	nominal	µg l^{-1}	Radiotracer Ag Uptake		Uptake since previous timepoint (µg g^{-1})	3		12318.50	±1425.7
2793				3.3 h	500	nominal	µg l^{-1}	Radiotracer Ag Uptake		Uptake since previous timepoint (µg g^{-1})	3		14435.60	±1018.2
2794				4.5 h	500	nominal	µg l^{-1}	Radiotracer Ag Uptake		Uptake since previous timepoint (µg g^{-1})	3		13452.30	±1019.6
2795				4.5 h	500	nominal	µg l^{-1}	Influx rate (*J*)		µg g^{-1} h^{-1}			3570.80	±439.5
2796				4.5 h	500	nominal	µg l^{-1}	Ag Tissue Content	Radiotracer method	Ag (µg g^{-1} dry weight)	3		13436.70	±2605.5
2797				4 h	500	nominal	µg l^{-1}	Ag Tissue Content	HNO_3 + AAS	Ag (µg g^{-1} dry weight)	3		9106.40	±1521.1
2798				6 h	80	nominal	µg l^{-1}	Ag content of gut		As a % of Total Ag Body Burden	3		74.60	±10.5
2799				4.5 h	1	nominal	µg l^{-1}	Influx rate (*J*)	Cysteine -	µg g^{-1} h^{-1}			1.10	±0.1
2800				4.5 h	2	nominal	µg l^{-1}	Influx rate (*J*)	Cysteine -	µg g^{-1} h^{-1}			2.00	
2801				4.5 h	4	nominal	µg l^{-1}	Influx rate (*J*)	Cysteine -	µg g^{-1} h^{-1}			4.30	
2802				4.5 h	1	nominal	µg l^{-1}	Influx rate (*J*)	Cysteine (1 µM)	µg g^{-1} h^{-1}			0.20	

Study Details | Particle Details | Assay Details | Study Outcomes

Normal View | Ready | Sum=44.98 | SCRL | CAPS | NUM

SEVENTH FRAMEWORK PROGRAMME

Statistical Methodology

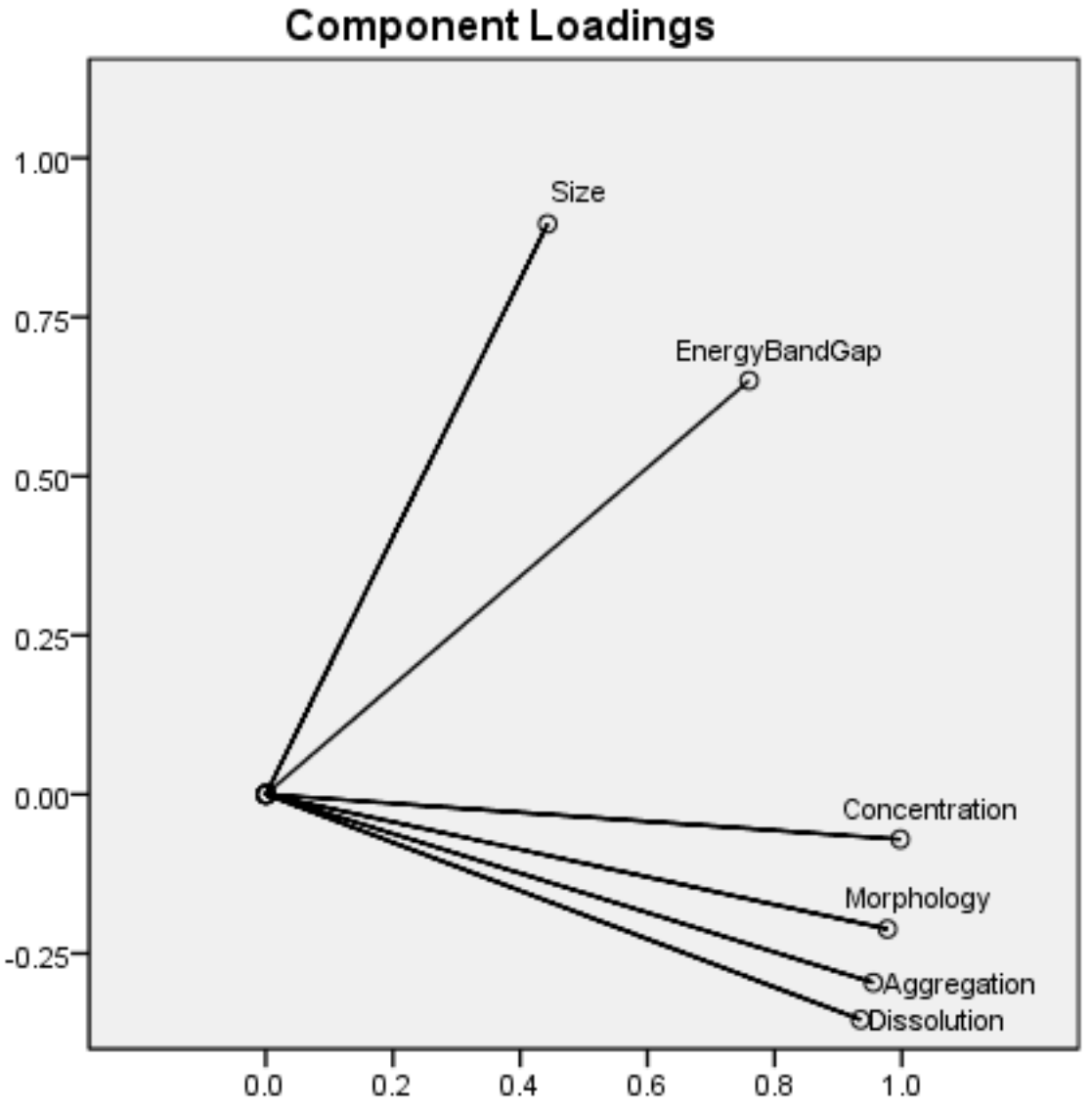
- Due to data non-linearity Categorical Principal Component Analysis (CatPCA) was used for analysis
- CatPCA transforms data in 3D space, brings out strong patterns in data sets and emphasises data variation
- CatPCA is useful for eliminating dimensions (data parameters), which have little or no effect on the overall dataset variation
- Data imputation was used for small amounts of missing data to avoid bias
- Two separate groupings were performed:
  - Total study score rating per year
  - Mortality (%) per ENM type and per species

ENMs Characterisation Rating – CatPCA

Model Summary

Dimension	Cronbach's Alpha	Variance Accounted For	
		Total (Eigenvalue)	% of Variance
1	.934	4.511	75.178
2	.394	1.489	24.821
Total	1.000[a]	6.000	100.000

a. Total Cronbach's Alpha is based on the total Eigenvalue.



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Conclusions – ENMs Characterisation

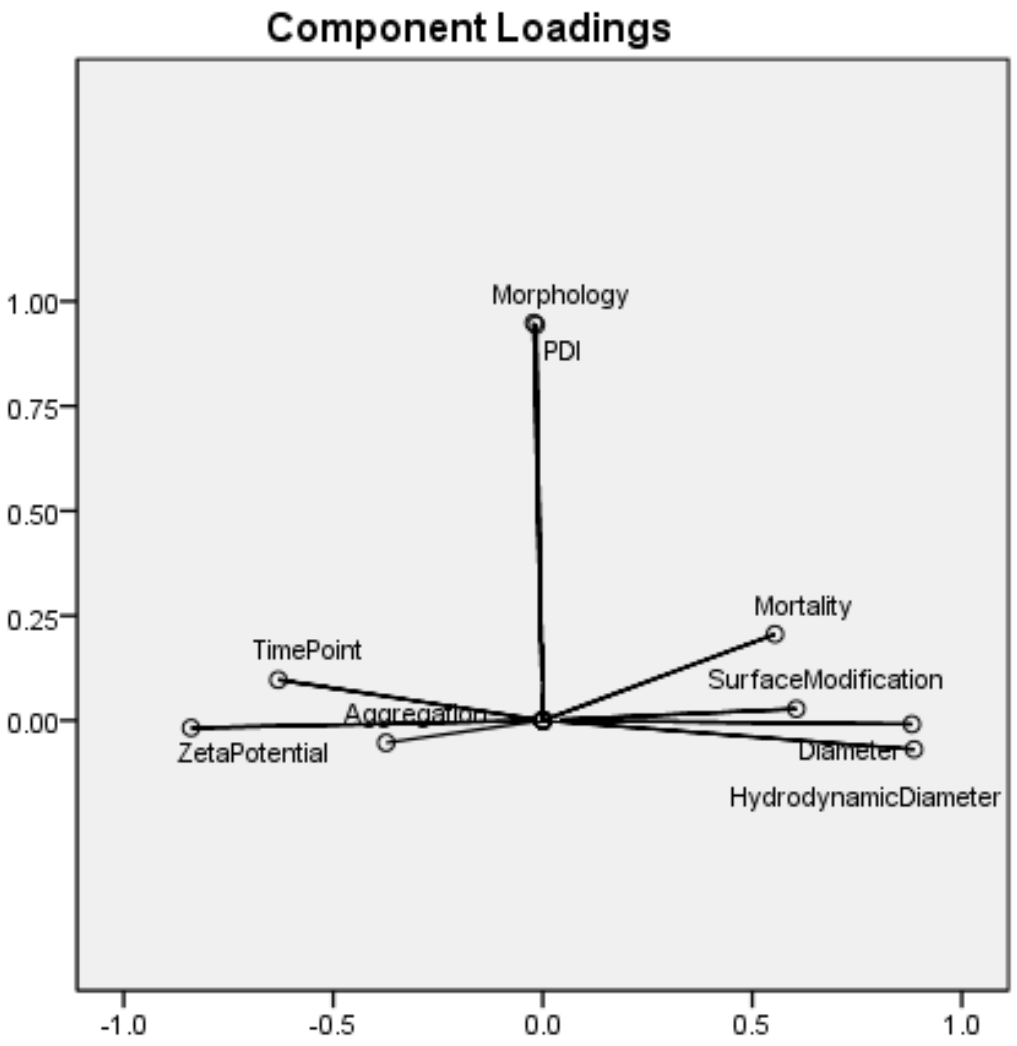
- ENM parameters studied in more detail over time are: size, morphology, concentration, dissolution, aggregation and energy band gap
- Cross-study discontinuity and lack of comparability exists due to different ENM characterisation protocols
- Analysis of extracted ENM parameters demonstrated the need for complete in-house characterisation in the case of commercial ENMs

SEVENTH FRAMEWORK PROGRAMME

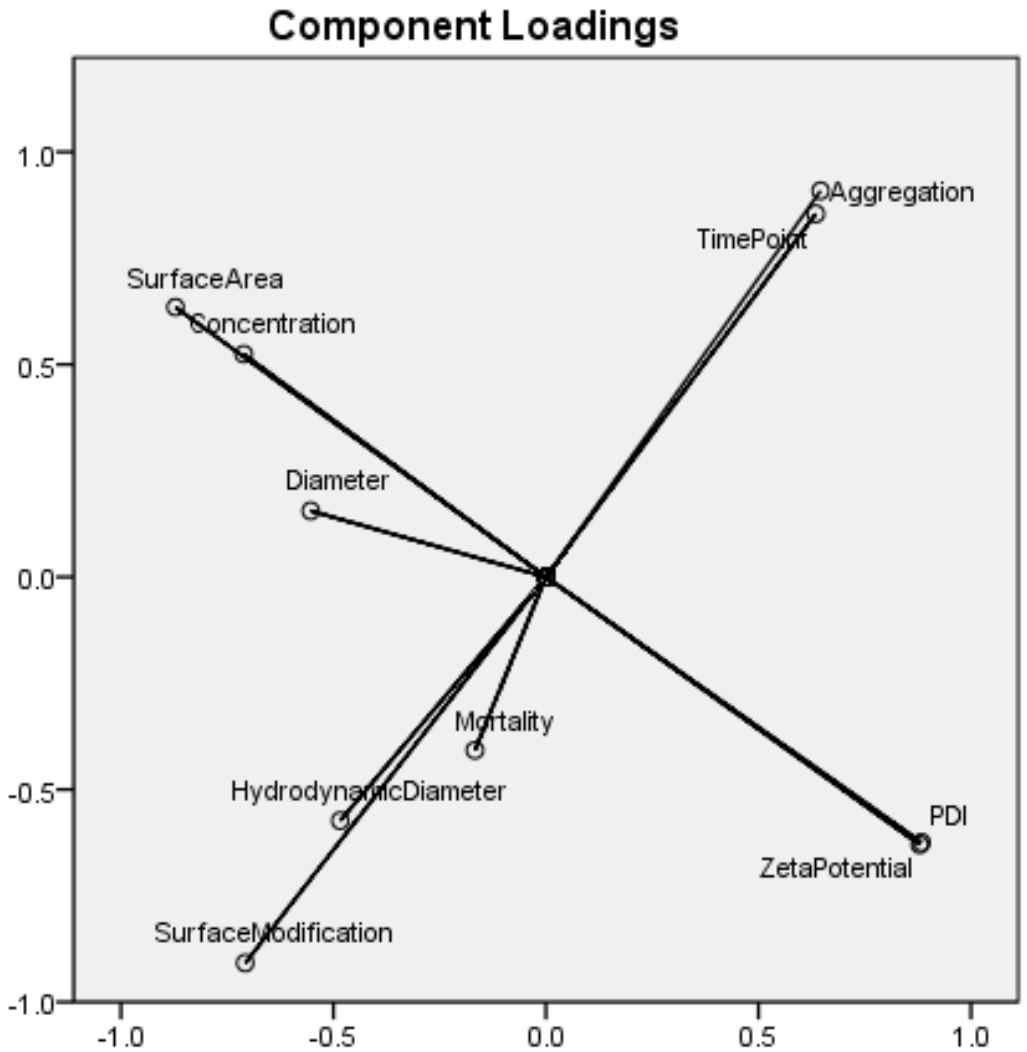
ENP parameters vs Toxicity
Ag ENMs case study

- Toxicity vs ENM physicochemical parameters analysis was performed using mortality (%) of the respective biotarget
- In total 1,118 mortality (%) entries for Ag NMs were included
- Analysis was segmented according to biotarget (*D. magna* (279), *D. rerio* (256), *C. elegans* (78), *O. latipes* (174), *E. coli* (173))
- Analysed data demonstrated no linearity

Ag Case Study – Mortality vs Species



D. magna



D. rerio

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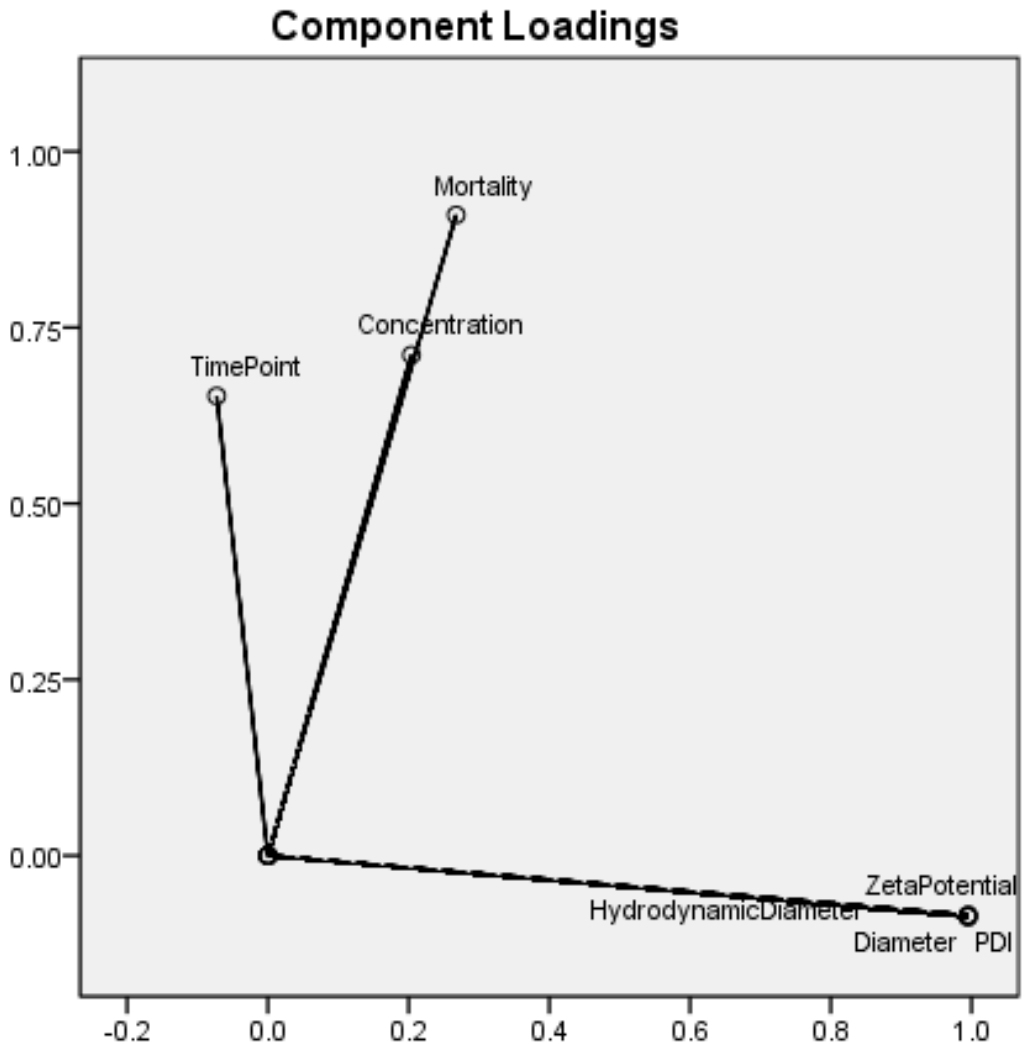
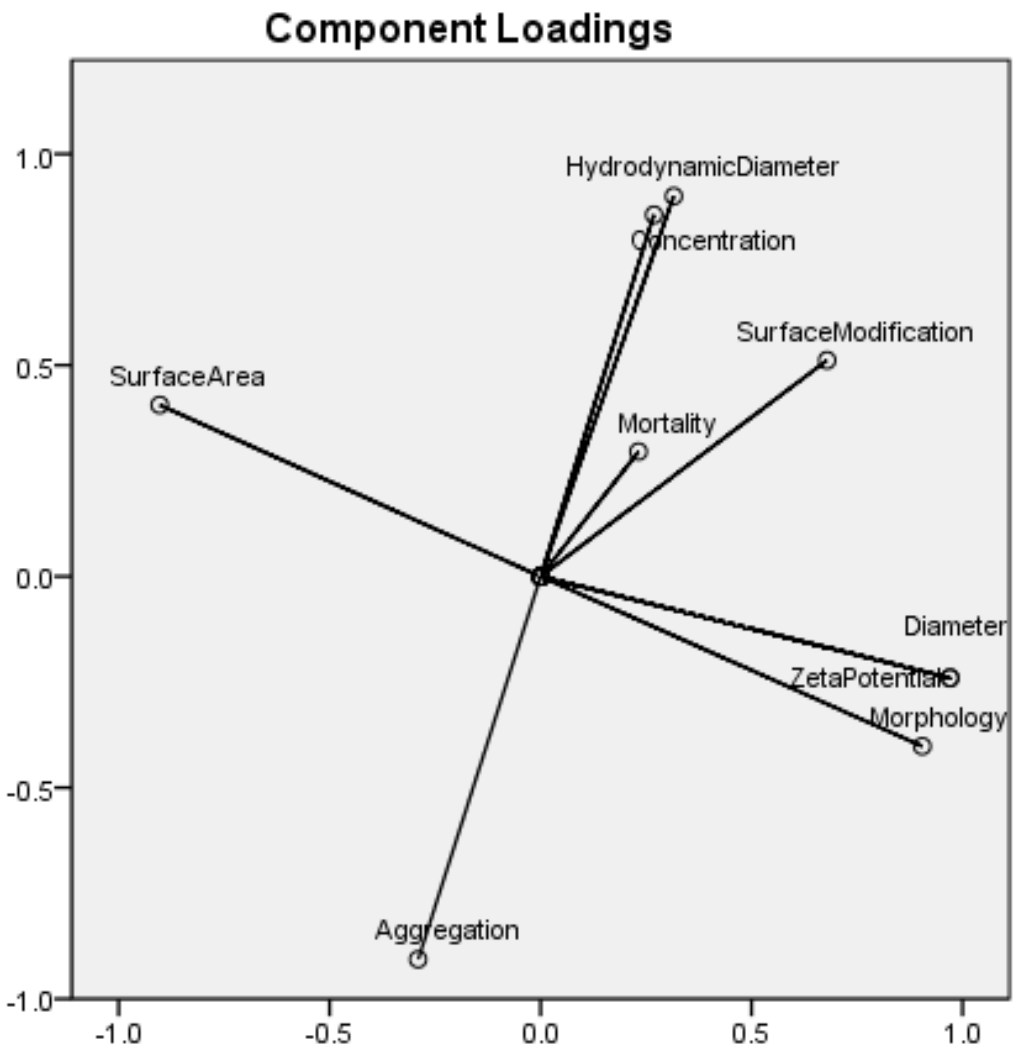
Ag Case Study – Mortality vs Species



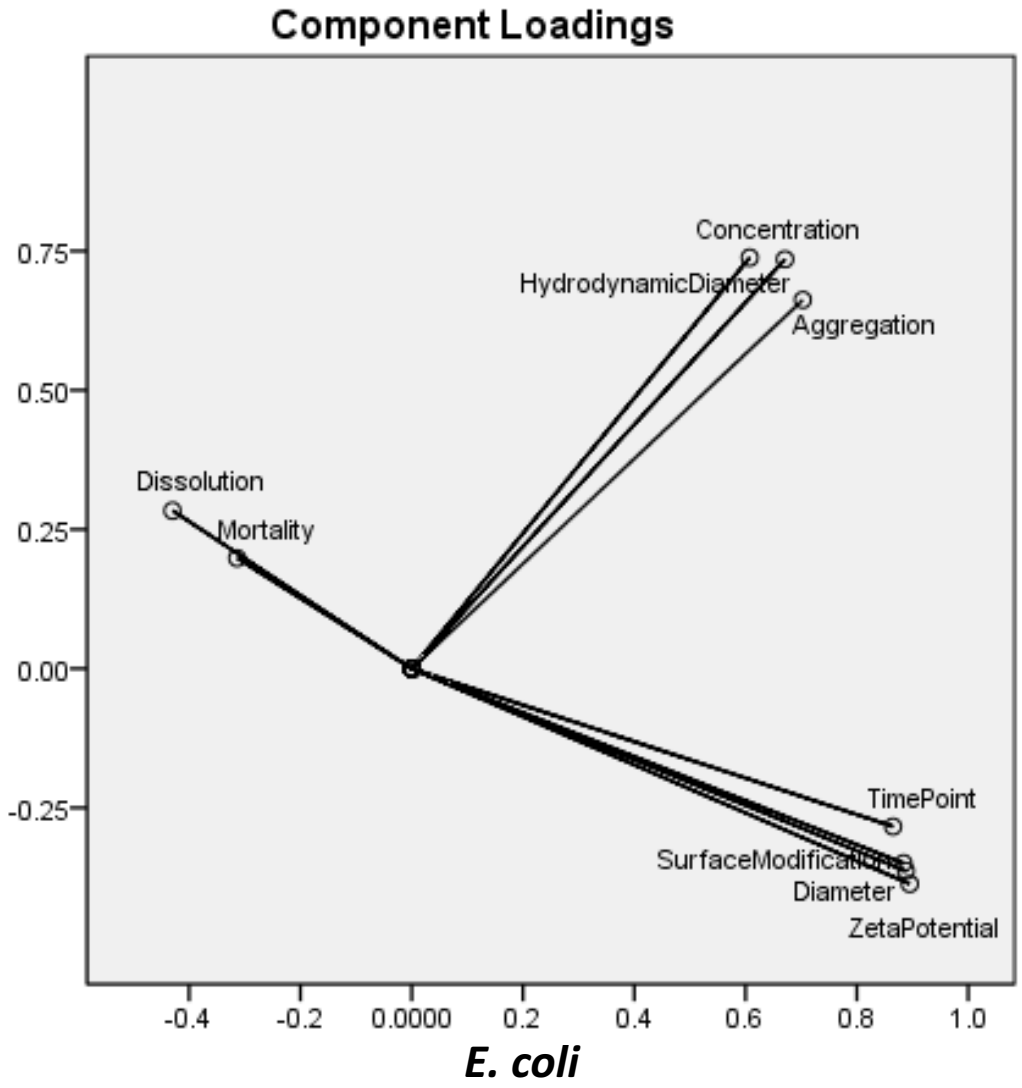
C. Elegans*



O. latipes

*No coated NP studies included in DB

Ag Case Study – Mortality vs Species



E. coli

Conclusions – ENMs parameters vs Toxicity

- The ENM parameters affecting toxicity are in general similar between biotargets
- Lack of data points could have a direct effect on the results and the conclusions reached
- A common ENM characterisation protocol would be desirable to ensure cross-study comparability and allow safer conclusions to be reached
- Further segmentation (e.g. per study media) would also help point out stronger or missed data patterns

SEVENTH FRAMEWORK PROGRAMME

Gap Identification & Statistical Analysis

- Significant gaps in characterisation (e.g. over-exposure duration, aggregation)
- In-house characterisation is essential for commercial ENMs
- A standardised characterisation protocol is needed to ensure cross-study continuity and comparability
- Statistical analysis needs to be based on appropriate non-linear models
- Uptake and depuration kinetics data
- Internal concentration of aquatic organisms

Thank you for your attention..

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