Task 1.2 An Update on Gap-filling Experiments: Bioaccumulation in Earthworms and Fish Gut Lumen Digestibility Assays

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Partners: UKRI (CEH), Associates: Defra, Environment Agency, UK.

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The Key Triggers for Environmental Risk Assessment

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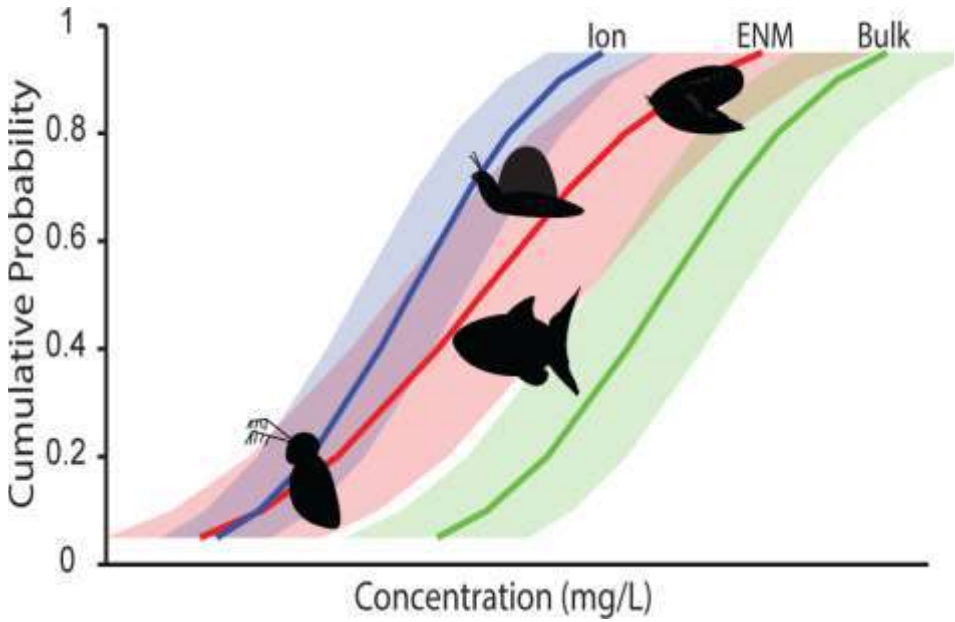
- Persistence in the Environment
- Bioaccumulation Potential
- Toxicity

Risk = Exposure x Hazard
PEC/PNEC ratios >1 presents a risk

Species sensitivity distributions for Nano?



Garner et al. Environ. Sci. Technol. 2015, 49, 5753−5759

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Bioaccumulation Testing of ENMs

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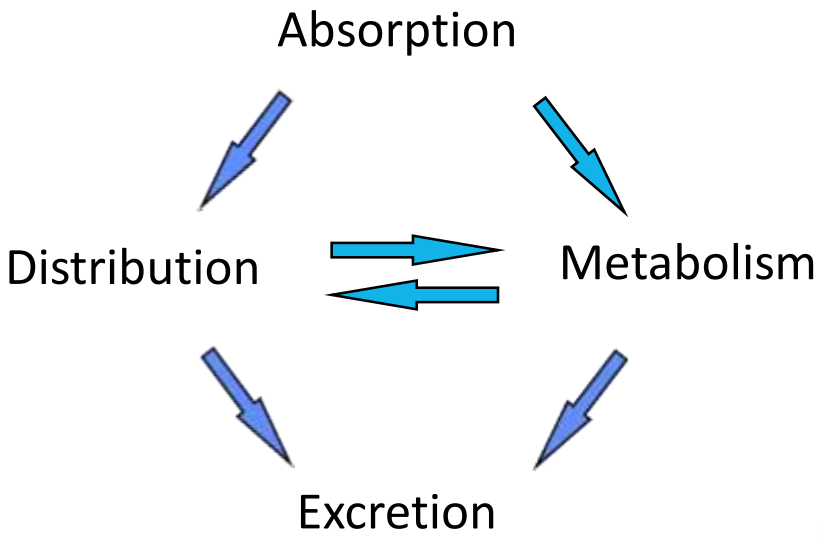
- Traditionally using a chronic exposure with fish over ~4 weeks.
- OECD TG 305
- Choice of exposure method: via the water or the food.
- Measure the uptake (k_1) to steady state in the tissues and water.
- Calculate the bioaccumulation factor, BCF.
- For dietary exposure method, calculate the biomagnification factor, BMF.



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Correlation of log BCF with log *P* in Octanol/Water

Veith et al. J. Fish. Res. Bd. Can. (1979) 36, 1040-1048

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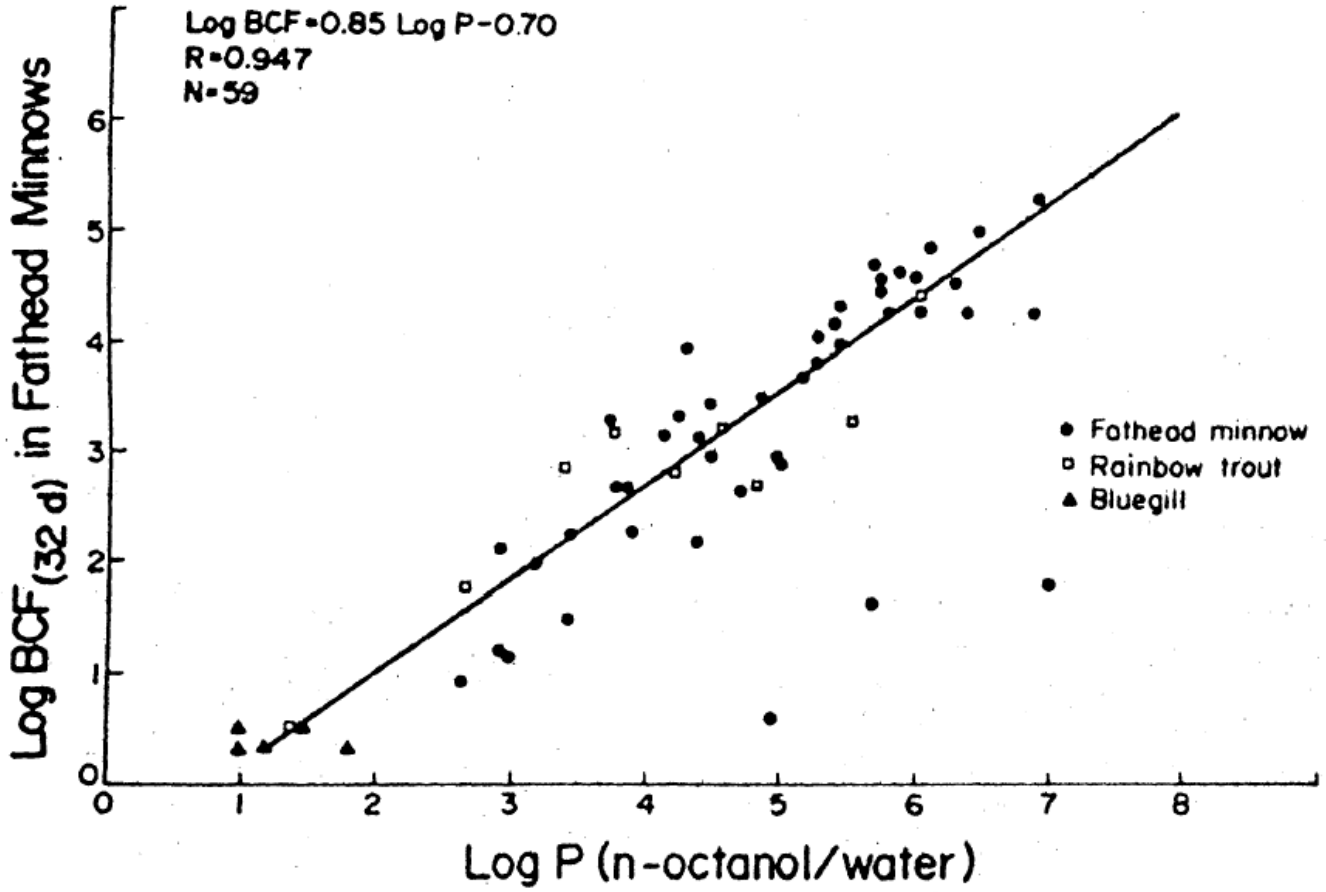

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Problems with the Current OECD Testing Strategy for Bioaccumulation Potential

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Handy et al (2018) Environ. Sci. Nano, 5, 2030–2046

- The (K_{ow}) test is not intended for nanomaterials, and does not work for many materials.
- Assumes solute chemistry – not relevant to nano.
- Under current test guidance at the OECD, if the (K_{ow}) test is not practical, then the work moves directly to the *in vivo* fish test, TG 305.

Testing all ENMS with TG305 is expensive and unrealistic.

Animal Welfare – no alterative methods in the OECD testing strategy for bioaccumulation

TG 305: Bioaccumulation in Fish

~150 fish, 28 day test
~€90-130k per test

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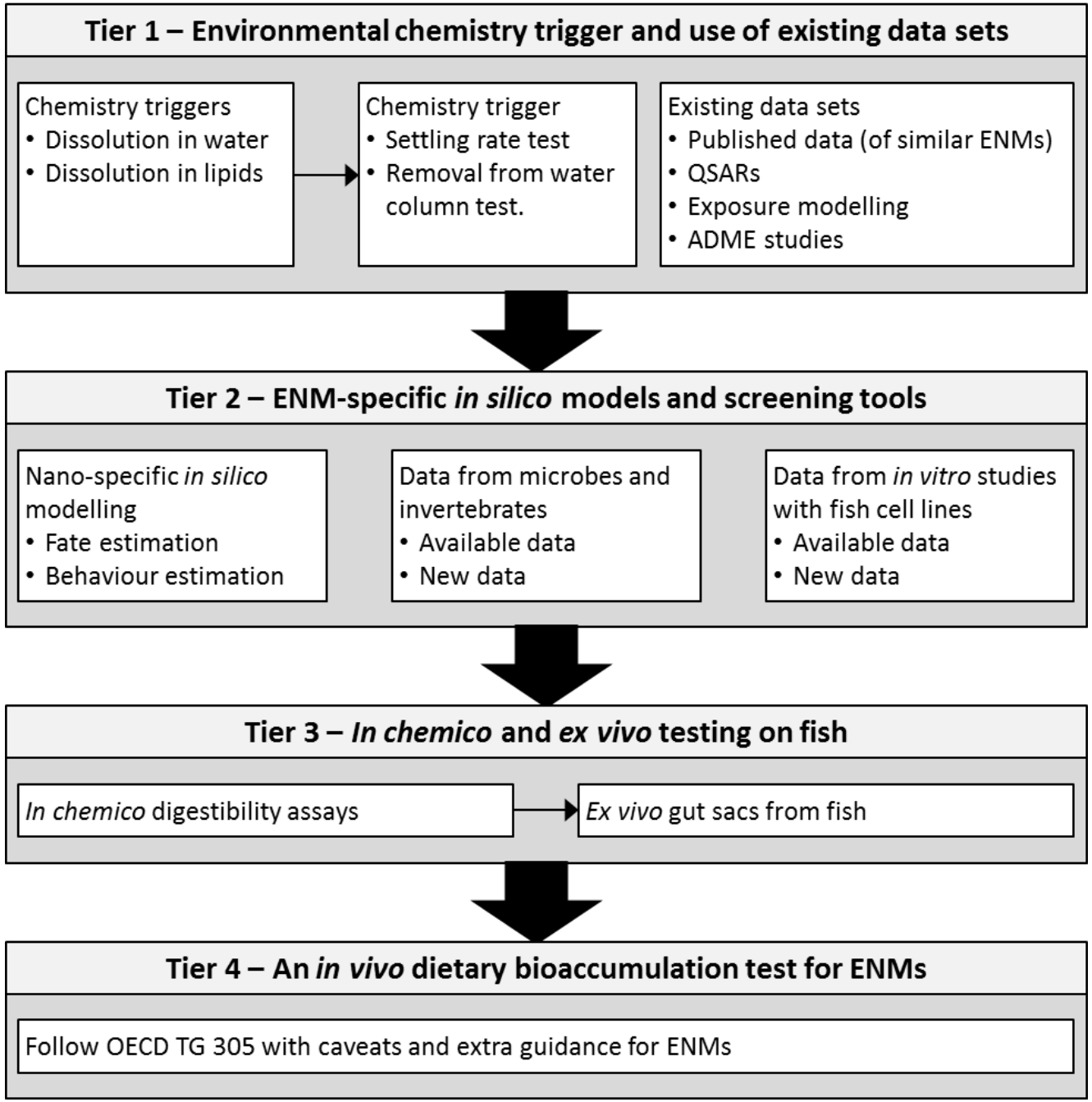

Proposed Dietary Bioaccumulation Potential Testing Strategy with Fish

Handy et al. (2018)
Environ. Sci.: Nano,
5, 2030–2046.

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Task 1.2 Scientific basis for a new GD on determination of bioaccumulation potential of ENMs - Deliverables

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Partners: University of Plymouth (Lead), UKRI-CEH, associated partners: Defra, EA

D1.2 Report on the existing data, test methods, and recommendations for a tiered approach for determination of bioaccumulation potential of ENMs (M12).

Completed. Supporting scientific papers with meta-analysis of data.

Handy et al., 2021, Environ. Sci.: Nano, 8, 3167-3185;

Handy et al. 2022 Environ. Sci.: Nano, 9(2), 684-701.

D1.3 Report on the data gaps analysis on methods for bioaccumulation of ENMs (M24).

Completed, but taking the opportunity to collect more data on earthworms and digestibility assays.

D1.4 Draft of the scientific document to support OECD activities on the development of GD on bioaccumulation of ENMs (M33 – UOP).

Scoping review on tiered approaches to bioaccumulation testing, OECD-NanHarmony Expert Group, revised version with ECHA, and then back to OECD.

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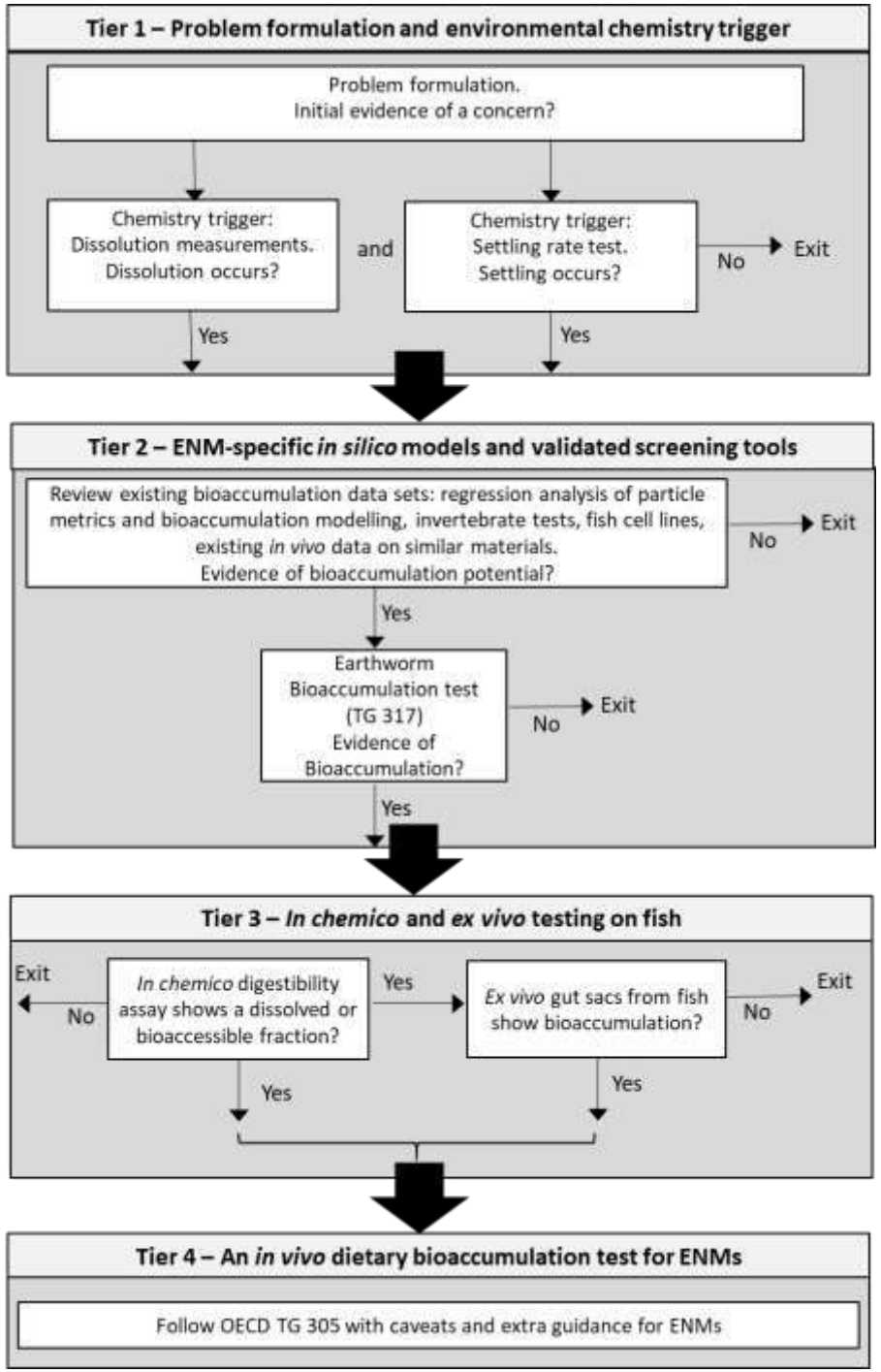

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Refinements to the Tiered Approach to Bioaccumulation Testing with Fish

Handy et al. (2021)
Environ. Sci.: Nano,
8, 3167-3185.

A meta-analysis of bioaccumulation data on earthworms versus fish, and review considering other invertebrate bioaccumulation tests.

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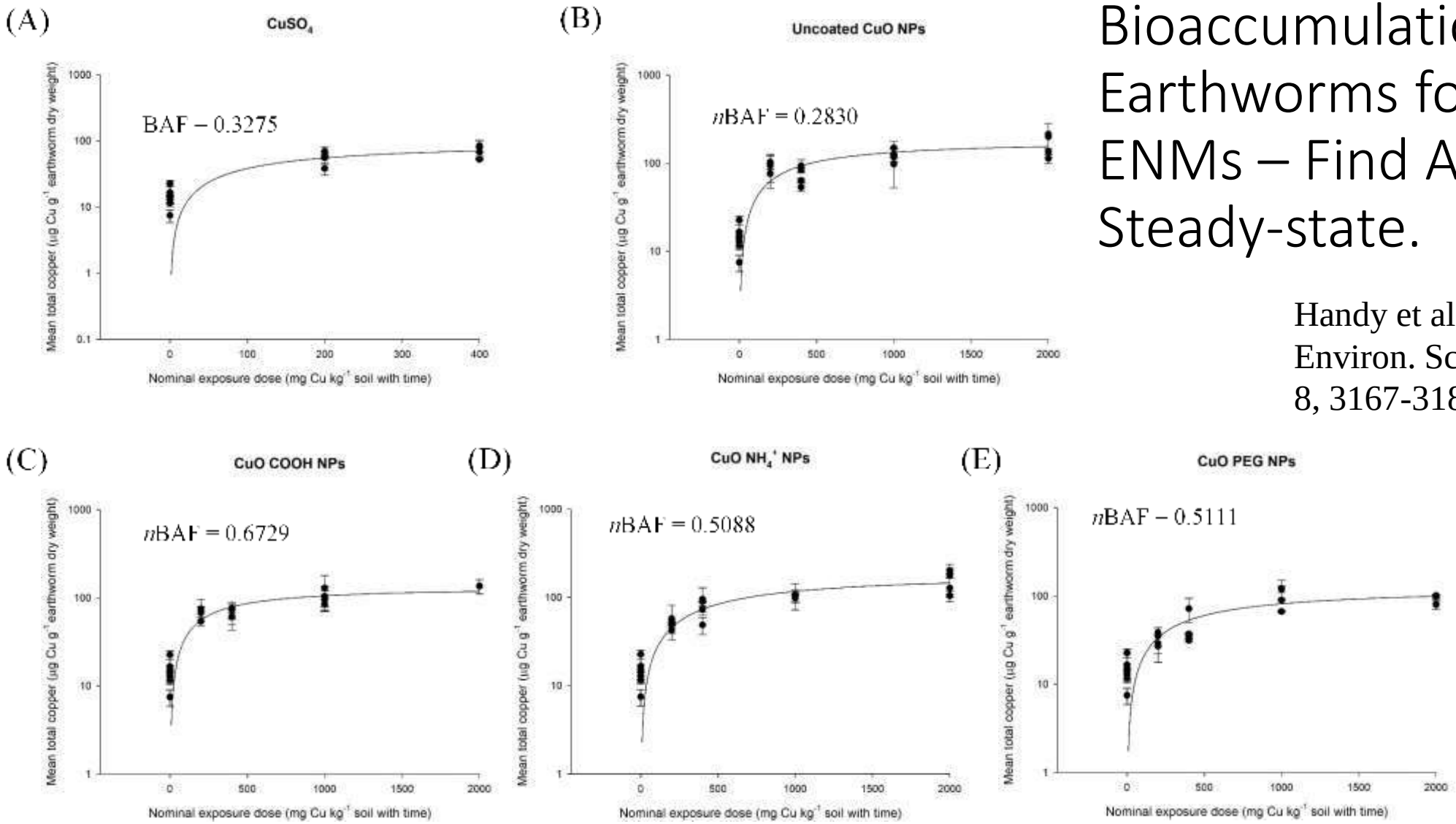
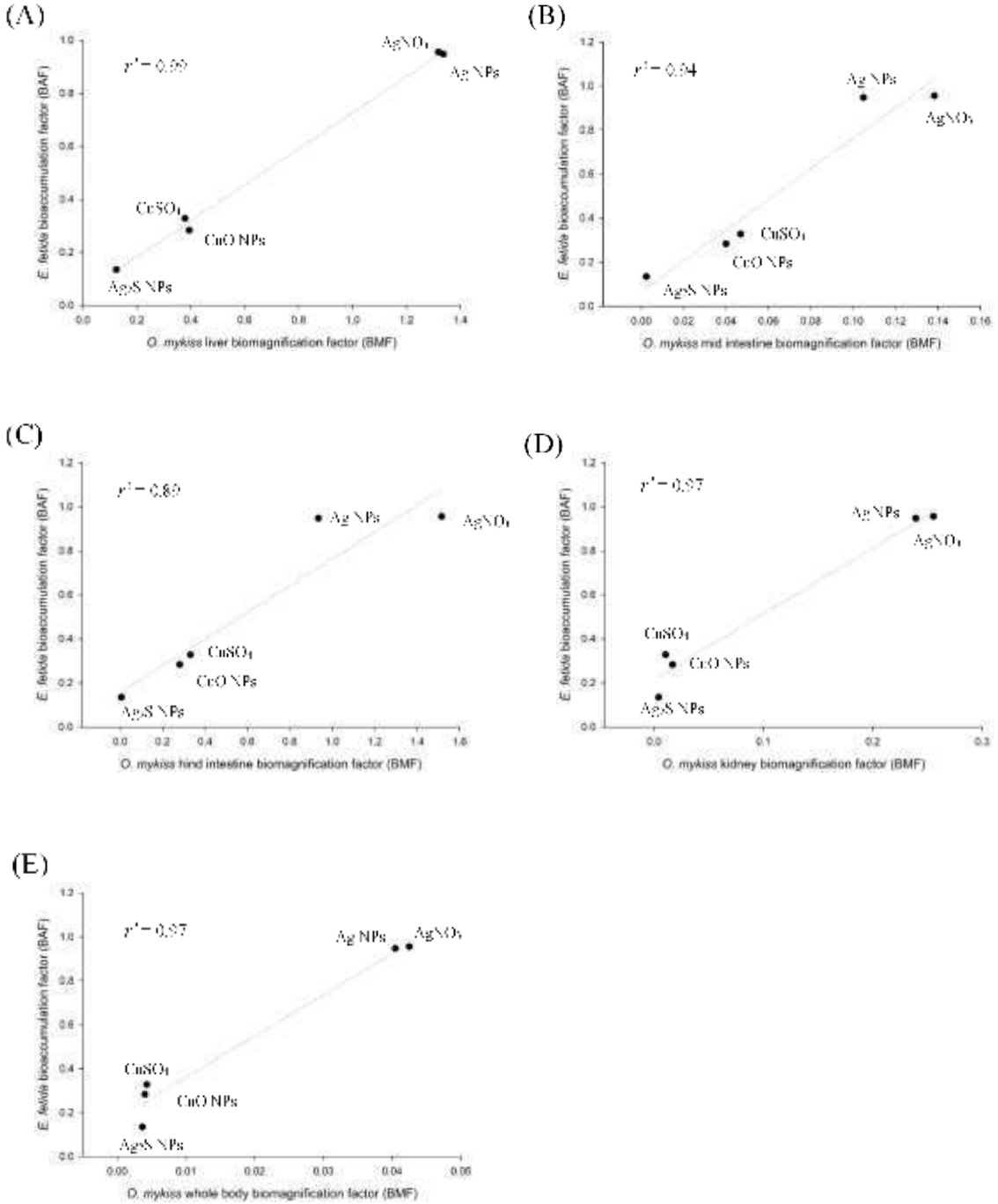
Bioaccumulation Plots of Earthworms for Different ENMs – Find Apparent Steady-state.

Handy et al. (2021) Environ. Sci.: Nano, 8, 3167-3185.





Correlate of BAFs in Earthworms with BMFs in Fish (TG 305).

Handy et al. (2021)
Environ. Sci.: Nano,
8, 3167-3185.

Pilot Study on 'Degussa' P25 TiO_2

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TiO_2 exposure (500, 1000 and 5000 mg TiO_2/kg) in Lufa 2.2 soil for seven days.

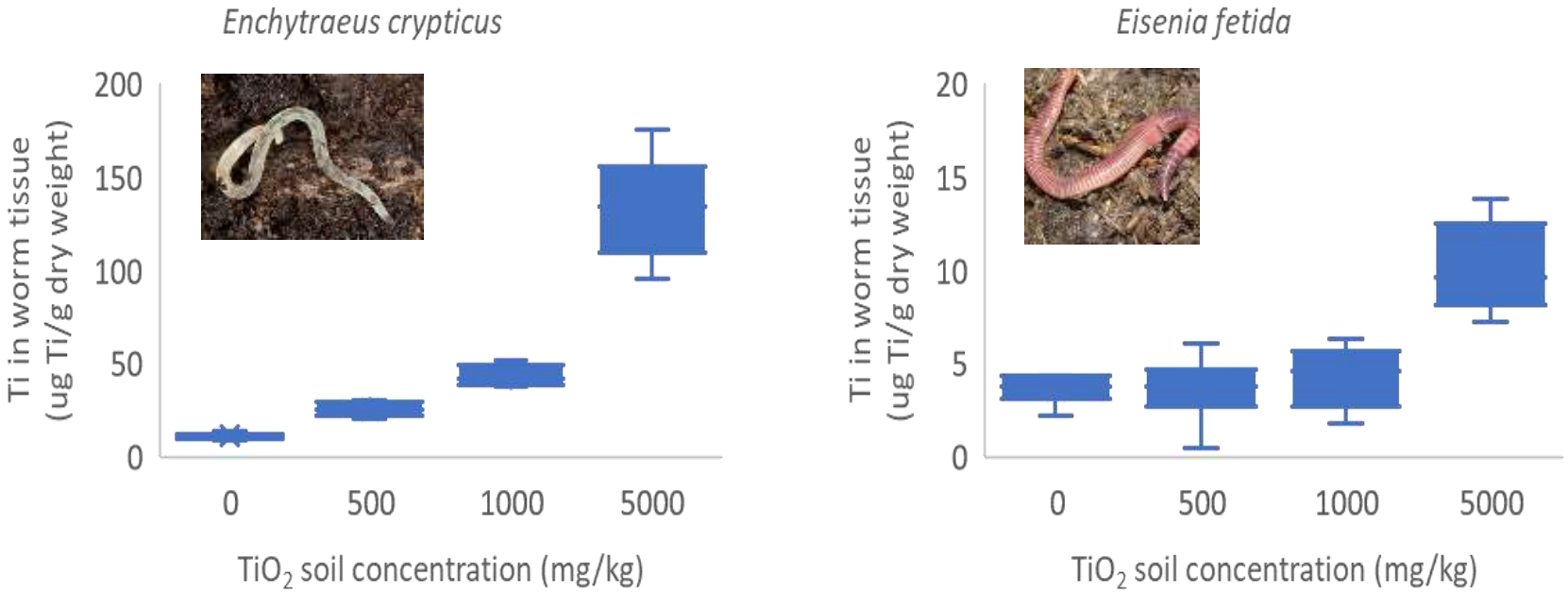

***Figure** - The total Ti concentration in worm tissues following seven days exposure to soils spiked with three difference concentrations of TiO_2.*

Data from Elma Lahive et al., UKCEH

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(Earth)worm Experiments with TiO_2 materials

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- Essentially followed the OECD TG317.
- Exposure of 4000 mg TiO_2/kg was selected
- Spiked soil, follow uptake period (14 days for *E. crypticus* and 21 days for *E. fetida*).
- Transfer worms to clean soil for a further 14- or 21-day period to allow for elimination.
- Samples taken for total metal bioaccmulation and other endpoints.

Material	**Size (nm)**
NanoSolutions (TiO_2_NSol)	4-8
Degussa P25 (TiO_2_P25)	25
Bulk (ACROS) (TiO_2_Acros)	>100 nm
Anatase (TiO_2_Anat)	Measured size TBC

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Total Ti Accumulation from TiO_2 Materials in Earthworms *E. fetida*

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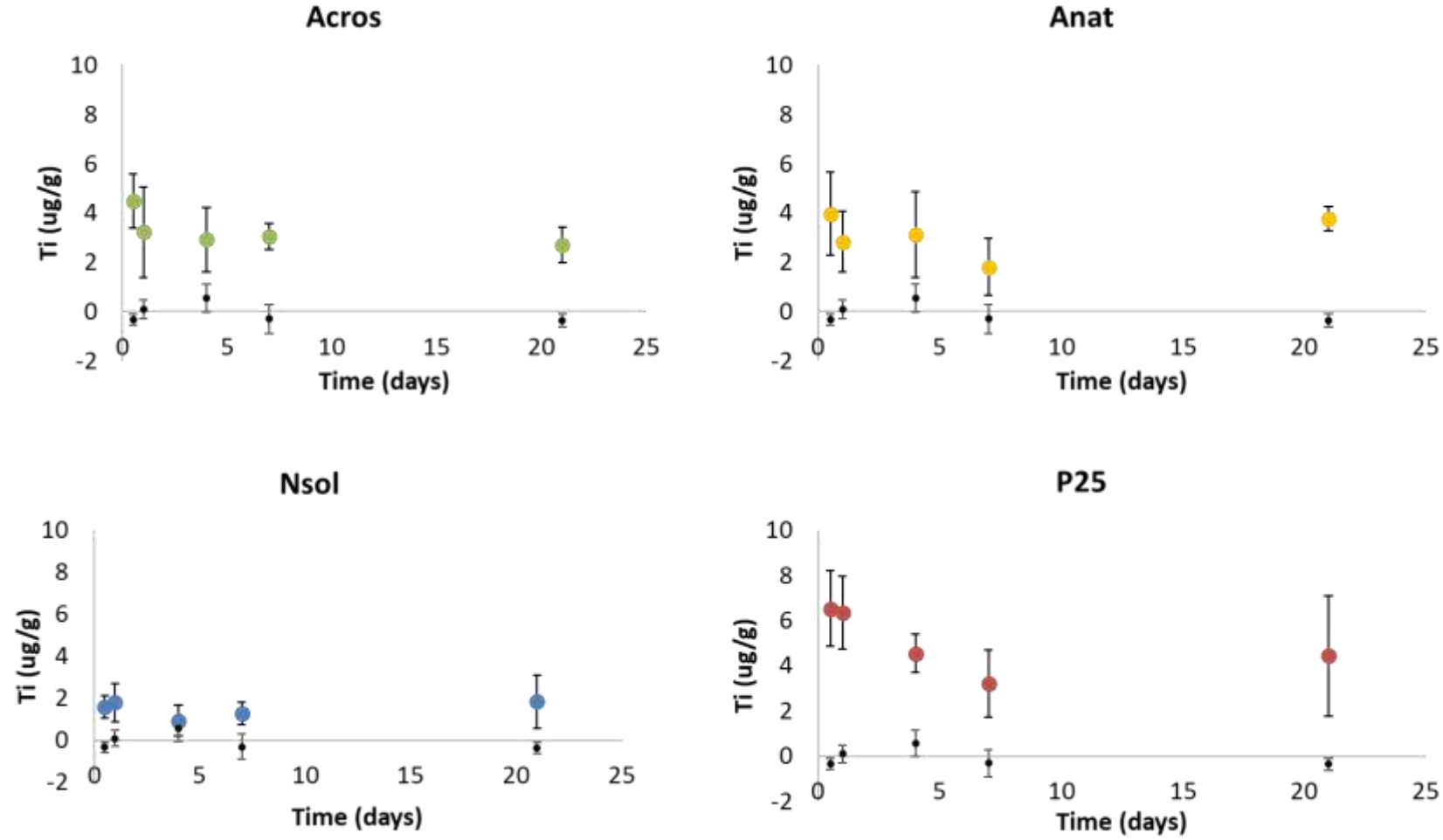

***Figure**: Total Ti concentration in E. fetida during the uptake phase of the experiment.*

Black circles = controls

Coloured dots = TiO_2 exposed worms.

Data from Elma Lahive et al., UKCEH

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Total Ti Accumulation from TiO_2 Materials in *Enchytraeus crypticus*

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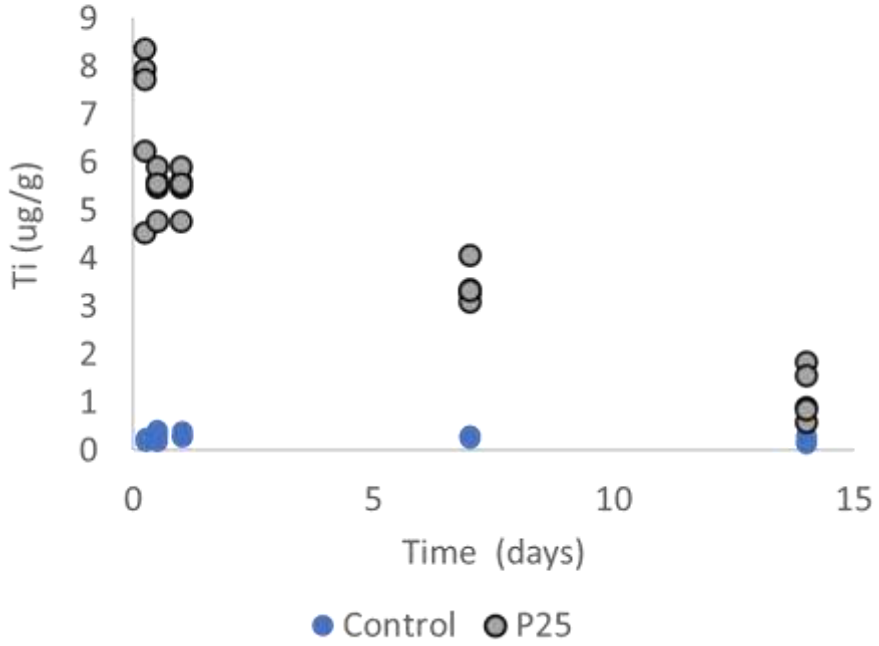

Figure: *Total Ti concentration in the E. crypticus tissue during the uptake phase of the experiment.*

Data from Elma Lahive et al., UKCEH

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“In vitro” Alternatives to Fish Bioaccumulation Test TG305.

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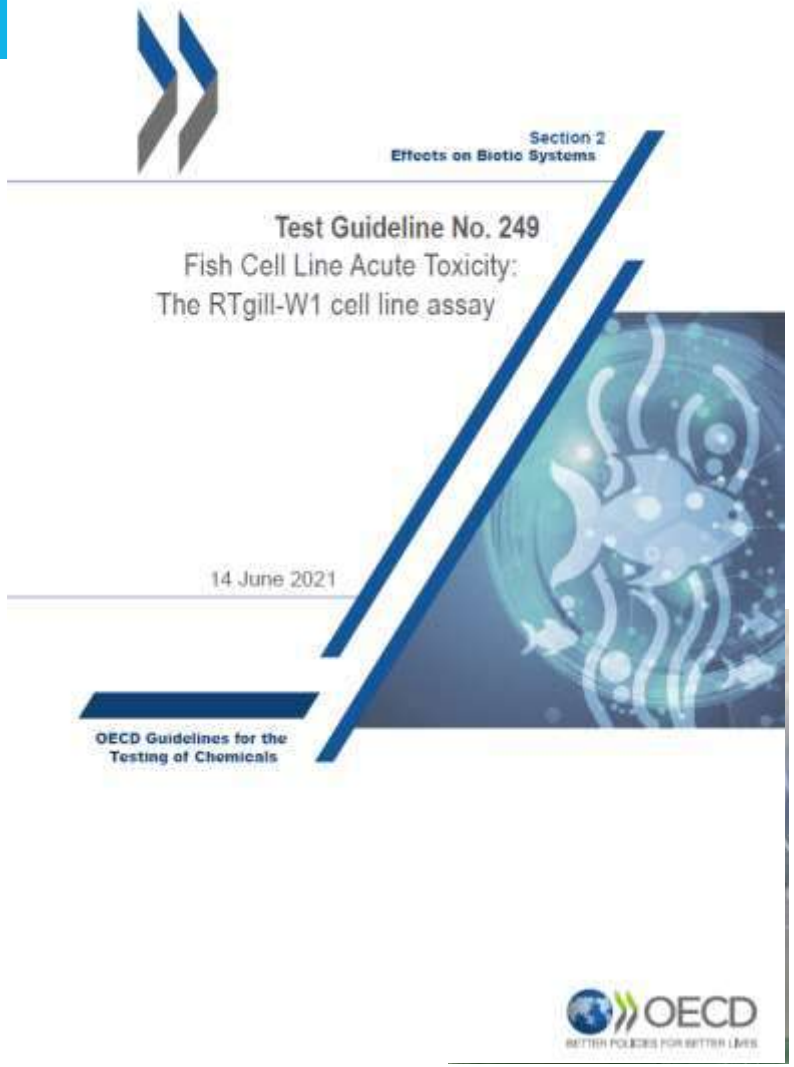
- Digestibility assay as a quick *in chemico* method to inform on bioassessible fractions of nanos in the gut lumen of fish.
- Agreement that digestibility assay is useful and works well for fish, test a wider range of ENMs.
- Fish gut sac preparation has utility, much closer to *in vivo*, and can report accumulation rates in different parts of the gut. Correlate with *in vivo*, but data on more ENMs.
- Fish cell lines
  - RTG cells are now used in an OECD acute toxicity test, OECD TG 249.
  - Rainbow trout gut cell lines.



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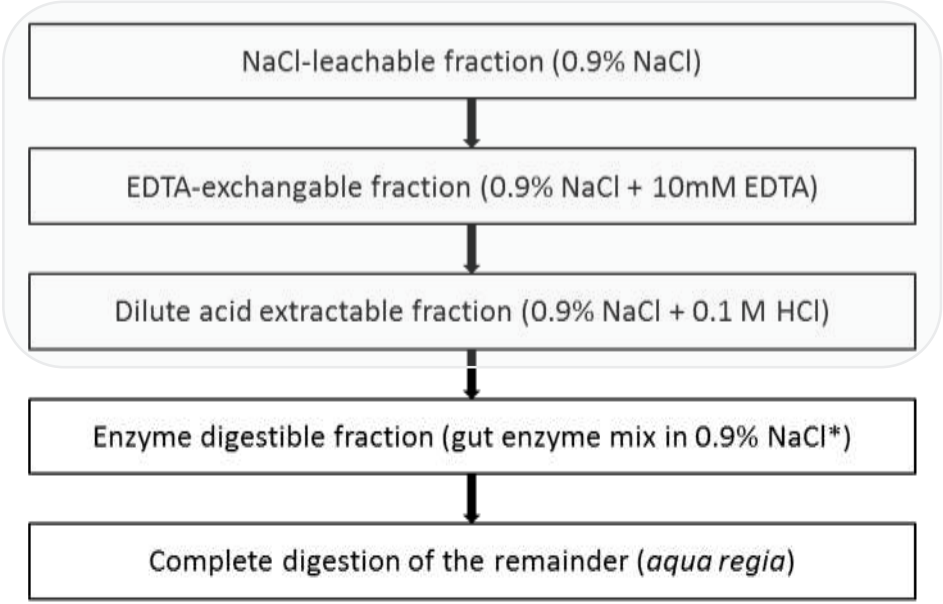
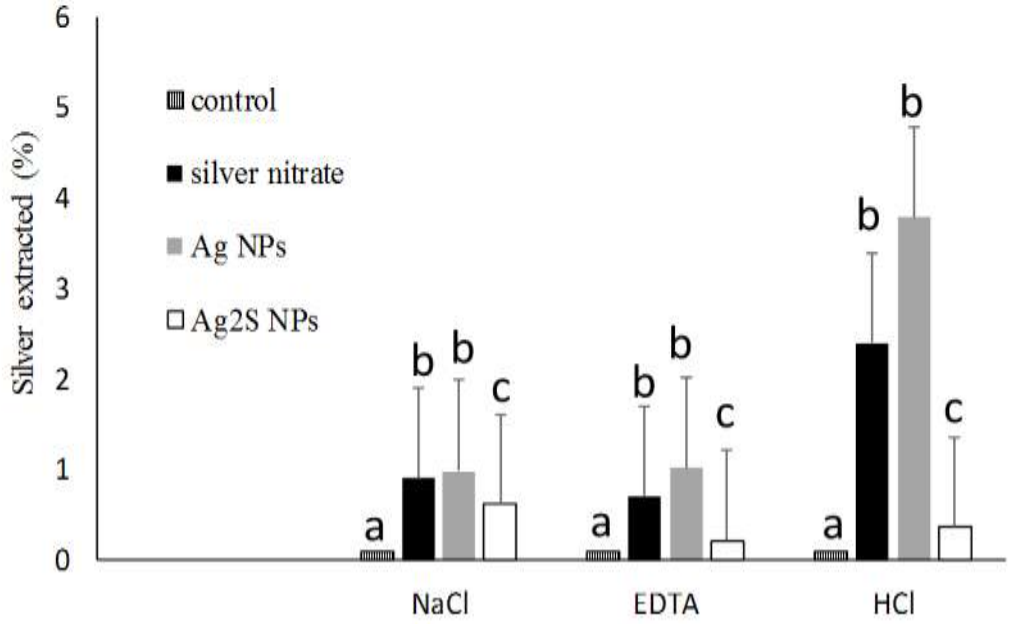
(A)



(similar to EFSA *in vitro* digestibility testing)

Tier 3A. New Trigger for TG305: *In Chemico* Digestibility Assay.

(B)



Handy et al. (2018) Environ. Sci.: Nano, 5, 2030–2046.

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Digestibility of Ce-containing ENMs – incubations of food pellets over 4h in 0.9% NaCl

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Table 1. Digestibility of fish feed supplemented with different Ce materials (Ce salt or CeO_2 NPs) at 100 mg Ce kg^{-1} compared to an unexposed control (no added Ce).

Compartment pH	Time (h)	Control	Ce salt (as $(NH_4)_2[Ce(NO_3)_6]$)	CeO_2 NP
2 ('Stomach')	1	5.2 ± 0.1 ^{Aa}	14571.5 ± 1728.9 ^{Aa}	4845.9 ± 310.4 ^{Aa}
	2	108.8 ± 180.4 ^{Aa}	20545.7 ± 1993.5 ^{Aa}	8362.8 ± 2112.9 ^{Aa}
	4	101.8 ± 169.2 ^{Aa}	30647.6 ± 3703.2 ^{Ba}	23559.1 ± 4408.3 ^{ABa}
7.8 ('intestine')	1	7.3 ± 2.1 ^{Aa}	7980.6 ± 218.6 ^{Ba}	2669.6 ± 197.3 ^{Ca}
	2	6.2 ± 1.5 ^{Aa}	14106.8 ± 868.0 ^{Bb}	6412.1 ± 932.7 ^{Cb}
	4	8.7 ± 1.5 ^{Aa}	29199.8 ± 513.2 ^{Bc}	17827.4 ± 1866.4 ^{Cc}

Data are mean ± SD, n = 3. Different upper case letters denote significant difference between treatments (rows). Different lower case letters denote significant difference between time points (columns).

The order of Ce release (digestibility): control < CeO NPs < Ce salt.

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The *ex vivo* gut sac technique

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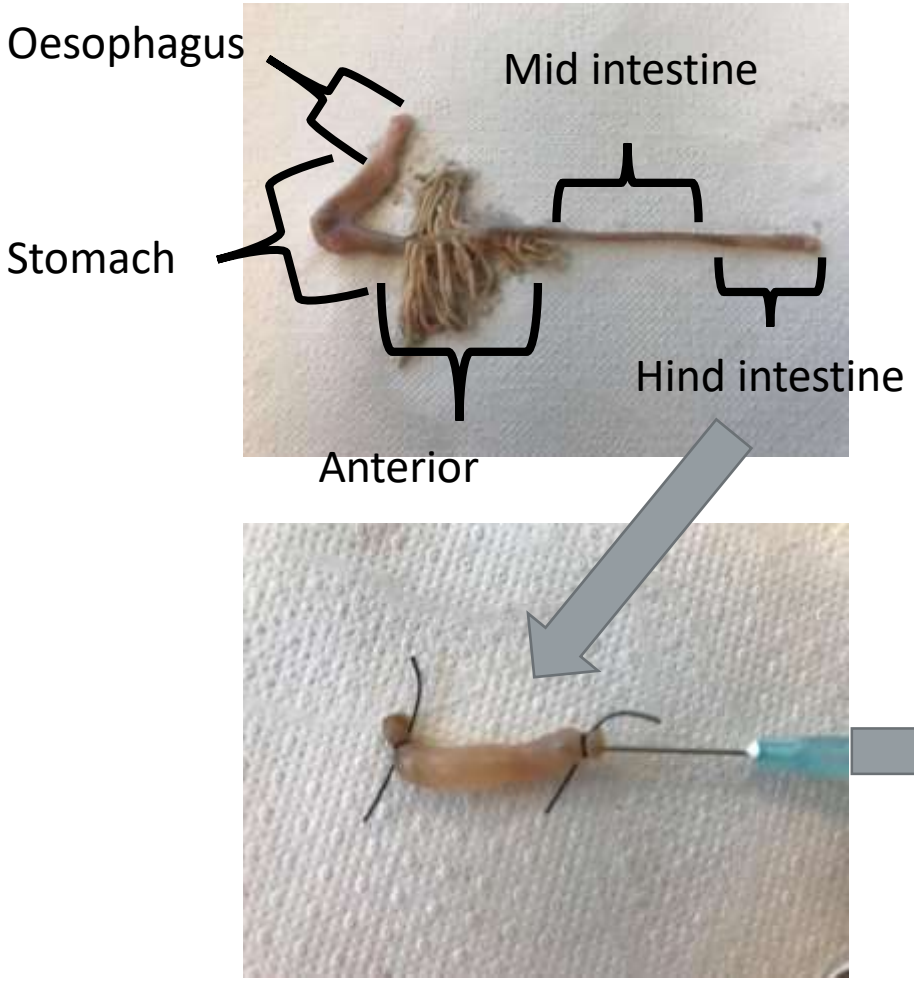

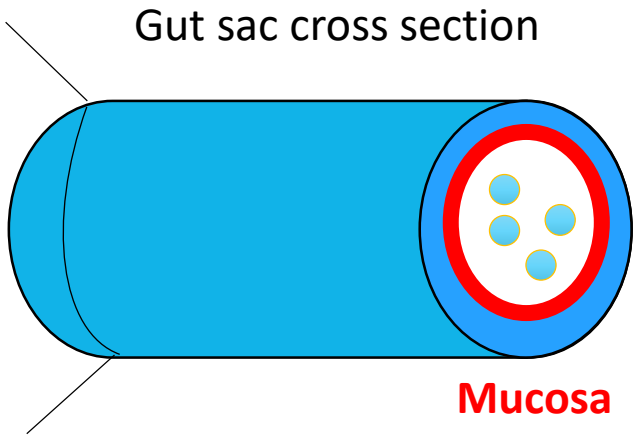

- 5 compartments of the gastrointestinal tract.
- Separate into compartments.
- Fill with materials of interest.
- Incubated for 4 h.
- Separate into mucosa, muscularis and serosal saline.

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Gut Sac Studies: New data on Ce-Materials

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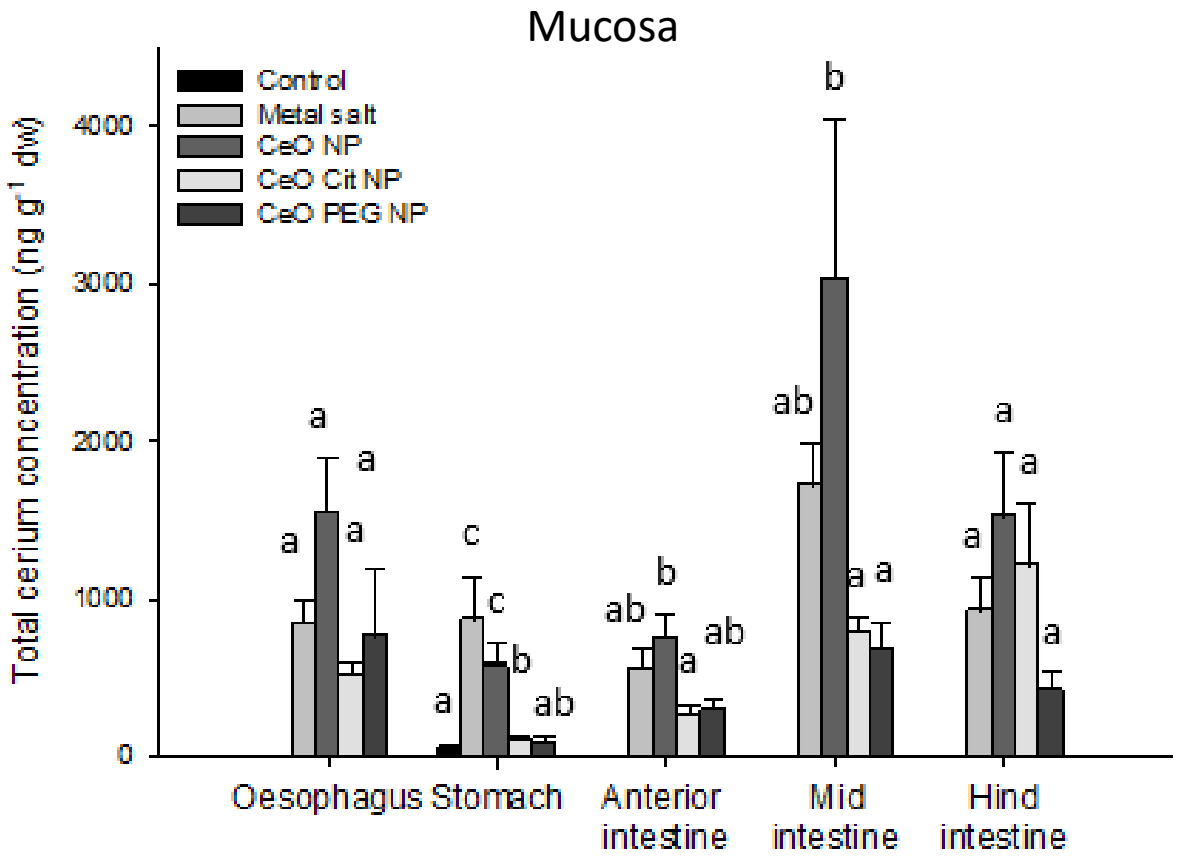
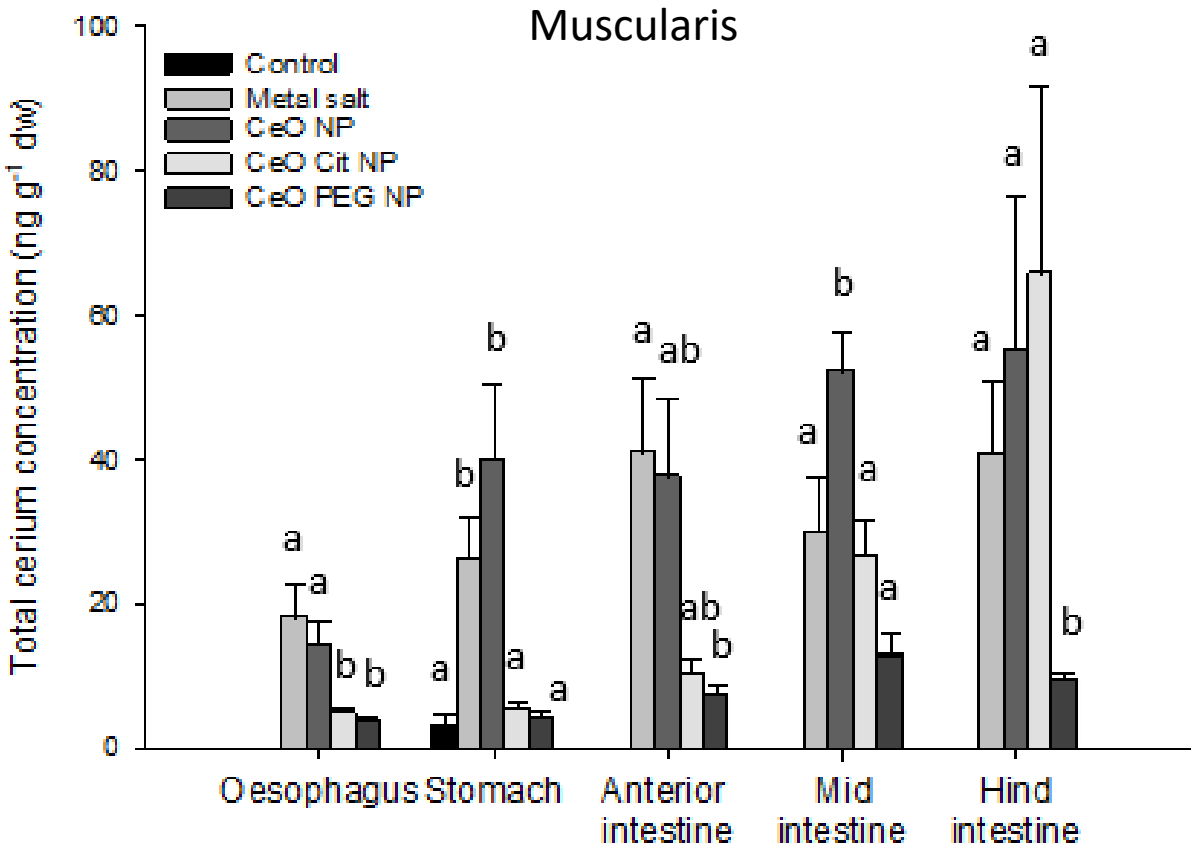
Data slide from: Nathaniel Clark, UoP





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Key New Findings on Ce-materials

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- **Digestibility Assay** - The order of Ce release: control < CeO NPs < Ce salt. Nanoform less bioaccessible than the dissolved metal.
- **Gut Sacs** - Ce was detected in all gut regions of the gut, also mucosa and less in the muscularis.
- Mucosa - Overall, the uncoated CeO_2 NPs had a higher bioavailability, especially in the mid intestine, compared to the coated CeO_2 NPs, but the CeO_2 NPs did not differ from the metal salt.
- Mucosa - There were no coated related differences between the citrate or PEG materials, with both of these typically lower than the uncoated material.
- Muscularis - Overall, the uncoated CeO_2 NPs had a higher bioavailability compared to the citrate and PEG coated CeO_2 NPs, but the CeO_2 NPs did not differ from the metal salt, except in the mid intestine.
- Coating effect – coatings less bioavailable than uncoated.
- Metal salt v nano – not much difference in bioavailability.

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Gap Filling: Data Collection Progress.

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ENM	Characterisation	Earthworm data	Digestibility assay fish	Gut sacs fish	TG305 Fish in vivo.
Ag NPs & Ag2S NPs (Applied Nanoparticles)	✓	✓	✓	✓	✓
CuO NPs (PlasmaChem)	✓	✓	✓	✓	✓
CdTe QDs (PlasmaChem)	✓	✓	✓	✓	(in progress - Spain)
TiO_2 (P25 Degussa)	✓	(in progress CEH)	✓	✓	✓
ZnO (Spain)	✓	✗	(in progress Plymouth)	✓	✓
CeO_2 (Spain)	✓	✗	✓	✓	✓ (data Spain)

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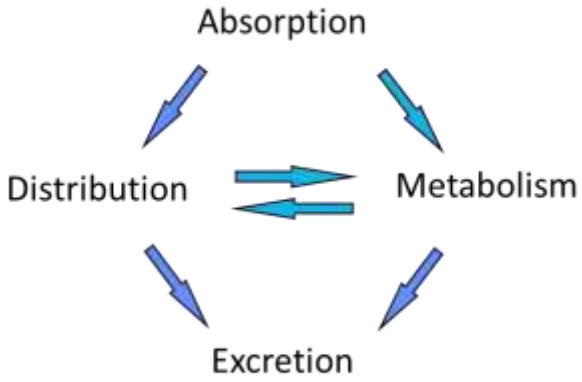
Conclusions

- Earthworm bioaccumulation tests are good predictors of bioaccumulation in fish.
- All these approaches have utility with a variety of materials:
  - *Earthworm tests*
  - *In chemico* digestibility assay.
  - *In vitro* fish.
- OECD work – the “scoping review” will show what and how tools/techniques could be used.
- Good prospect of a tiered approach to reduce testing (burden of work) and enable 3Rs (animal welfare).
- Revised scoping review back with ECHA and the OECD.



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