

International Workshop on Gap Analysis and Data Requirements to support Test Guideline and Guidance Document Development.

# Modifying the Bioaccumulation Testing Strategy with Fish for Nanomaterials

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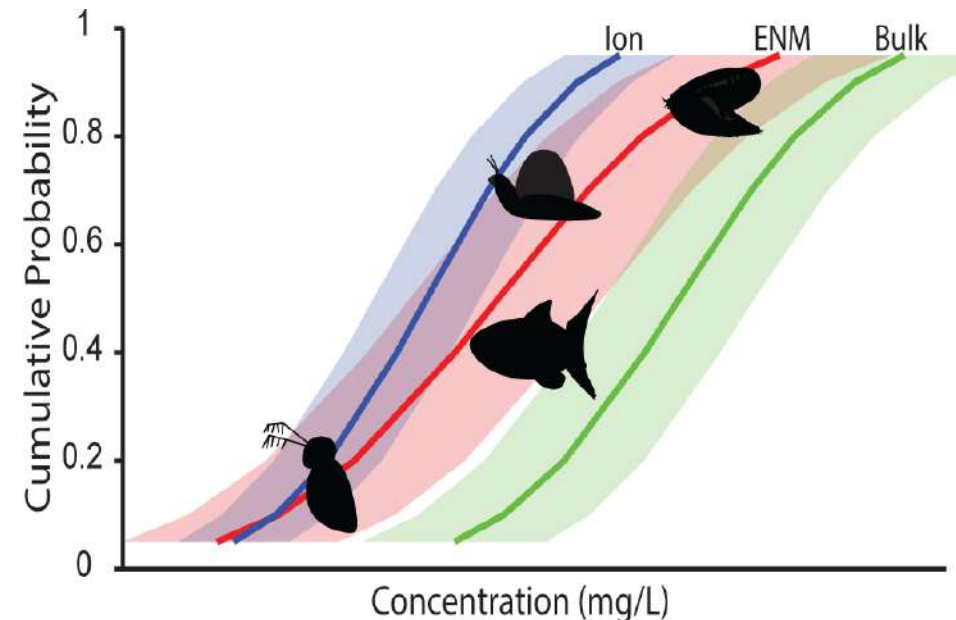
# The Key Triggers for Environmental Risk Assessment



- **P**ersistence in the Environment
- **B**ioaccumulation Potential
- **T**oxicity

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

Species sensitivity distributions for Nano?

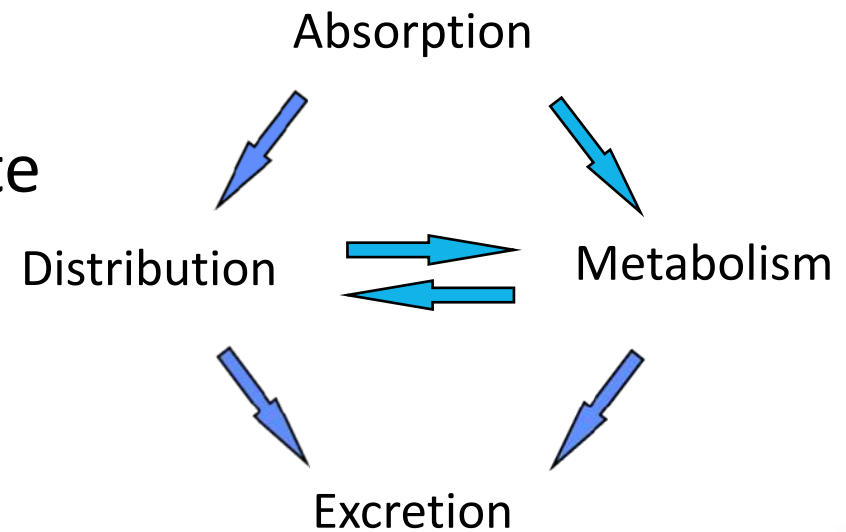


Garner et al. Environ. Sci. Technol. 2015, 49, 5753–5759





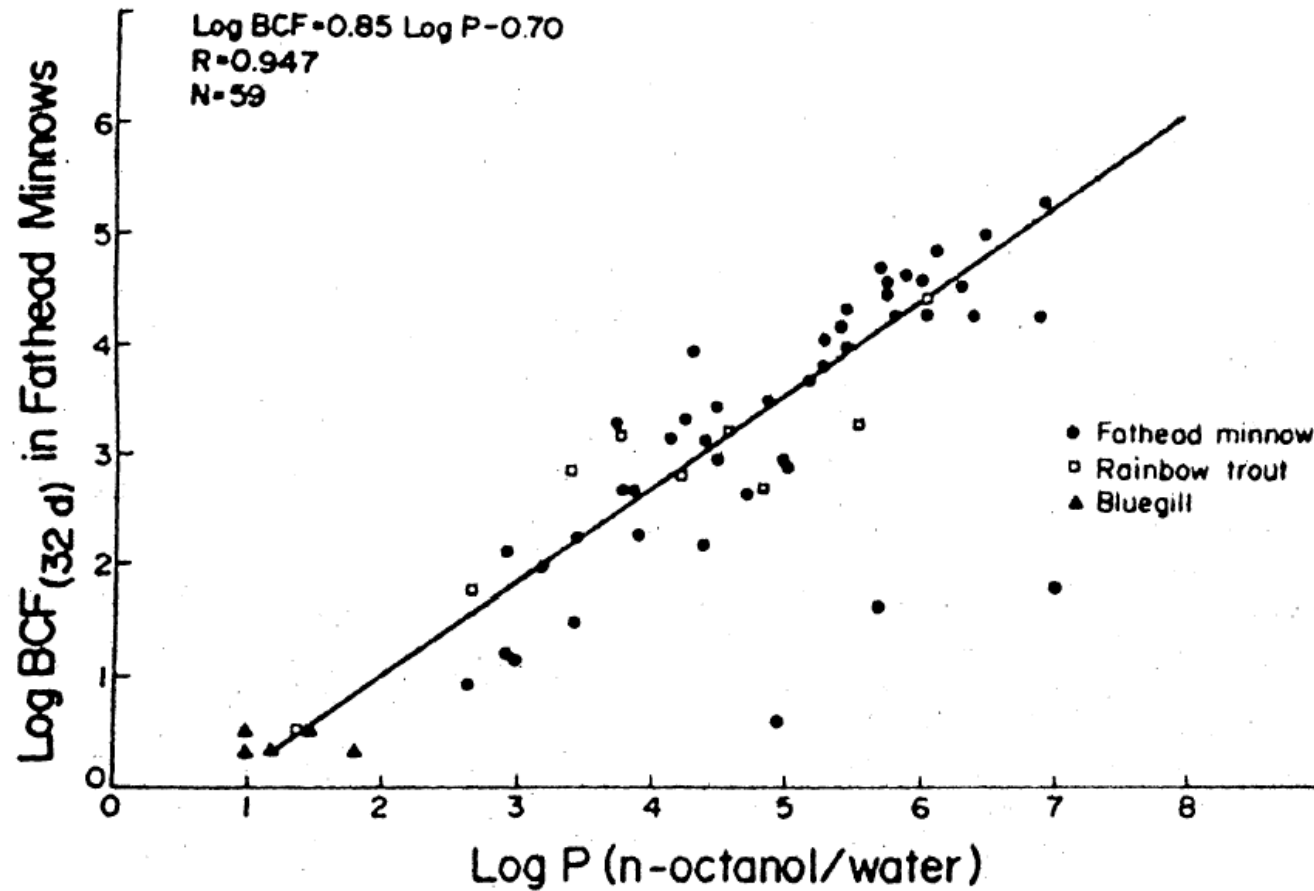
- Traditionally using a chronic exposure with fish over ~4 weeks.
- OECD TG 305
- Choice of exposure method: via the water or the food.
- Measure the uptake ( $k_1$ ) to steady state in the tissues and water.
- Calculate the bioaccumulation factor, BCF.



# 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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WITH  
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# Problems with the Current OECD Testing Strategy for Bioaccumulation Potential



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 alternative methods in the OECD testing strategy for bioaccumulation

## TG 305: Bioaccumulation in Fish

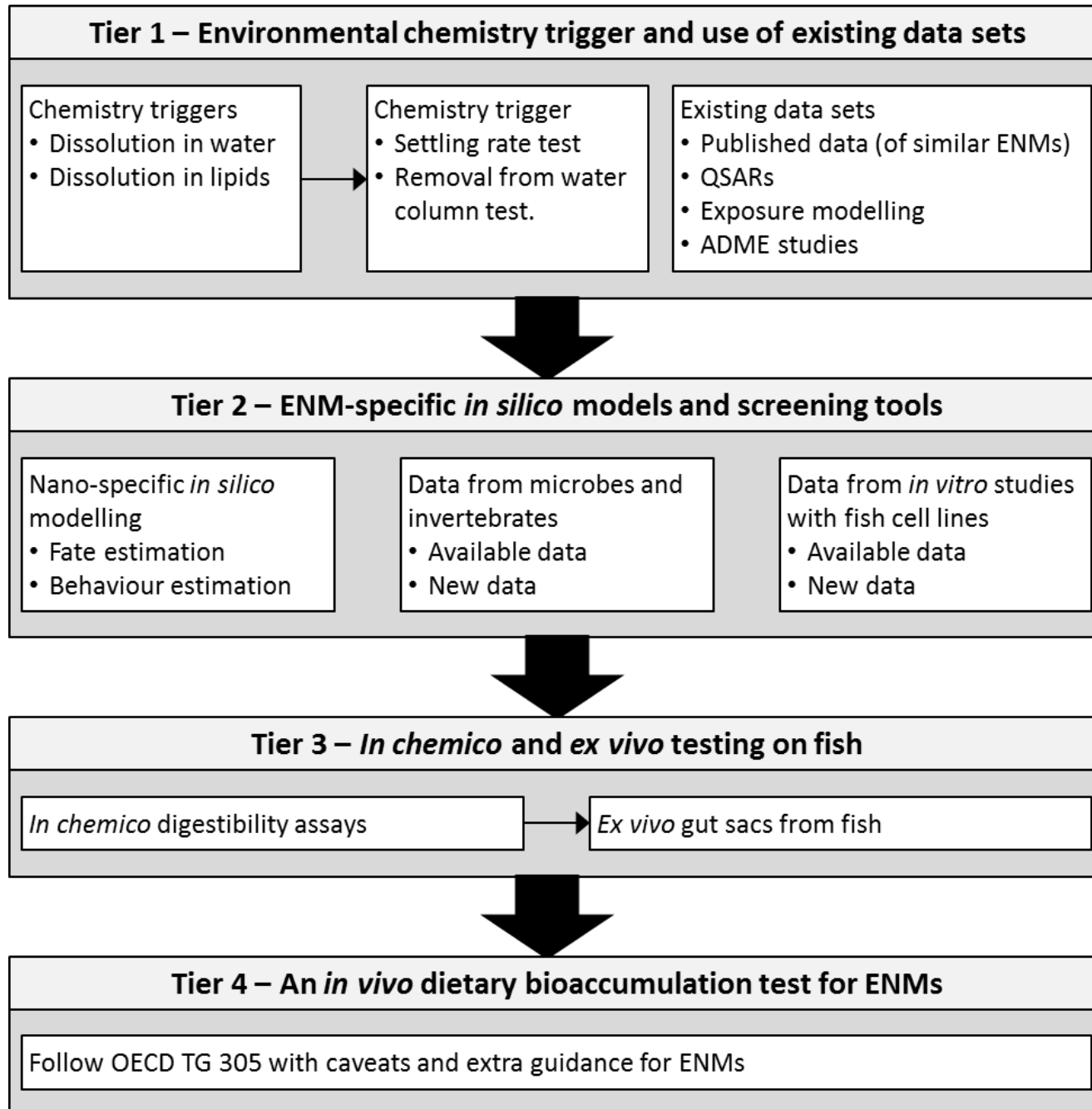
~150 fish, 28 day test  
~€90-130k per test





# Proposed Dietary Bioaccumulation Potential Testing Strategy with Fish

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





- **The overall aim** of the workshop sessions was to discuss how best to proceed with the bioaccumulation testing strategy for nanomaterials in a regulatory setting (e.g., OECD guidance).
- **Q1.** Alternative chemical triggers for TG 305 bioaccumulation test when the Log Kow does not work/not relevant to ENMs.
- **Q2.** Should we use earthworm bioaccumulation tests, or some other invertebrate test to waive TG305 or as an alternative to tests with live fish?
- **Q3.** *In vitro* alternatives to fish testing to introduce the 3Rs into the strategy and screen out nanomaterials/rationalise workload?



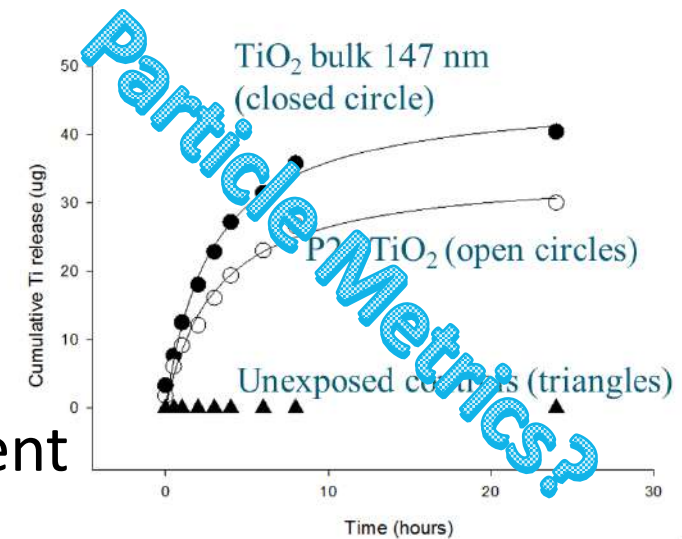


# Chemical “Trigger” for Bioaccumulation Testing Strategy



- Log Kow is not working, better to use particle properties that predict bioaccumulation.
- Particle size, hydrodynamic diameters (aggregation state), dissolution rate, are possible triggers.
- No single metric that is the best predictor, but simple multiple regressions can give strong prediction equations.
- Attachment efficiency ( $\alpha$ ) may be an inherent property linking to bioaccumulation. GD 318 provides a measurement method for  $\alpha$ .
- Develop a hydrophobicity test that works for nano.
- Inherent properties of metals can predict bioaccumulation potential.

Fish Gut Saline





# Invertebrate Tests and Other Tools to Waive TG 305 on Fish?

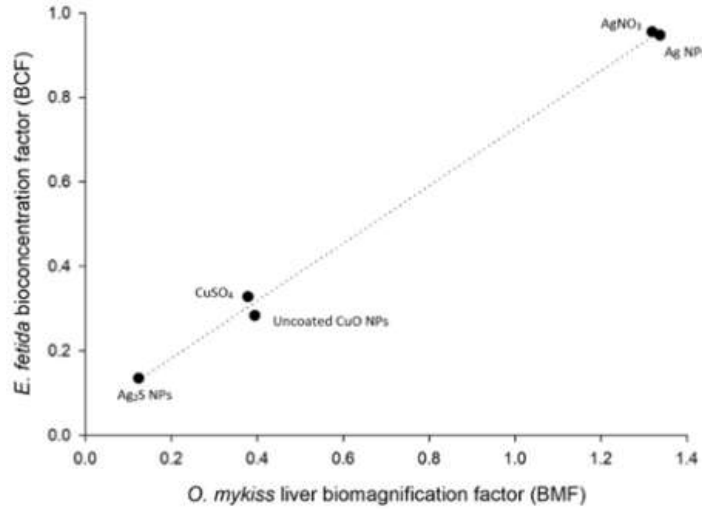
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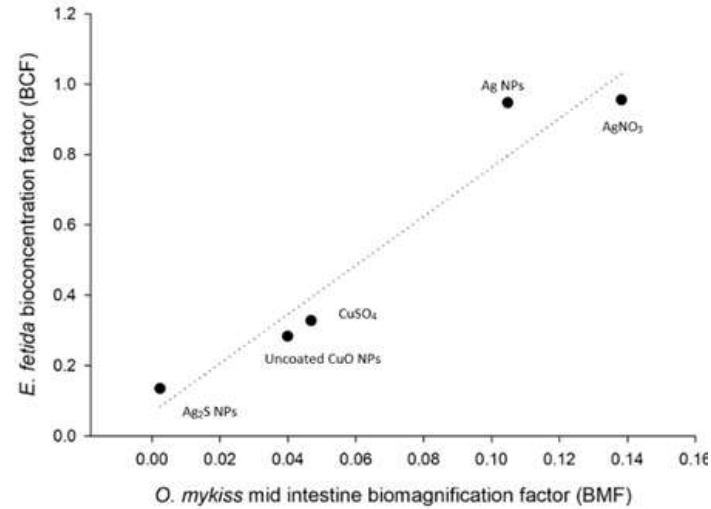
- *Hyalella* test with some modification should be strongly considered.
- Earthworm BCFs correlate well with fish BMFs for nanos so far, but maybe less regulatory acceptance of terrestrial test in an aquatic scheme.
- Aquatic sediment tests.
- Can normalise data to tissue carbon or lipid content so that invertebrates can be compared with fish.
- Uptake by bivalves (mussels) could also be used (marine).
- Consensus – use an aquatic invertebrate test if possible, and drive toward sediment invertebrate test...
- Wide agreement that nano-QSAR is in infancy
- A toxicokinetic/toxicodynamic model could be developed.



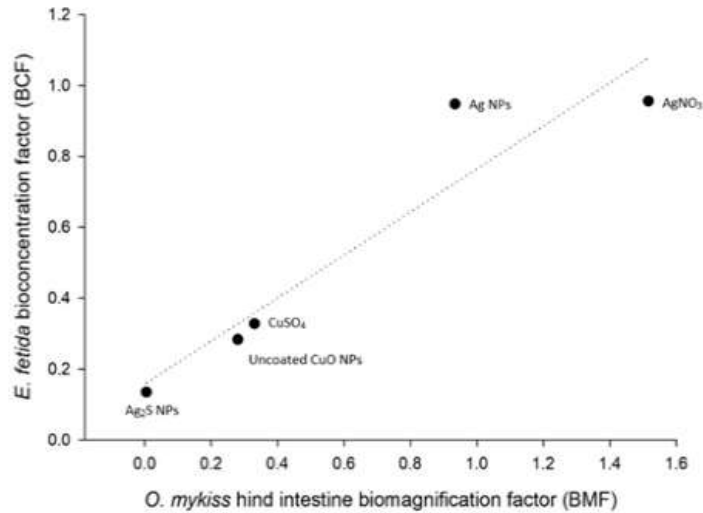
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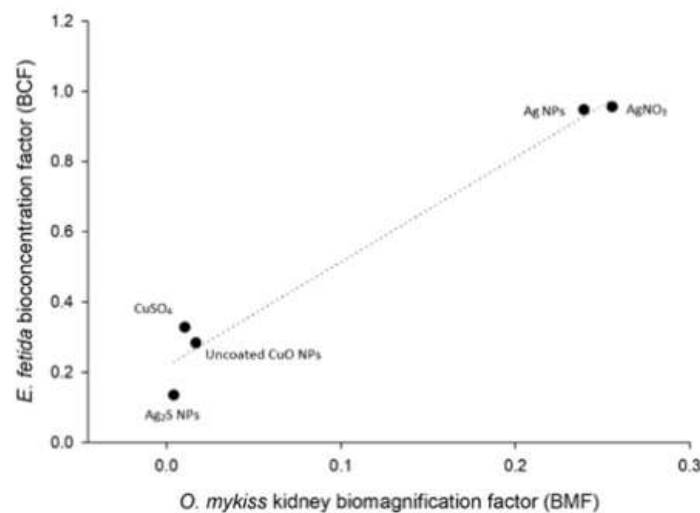
B



C



D



# Correlations between fish BMF and Earthworm BCF for nanomaterials

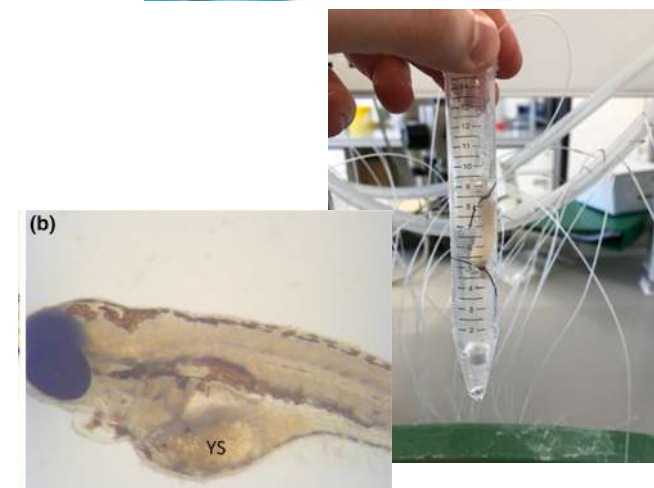
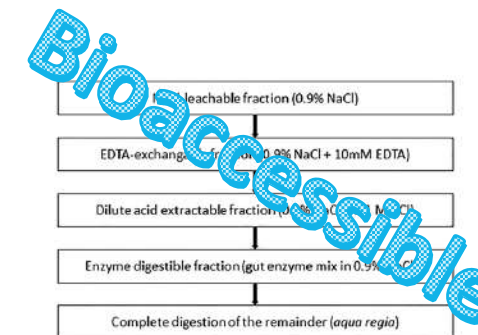
Handy, Clark, Vassallo et al. unpublished

# “In vitro” Alternatives to Fish Bioaccumulation Test TG305.

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- Digestibility assay as a quick *in chemico* method to inform on bioaccessible fractions of nanos in the gut lumen.
- Agreement that digestibility assay was useful, but need to show this works with herbivorous fish, wide range of ENMs.
- Gut cell lines, RTG cells, RT liver cells discussed. Concerns around whether a cell culture was a good model for measuring accumulation.
- REACH – demonstrate the substance does/not cross “biological membranes”.
- Gut sacs are 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 embryo/ELS test could be adapted for bioaccumulation measurement.



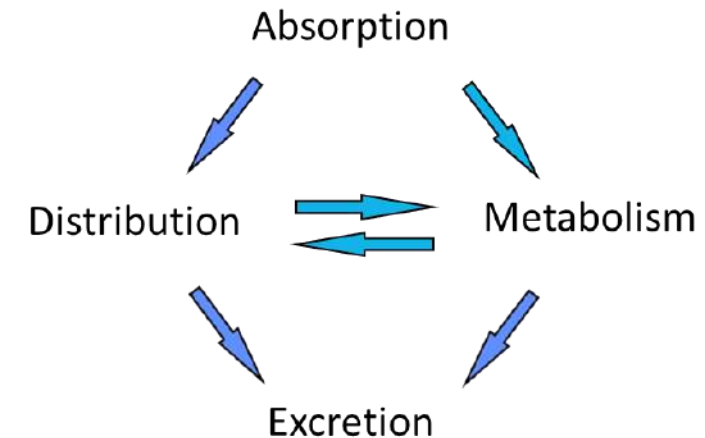
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# The Way Forward on the Bioaccumulation Testing Strategy

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- We can develop a chemical trigger to replace the log Kow measurement for nano.
- Keep it simple, not too many protocols to include.
- We can simplify the proposals for the overall scheme further
  - Waive TG305 with an aquatic invertebrate test.
  - *In chemico* digestibility assay.
  - Maybe other in vitro fish methods not needed to show a negative.
- Decision tree that protects against false positives and negatives in the scheme. Precautionary.
- Test more ENMs, but measurement methods for carbon-materials in tissue are needed.
- Good prospect of a tiered approach to reduce testing (burden of work) and enable 3Rs (animal welfare).







# Questions?

## Thank You!



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