Blue Frog Scientific

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A Nanomaterial Testing Strategy for Achieving REACH Regulatory Compliance

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Innovation in Chemical Regulation

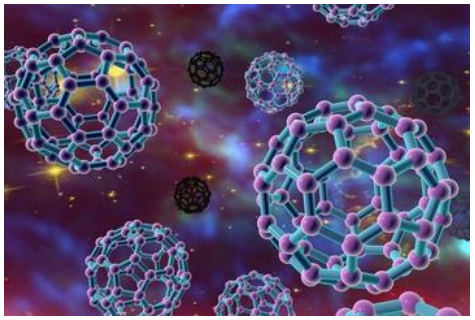
Blue Frog Scientific

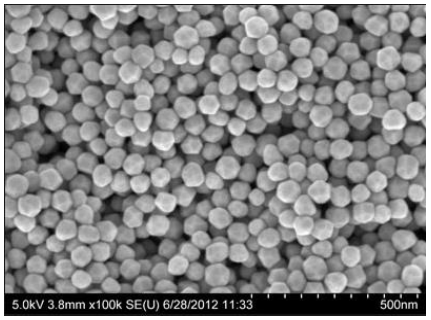
Outline

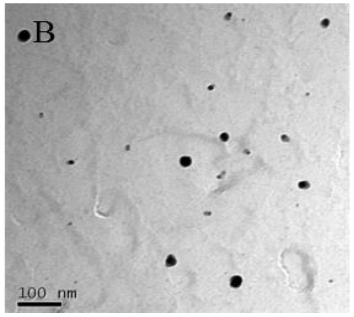
- Take an example NM and take it through regulatory processes
- Look at key physical chemical data requirements for nanomaterials and a testing strategy for one endpoint
- Ecotoxicological data requirements for the example NM, testing strategy and potential pitfalls, considerations
- Brief detail on environmental hazard and risk assessment

First step

- Do we have a nanomaterial:
- Is it:
  - 1-100 nm
  - 1-50 % of substance in the nanorange (or 0.1 % for nanomaterials of very high concern)
  - Surface area > 60 m^2/m^3







What are we going to register and what is our volume?

- PVP-coated silver nanoparticles as an anti-foulant

- Under REACH Regulation Annexes VII to X set out specific data requirements for successive tonnage bands.
  - Higher tonnage chemicals will generally require more comprehensive testing programmes.

- Annex VII 1-10 T
- Annex VIII 10-100 T
- Annex IX 100-1000 T
- Annex X > 1000 T

- Possible life-cycles
  - Manufacture
  - Formulation
  - Industrial sector end use
  - Professional use
  - Consumer use

What are we going to register and what is our volume?

- PVP-coated silver nanoparticles as an anti-foulant supplied as a dispersion

- Within REACH legislation Annex VII to X set out specific endpoint requirements for successive tonnage bands
  - Higher tonnage chemicals will generally require more comprehensive testing programmes

- Annex VII 1-10 T
- Annex VIII 10-100 T
- Annex IX 100-1000 T
- Annex X > 1000 T

- Possible life-cycles
  - Manufacture
  - Formulation
  - Industrial sector end use (Substance is imported only)
  - Professional use
  - Consumer use

Physical chemical endpoints of priority or specific to NMs:

PHYSICAL-CHEMICAL PARAMETERS: MEASUREMENTS AND METHODS RELEVANT FOR THE REGULATION OF NANOMATERIALS (OECD 2016)

- Size & Shape (can include crystal structure) and aspect ratio
- Dispersability
- Agglomeration/ Aggregation
- Specific surface area
- Surface charge
- Dissolution
- Photocatalytic Activity

Must be taken: as-made, as- dosed, as-exposed

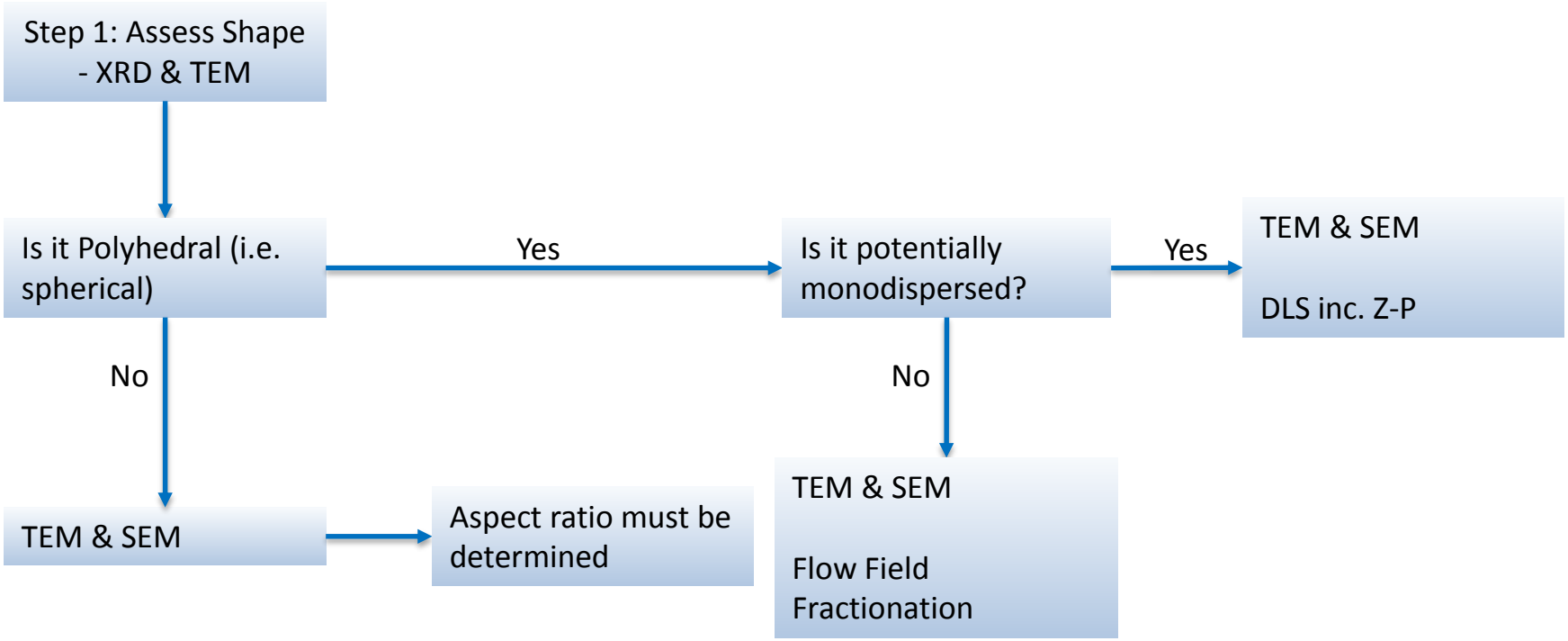
Who can do it?

Most likely a well established academic laboratory, does not need to be GLP.

CROs are not generally equipped to deal with the specific requirements of NMs under REACH as there has currently not been high demand for the testing.

Testing Strategy for Size and Shape:

What's the importance of size and shape?



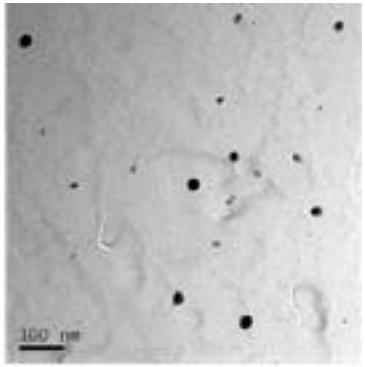
Considerations/Detail:

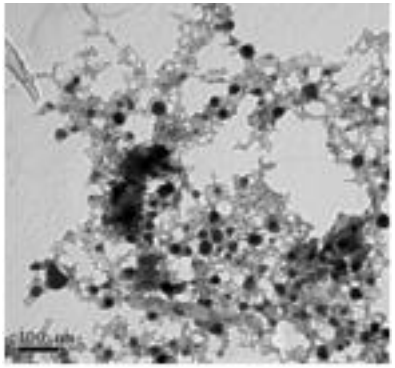
- DLS
  - Only suitable for monodisperse, spherical particles – not an issue here
  - Sensitivity of technique depends on substances refractive index. >=1 mg/L – providing the sample does not begin to homoaggregate/homoagglomerate this is not a concern
  - Low resolution and small sample size make it indicative only – replicate, use reference material e.g. Au RM 8013 - Gold Nanoparticles (Nominal 60 nm Diameter; NIST) to validate and calibrate method.
  - Make note of PdI for dispersability measure, can measure zeta-potential!

Physical-Chemical Properties of Nanomaterials: Evaluation of Methods Applied in the OECD-WPMN Testing Programme, Doc 65.

Considerations/Detail:

- TEM
  - Only quantitative when not agglomerated
  - Small sample size and small share of evaluated particles → poor statistical precision – at least 200 particle measures, 3-10 images. Multi-method approach confirm with DLS. Use reference material e.g. Au RM 8013 - Gold Nanoparticles (Nominal 60 nm Diameter; NIST) to validate and calibrate method.
  - Preparative techniques may cause changes to particle, and pseudo-aggregation – possibility to use techniques such as Cryo-TEM
  - Bias due to orientation – not an issue for spherical particles





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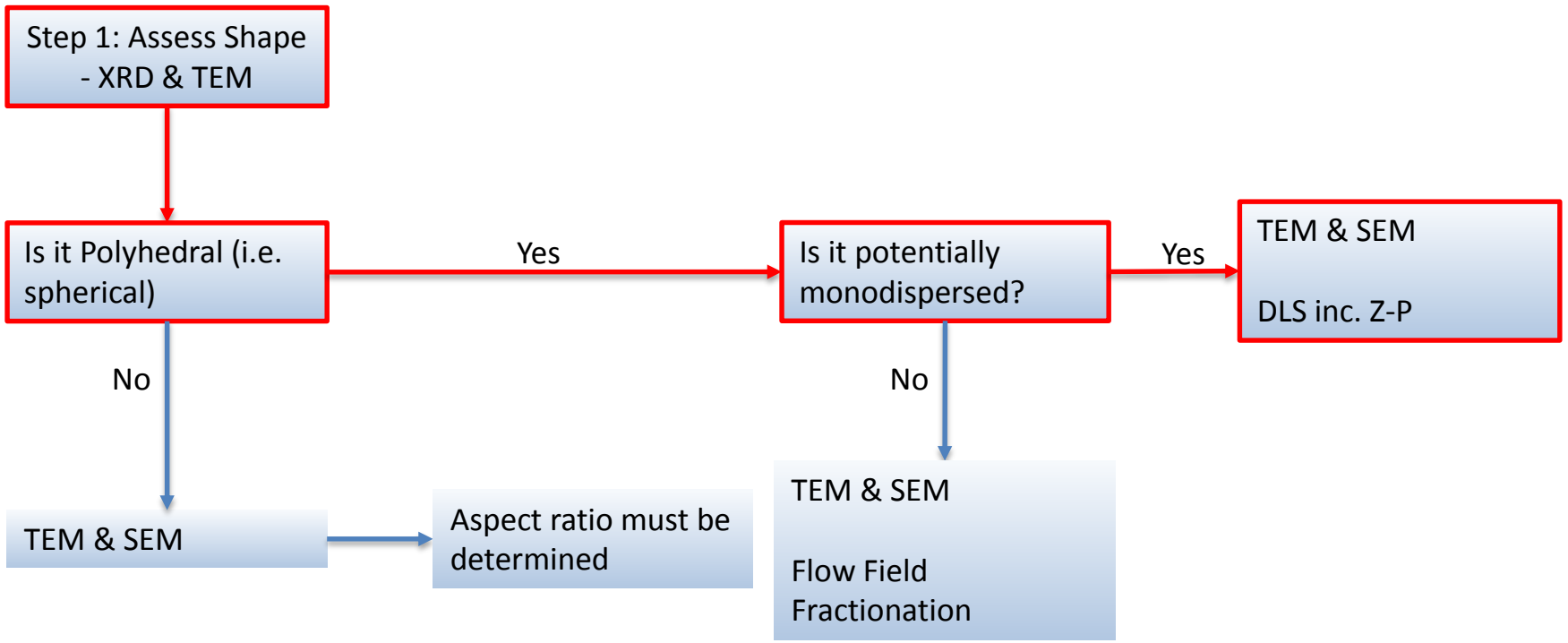
Testing Strategy for Size and Shape:



- Validated SOPs for TEM and DLS methods exist under NanoValid
- Dustiness – Not required, no potential for inhalation exposure during use.

Toxicology and Environmental Fate:

Endpoint specific guidance (Chapter R.7a). Appendix to Chapter R.7a: recommendations for nanomaterials

Endpoint specific guidance (Chapter R.7c). Appendix to Chapter R.7c: recommendations for nanomaterials

Characterisation of dose [concentration] - response for human health. Appendix to Chapter R8: recommendations for nanomaterials

https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment

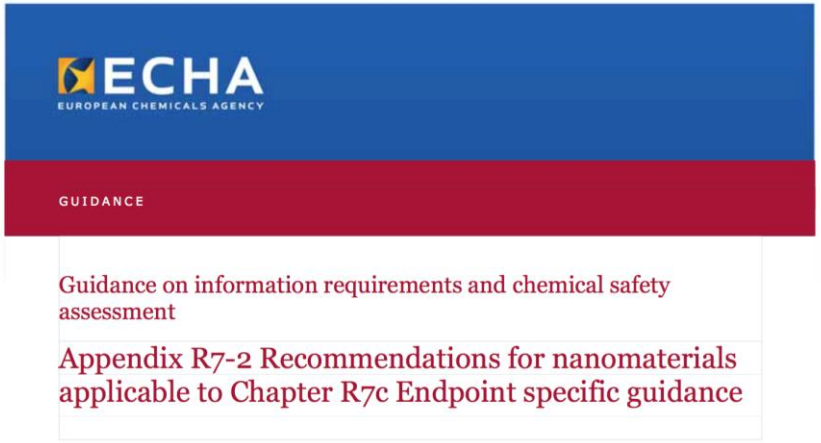
Genotoxicity of Manufactured Nanomaterials : Report of the OECD expert meeting

http://www.oecd.org/science/nanosafety/latestdocuments/

Ecotoxicology and environmental fate of manufactured nanomaterials: test guidelines. Doc. 40

http://www.oecd.org/science/nanosafety/latestdocuments/

Ecotoxicology:



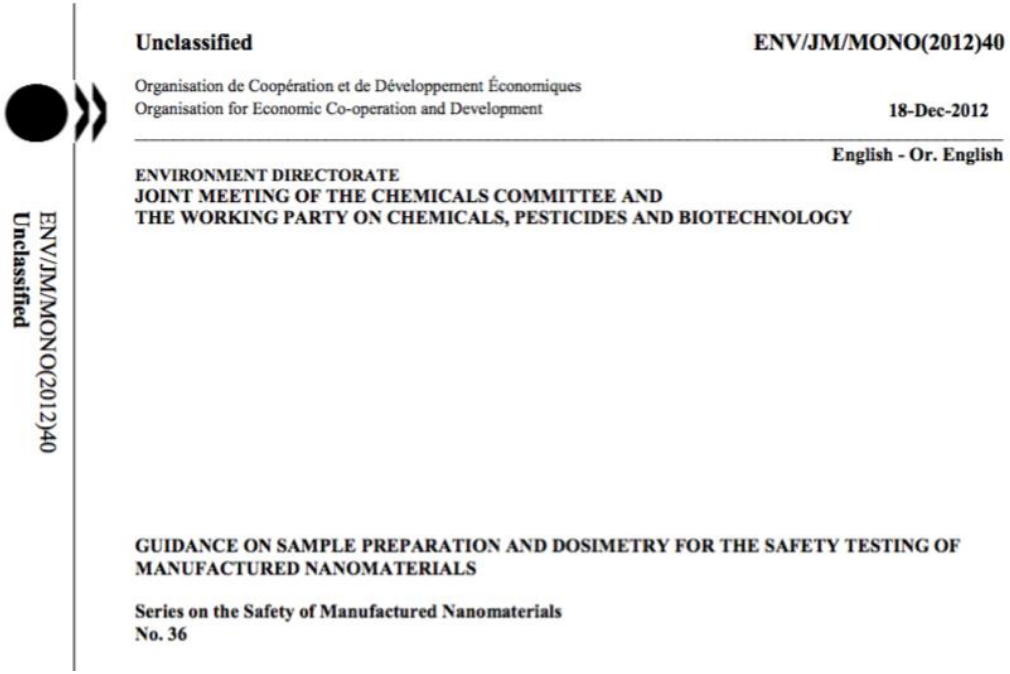
Group assessing already registered nanomaterials (GAARN) have some more specific recommendations.

Currently minimal guidance is given within ECHA R7-2 and R7c with regards to nanomaterials, this was based on some GAARN recommnedations/findings.

Academic literature produced from EU funded projects which feed directly into regulation have started to point to the need to update or create nano-specific OECD guidelines.

Ecotoxicology sample preparation and dosimetry:

Preparation and dosimetry will ensure the longevity of the study as guidance updates occur. Ensures validity and stands up to scientific scrutiny.



Unclassified ENV/JM/MONO(2012)40

Organisation de Coopération et de Développement Économiques
Organisation for Economic Co-operation and Development

18-Dec-2012

English - Or. English

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

ENV/JM/MONO(2012)40
Unclassified

GUIDANCE ON SAMPLE PREPARATION AND DOSIMETRY FOR THE SAFETY TESTING OF MANUFACTURED NANOMATERIALS

Series on the Safety of Manufactured Nanomaterials
No. 36

Dose measures:

Must be taken: as-made, as-dosed, as-exposed

Concentration of NM

Particle number

Size and agglomeration/ aggregation

Surface area

Concentration of soluble fraction

Ecotoxicology:

Required tests:

- Short-term toxicity testing on invertebrates (preferred species *Daphnia*); Annex VII
- Growth inhibition study aquatic plants (algae preferred); Annex VII
- Short-term toxicity testing on fish: the registrant may consider long-term toxicity testing instead of short-term; Annex VIII
- Activated sludge respiration inhibition testing; Annex VIII

CROs will have the capability (but is there sufficient experience?), and currently studies must be GLP. Samples for further physical chemical work may need to be sent to collaborating laboratories for specialist equipment.

Ecotoxicology:

Daphnia considerations:

Short-term toxicity testing on invertebrates (preferred species *Daphnia*); Annex VII

Important physical chemical property findings
- dissolution in water = 2 %
- Stable, 50 nm NPs

Stability/ dissolution in medium must be considered prior to testing

Current recommendations

- Modification of medium to a lower ionic strength to improve stability and dispersability
- Health depends on suitable levels of cations such as Ca^{2+}, how successful would they be long term
- Comparability with other tests where such modifications to medium is not recommended (i.e. algae test or existing data)
- Read across from existing data i.e. Ag^{+} → higher toxicity in “weaker” medium
- Risk Assessment based on most sensitive species, may skew result. Further implications on MoA/AOP

Ecotoxicology:

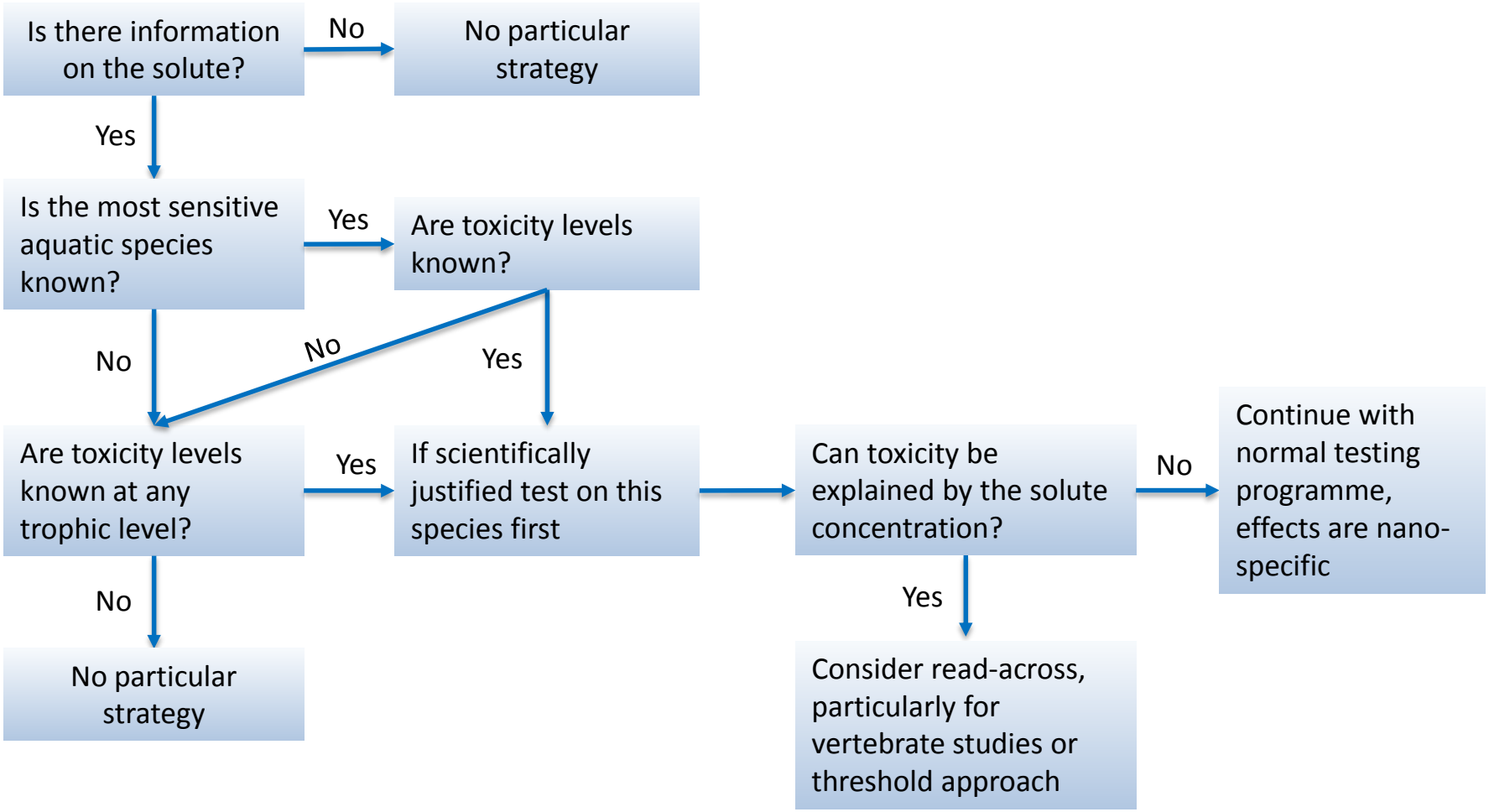
Considerations/Strategy:

Strategy:

- Conduct test over 48 hours in medium only to determine stability/ sedimentation and dissolution in M7 medium with removal of EDTA/ ISO medium (as dosed)
- Centrifugal filtration to separate dissolved Ag and Ag NP (as exposed) no validated SOP for dissolution → ICP-MS
- Result: Sedimentation of around 10 % of particles, dissolution/free Ag ~0.1 %
- Strategy, perform analytics on middle of water column and base of testing vessel samples, water renewal at 24 hours.
- Important to assess behaviour of organism, do they scrape the bottom which is an observed behaviour for daphnids looking for food. (as exposed)
- Toxicity shown at = 50 µg/L. Could it be the dissolved fraction? Ag^+ EC_{50} ~1 µg/L
- Only 0.1 µg/L of Ag^+, must be nanospecific, no possible read across or knowledge from Ag^+

Ecotoxicology:

Overveiw: Strategy flow chart for NMs which undergo dissolution:



Ecotoxicology:

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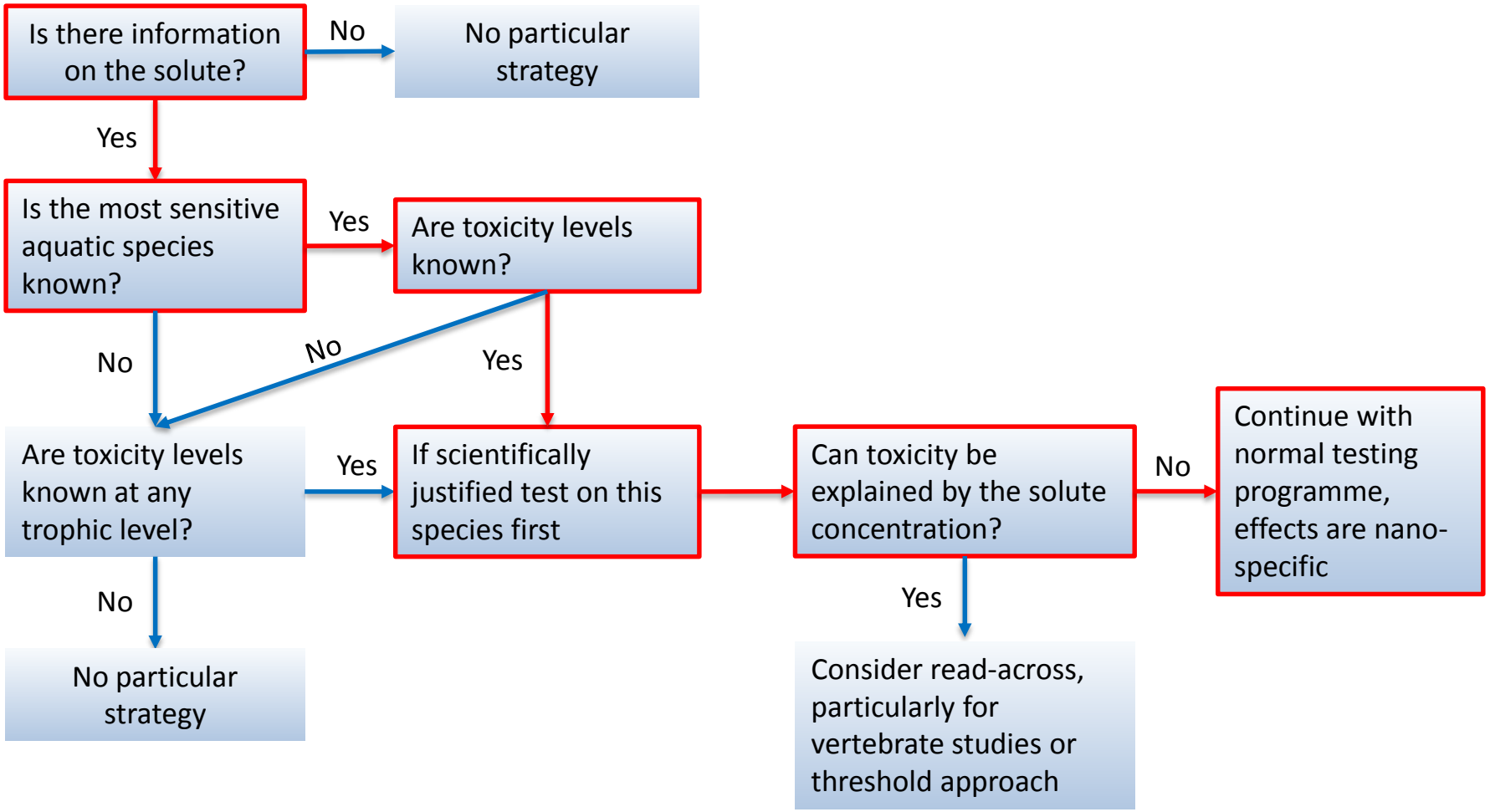

Environmental hazard and risk assessment:

Using the information gained from the testing strategy it should be possible to complete an environmental hazard and risk assessment in the usual manner.

Predicted No Effect Concentrations (PNECs) are derived by using an assessment factor on the most sensitive ecotoxicological endpoint, this could be derived in the usual way and should the dose-metric change then the Predicted Environmental Concentration (PEC) only need change to reflect this i.e. same measurand.

PECs will arise from exposure models, however only a few probabalistic models exist, with no standard landscape model. In the absence of such models environmental modenitoring may be the first choice. Where, differentiation between natural and anthropogenic materials can be discerned due to differences in impurities/impurity ratios

During landscape modelling Environmental Release Category (ERC) inputs as determined within EHCA R.16 which may not be relevant for NMs Need for nano-specific ERC

Refinements & risk management measures, can still be made in the same manner using sound scientific justification and knowledge of the chemical and the use/production processes

6. Discussion

- There are nano-specific physical-chemical endpoints beyond that of the original Annexes which are yet to be integrated into Column 2 of the legal text for REACH
- Physical chemical property measurements must not only be fulfilled for the pristine material but also at several stages of the life-cycle
- Further detail to decision trees and methodologies may provide clarity and reduce testing needs
- Current recommendations for test guidelines require integration within the current framework not just through academic journals. These guidelines must consider instances where there is sound rationale and scientific justification for deviation from the “nano-guideline”. Such as: how results feed into an ITS
- There is currently a need for appropriate landscape modelling particularly due to the difficulties of environmental modelling, which include speciation
- Need for nano-specific release factors

THANK YOU FOR LISTENING

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