Approaches to the safety assessment of engineered nanomaterials (ENM) in Food

An ILSI Europe Expert Group Opinion

Novel Food and Nanotechnology Task Force

Background

- Food + biological systems are composed of nanomaterials
- Wide group of materials
- Properties that are characteristic to the nano scale ?
- ‘Unexpected’ effects due to size ?
- Toxicological uncertainties being debated
- Lack of prior experience/historical database
- Problems of detection/description/dosimetry

REMIT 2008

Develop a procedure for the safety assessment of potential direct applications of ENM in foods, focusing on consumer safety, and publish….. ….

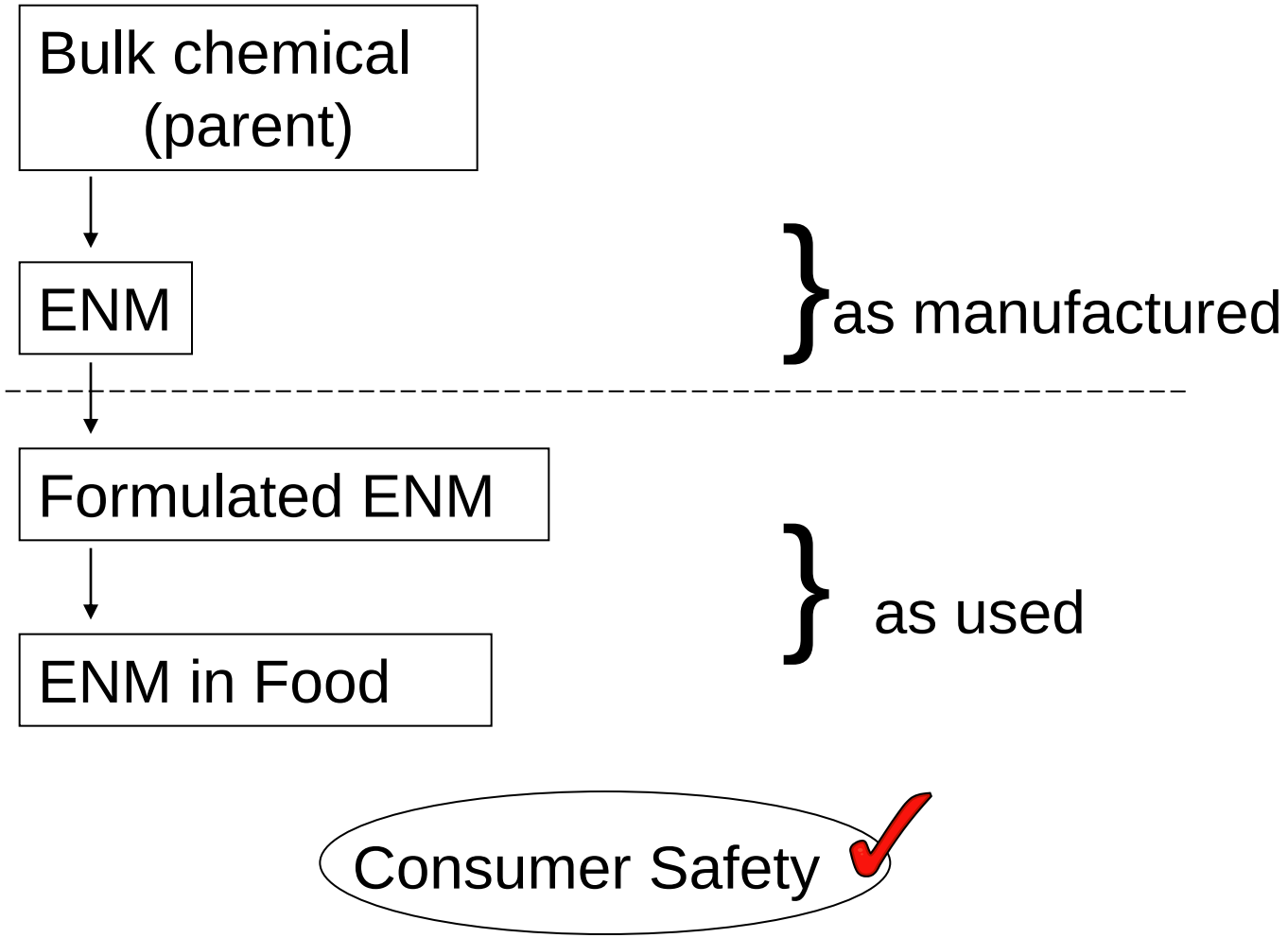
- Identify characteristics which might require specific attention
- Classify food related nanomaterials
- Guide data requirements for a risk assessment
- Provide a structured framework

➢ **Guidance on how to conduct a PRACTICAL, systematic & robust Safety Assessment**

Time Frame

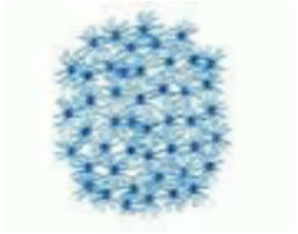
Feb 2009:	1st EG meeting
Oct 2010:	DRAFT manuscript
April 2011:	ILSI Peer Review Workshop
Summer 2011:	Final manuscript submitted to scientific journal

SCOPE


Bulk chemical (parent)
ENM
as manufactured
Formulated ENM
as used
ENM in Food
Consumer Safety

Exposure to ‘natural’ nanoforms

- Natural, e.g soil erosion, sea spray
- Anthropogenic eg fuel combustion, cooking
- Food and food processing e.g casein micelles (~100nm), whey (~3nm)



- Calcium phosphate re-secreted in the gut (~20 – 200 nm)
- Ferritin from meat and plant based foods (~12 nm)

Potential Applications of ENM in Food

- Improved taste, colour, flavour, texture and consistency
- Increased availability of nutrients and bioactives
- Improved quality, shelf-life and safety of food products due to new food packaging materials with improved mechanical, barrier and antimicrobial properties
- Nanosensors for traceability and monitoring the condition of food during transport and storage.

Basic Considerations

- Risk assessment
  - Existing risk assessment paradigm : Hazard identification, hazard characterisation, exposure assessment, risk characterisation
  - Safety assessment procedures for foods (as opposed to chemicals) and requirements for novel foods are available
  - Food safety testing is ‘case by case’ and where practical uses a traditional comparator
- Nature of materials
  - ‘ Bottom up’ technology – larger nanostructures formed by self-assembly of smaller organic components
  - ‘Top down’ technology – mechanical processing of inorganic materials
  - Bulk materials could be used as comparator?

Physicochemical and Biological Considerations

- ENMs subject to digestive processes in the gut
- GIT can be selective barrier – excreted, local GI effects, systemic exposure
- The potential for ENM toxicity depends on how the body 'sees', and hence reacts with, the ENM at any given place in time – dynamic situation
- Fate of an ENM is determined by **particle size**, but also **solubility, surface coating, shape, reactivity, dose** – affects potential for **translocation**

- If ENM **solubilises** in digestive fluids as it transits GIT, it would have the same biological properties as non-nanoform (bulk).

➢ **The body is unlikely to handle a soluble ENM differently from a soluble bulk counterpart**

- **Biopersistence** of **internalised, relatively insoluble, reactive material** has greater potential to lead to cellular reactions such as **cytotoxicity,** promotion of **inflammatory responses,** production of **intracellular reactive oxygen species (ROS), genotoxicity** (not unique to ENMs).

➢ Particles become focus of concern : **Dissolution/ dissolution rate** is a key factor

Safety Assessment strategy

- ***Standard definitions*** of nano terms
- ***4-step risk assessment paradigm*** is appropriate
- ***Comparison*** where possible between ENM and its bulk and/or nano reference material(s) : ***Characterisation***
- A ***decision tree*** to select for ENM specific testing
- ***Tiered approach*** to guide generation of further data, to look for alerts, to allow for decision making
- Use ***validated protocols*** as appropriate
- Alternative approaches must be '***fit for purpose***'
- Assessment of ENM '***as manufactured***' and '***as consumed'***

➢ 5 STEP APPROACH

(1) Characterisation of bulk material

- Is the ENM derived from a known bulk material(s)?
- What are the physico-chemical properties, including dissolution rates?
- What is its known toxicology profile including effects in humans?
- What are the intended functional (technological + biological) properties?

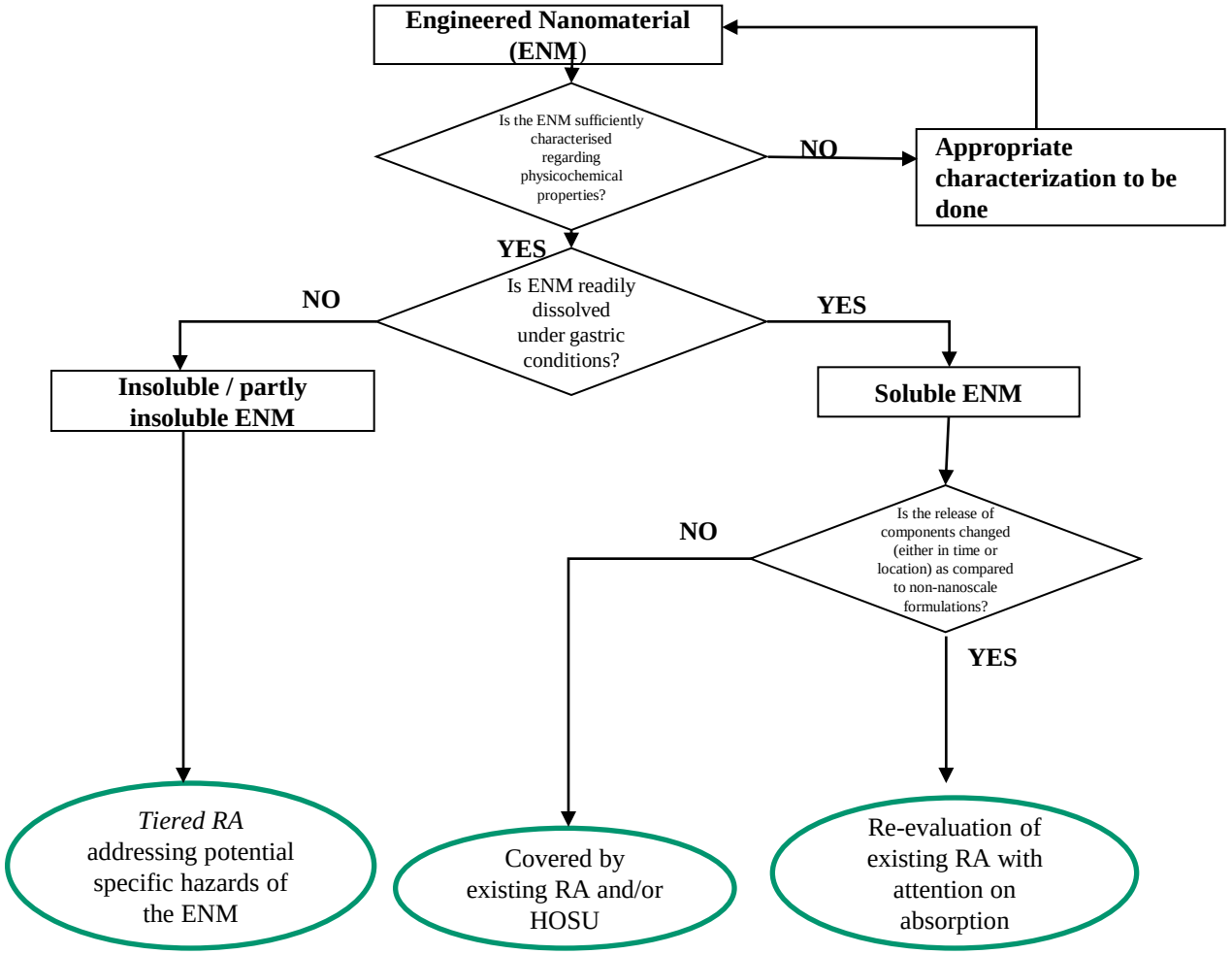
• Existing uses + functional properties	• Existing expert risk assessments
• Identity/Chemical nature	• Toxicological and nutritional data
• Specification	• Exposure

(2) ENM Phys-Chem characterisation

- Chemical ID/Composition/Purity
- Solubility *
- Particle size distribution
- Agglomeration/aggregation
- Shape (aspect ratio) + flexibility
- Crystal structure/crystallinity
- Surface properties
  - area
  - charge
  - reactivity
  - Chemistry/coatings

* *alone and in different environments eg food, GIT fluid, mucus, plasma, lymph & in vitro media*

(3) Decision tree for prioritisation



(4) Tiered testing strategy

Tier 1 (screening, alerts, translocation)

- Data and information mining, (+ comparator and references)
- *In silico* modelling : PBPK, (Q)SAR, read across and grouping
- *In-vitro* investigations for mechanistic insights and alerts: dissolution, genotoxicity, inflammatory potential, ROS induction, cytotoxicity, translocation potential
- *In-vivo* 14/28 day subacute oral repeat dose rodent study including elements of AD(ME) and genotoxicity, as appropriate
- Re-evaluation of all data and decision of further procedure

Tier 2 (toxicological effects , dose response)

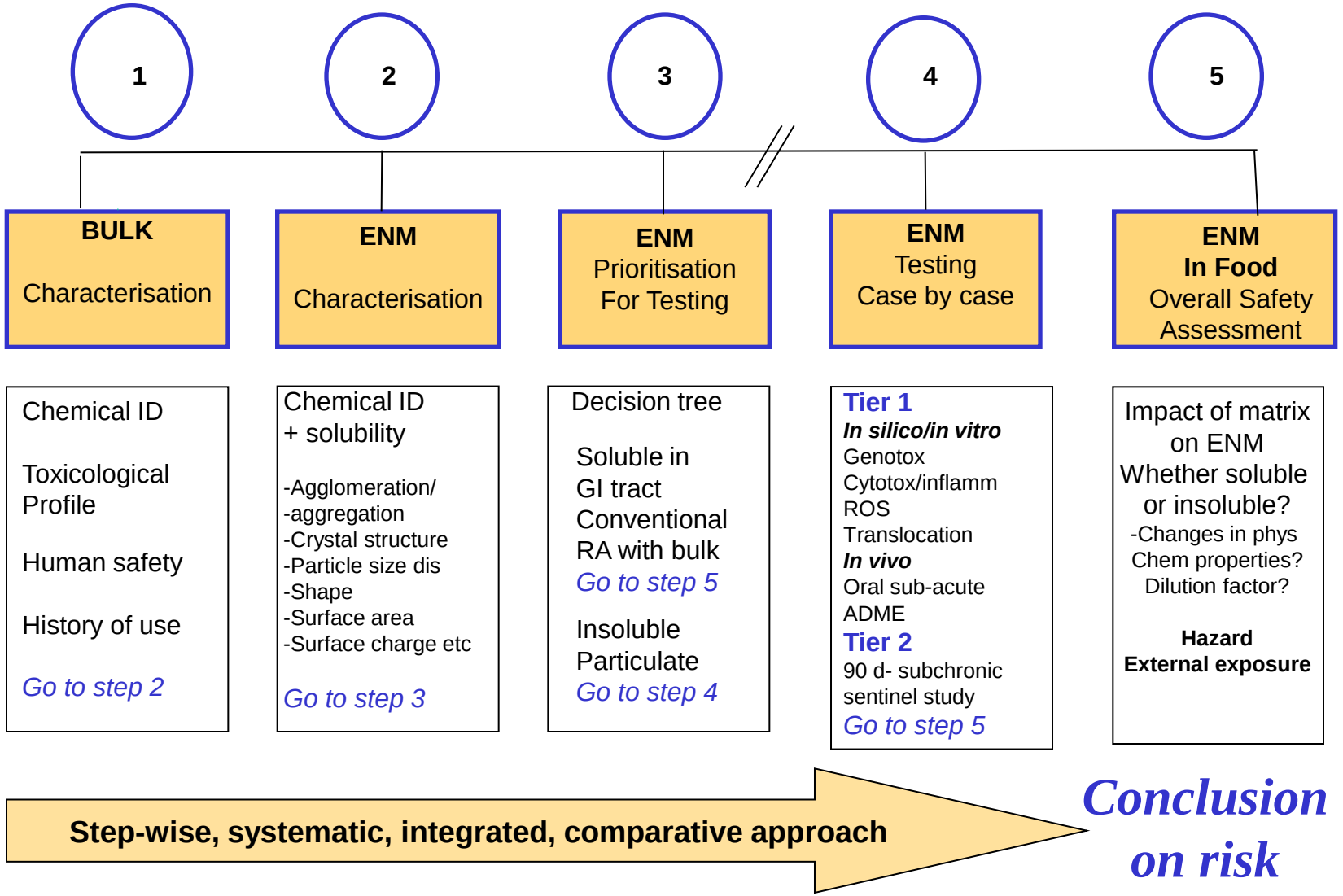
- 90 day repeat dose oral rodent study with possible additions
- Other focused studies eg reproduction, if indicated
- Mechanistic studies, if indicated

  - Consideration of dosimetry relevant to both tiers
  - Material to be tested should be the ENM compared to an appropriate bulk and nanoform reference material if available
  - Testing performed to OECD protocol guidance as available

(5) ENM ingredient in food

- Interaction with food matrix , i.e form of ENM in food ‘as consumed’
  - Impact on solubility/bioavailability
  - if not possible, default is “as manufactured”,
- Exposure from ENM in food
  - exposure assessment of ENM-containing foods is essentially the same as for other food additives/novel foods
  - type of food product, food consumption, maximum use levels
- To be considered as a paper exercise, supplemented with any necessary testing across a range of foods (matrices) of interest

Framework


1
2
3
4
5
BULK
Characterisation
ENM
Characterisation
ENM
Prioritisation
For Testing
ENM
Testing
Case by case
ENM
In Food
Overall Safety
Assessment
Chemical ID
Toxicological Profile
Human safety
History of use
Go to step 2
Chemical ID
+ solubility
-Agglomeration/
-aggregation
-Crystal structure
-Particle size dis
-Shape
-Surface area
-Surface charge etc
Go to step 3
Decision tree
Soluble in
GI tract
Conventional
RA with bulk
Go to step 5
Insoluble
Particulate
Go to step 4
Tier 1
In silico/in vitro
Genotox
Cytotox/inflamm
ROS
Translocation
In vivo
Oral sub-acute
ADME
Tier 2
90 d- subchronic
sentinel study
Go to step 5
Impact of matrix
on ENM
Whether soluble
or insoluble?
-Changes in phys
Chem properties?
Dilution factor?
Hazard
External exposure
Step-wise, systematic, integrated, comparative approach
Conclusion
on risk

Summary

- Physicochemical properties determine extent ENM testing
- Exposure – dosimetry; assume 100% bioavailability as a default
- Materials of highest concern are likely to be non-biodegradeable, persistent particles that can translocate/penetrate (cell) membranes
- Procedure provides a rigorous testing paradigm based on new & conventional methods and protocols that take into account the potential for ‘ENM-specific’ effects
- Testing framework will evolve in the light of examples/Case Histories/Experience
- Acknowledge uncertainties and continue research

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