GUIDELINES FOR EVALUATION OF NANO-AGRIINPUT PRODUCTS AND NANO-AGRIPRODUCTS IN INDIA

Department of Biotechnology
Ministry of Science & Technology
Government of India

……..2019

GUIDELINES FOR EVALUATION OF NANO-AGRIINPUT PRODUCTS AND NANO-AGRIPRODUCTS IN INDIA

INDEX

1. **Abbreviations**

2. **Introduction**

3. **Scope of the Guideline**

4. **General Considerations of the Guideline**

5. **Nano-agriproducts: Definition and Categorization**

5.1.1 Definition of nano-agriinproducts (NAIPs)
5.1.2 Definition of nano-agriproducts (NAPs)
5.2. According to degradation nature of nanomaterial
5.2.1 Biodegradable
5.2.2 Nonbiodegradable
5.3 According to chemical nature of NMs
5.3.1 Organic
5.3.2 Inorganic
5.3.3 Composite NMs
5.4 According to nanoform of the ingredient
5.4.1 Nanocarriers loaded with Active Ingredient (AI)
5.4.2 Active ingredient converted to nanoform
5.5. According to the synthesis
5.5.1 Biologically synthesized NMs
5.5.2 Chemically synthesized NMs
5.5.3 Physically synthesized NMz

6. **Scientific rationality of making nano-agriproducts**

7. **Specific considerations for evaluation of nanoagri-products**

8. **Excipients used in nanoformulations**
9. **Stability testing of nano-agriproducts**

9.1.Consideration of test parameters for stability testing of NAIPs and NAPs:

10. **Safety of manufactured nano-agriproducts**
11. **Residue analysis (nanoactive ingredients, additives, and nanocarrier materials of nanomaterials in food**
12. **Information required for evaluation of nano-agriproducts**
13. **Nano Agriproducts: Nanofood and nanofeed products**

13.1 Exposure risk
13.2 Hazard characterization
13.3 Uncertainty analysis

14. **Conclusions**
15. **References**

GUIDELINES FOR EVALUATION OF NANO-AGRIINPUT PRODUCTS AND NANO-AGRIPRODUCTS IN INDIA

1. Abbreviations

	Abbreviations
AI	Active ingredient
NAPs	Nano-agri products
APVMA	Australian Pesticide and Veterinary Medicine Authority
BIS	Bureau of Indian Standards
BrDU	Bromodeoxyuridine / 5-bromo-2'-deoxyuridine
CIB	Central Insecticide Board
OECD	Organisation for Economic Co-operation and Development
EU	European Commission
EDX	Energy Dispersive X-Ray spectroscopy
FAO	Food and Agricultural Organization
FCO	Fertilizer (Control) Order
LDH	Lactate dehydrogenase
ICP-MS	Inductively coupled plasma mass spectrometry
ISO	International Organization for Standardization
MTT	[3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide]
NAIP	Nano-agriinput product
NAP	Nano-agriproduct
NMs	Nanomaterials
PEG	Poly-ethylene glycol
PLA	Polylactic acid or polylactide
PLGA	poly(lactic-co-glycolic acid)
PMRA	Pest Management Regulatory Agency
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
TG	Testing guidelines
TSCA	Toxic Substances Control Act
USFDA	United States Food and Drug Administration
USEPA	US Environmental Protection Agency
WHO	World Health Organization
WST-1	Water Soluble Tetrazolium Salts
XRD	X-ray powder diffraction

XRF	X-ray fluorescence
XTT	(2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5-Carboxanilide)

GUIDELINES FOR EVALUATION OF NANO-AGRIINPUT PRODUCTS AND NANO-AGRIPRODUCTS IN INDIA

2. Introduction

Nanoparticles acquire unique properties due to their small size to large surface area ratio. It thus supports in development of novel products and processes as well as enhances the performance of existing ones across several disciplines. Nanotechnology has recently been introduced for improvement of agricultural systems through higher crop yield and better crop protection in order to meet the changing needs and domains of providing food to the growing population of the world. The innovative nano-intervention in agriculture and food sector could generate low-cost, high-efficacy solutions in terms of products and processes, especially suitable for developing countries. However, the unique properties can also lead to nanoparticle-related toxicity in humans and environment. The guidelines for evaluation of nanoproducts in agriculture and food are more challenging than the existing procedures for assessment of fertilizers or safety evaluation of pesticides or toxicity evaluation in food. The activity, efficacy and impact of nanomaterials (NMs) depend upon interaction of their physico-chemical parameters with diverse environmental factors and, therefore, require a multidisciplinary approach for development of new alternative strategies and methods for evaluation.

It is imperative to modify the existing policies and also develop certain new standard guidelines for evaluation of novel products on the basis of current scientific understanding. The multidisciplinary nature of nanotechnology and its rapidly increasing scope for development of commercially viable applications pose a huge challenge to regulatory bodies across the globe. Nanotechnology involves an amalgamation of knowledge from various disciplines of science, including chemistry, materials science, physics, biology, engineering and medicine. Such an interdisciplinary nature makes nanoscience an important domain to facilitate enhanced scientific and technological prospects and development of novel applications.[1] Moreover, nanotechnology and nanoproducts are dealt by different ministries and different departments, and thus interdepartmental and inter-ministerial convergence is also required (Annexure 1). These guidelines have the aim to ensure not only the quality and efficacy to encourage the commercialization of nanotechnology-based innovations but also safety of novel products by emphasizing high benefit to low risk ratio compared to bulk counterparts.

There are no unanimously acceptable international guidelines for nano-agriproducts (NAPs). A few provisions that are in place globally for nanomaterials include REACH, EPA, AVMPA, OECD, and FAO/WHO with certain specific guidelines for quality, safety and efficacy. However, new innovations with alteration of functionality of nanosystems make it difficult to apply a universal set of evaluation parameters for different nanoproducts with different applications. Many a time the case-by-case basis evaluation approach is advocated for NAPs.

3. Scope of the guidelines

These guidelines apply to the following two categories of products:

[1] Details available at https://www.teriin.org/sites/default/files/2018-03/zero-draft-policy.pdf, last accessed on 31 July 2019

i) Agri-input products in the nano form of finished formulation as well as active ingredients of a new material (inorganic/organic/composite) or an already approved material (inorganic/organic/composite) with altered beneficial properties, dimensions or phenomenon associated with the application of nanotechnology that is intended to be used in agriculture and allied sectors for crop production, protection, management, harvesting, post-harvesting and packaging. The applications include and may not be restricted to pest/disease prevention, control and management, fertilizers, agrochemical delivery, plant nutrients, anti-transpiration agents, plant growth regulators and biostimulants for crop benefits.

ii) Agriproducts in the nano form of finished food formulation, finished feed formulations, nanocarriers for nutraceuticals delivery, nano processing aids, nanocomposites for food packaging and nanosensors for food packaging and food safety applications.

These guidelines do not apply to the conventional products or formulations with incidental presence of natural nanomaterials. These guidelines also apply to sensors made from nanomaterials (as per the definition) and those that require direct contact with crops, food and feed for data acquisitions.

4. General considerations of the guidelines

The European Union along with Switzerland is the only part of the world where particular provisions to deal with nanoproducts are available in the legislation. In some countries, in the absence of specific regulations for nanoproducts, the existing legislative and regulatory frameworks (Annexure 2) also deal with nanoproducts, many a time with necessary adaptations to account for the specific properties of nanomaterials. In India, there are different government bodies and provisions that regulate different agriproducts. Since different NAPs are considered in the guidelines, their evaluation should be conducted as per the NAP type. However, in case any specific study is not included in the suggested regulatory framework, the principles of ICH guidelines for agriproducts or OECD guidelines for chemicals may be followed. This document may also serve as useful guidelines for manufacturers, importers of NAPs and other stakeholders involved in research and development of NAPs.

The following nano-agriinproducts are considered in these guidelines:

(i) Nanofertilizers (with or without nanocarriers): Safety, evaluation, functionality and other quality studies of nanofertilizers should be conducted under Fertilizer (Control) Order (FCO), 1985 with additional criteria for inclusion of nanofertilizers. FCO is administered by Department of Agriculture Cooperation, Government of India and issued under the Essential Commodities Act, 1955, which lays down registration requirement for fertilizers.

(ii) Nanopesticides (with or without nanocarriers): Safety studies on chemistry, bio-efficacy and residues, toxicity, packaging and processing of molecules for registration for manufacture or import of nanopesticides should be conducted as per the regulatory aspect provisions under section 9(3) specified in the Insecticides Act, 1968 with additional criteria for inclusion of nanopesticides and plant growth regulators as per the guidelines of Central Insecticides Board & Registration Committee under the Ministry of Agriculture (Insecticide Act, 1968).

The following nano-agriproducts are considered in the guidelines:

(i) Nanofood: The FDA guidelines (FDA, 2014a), (FDA, 2014b), (FDA, 2015) and Food Safety and Standards Act, 2006 may be adopted by FSSAI (Food Safety and Standards Authority of India) to address NAPs and develop final guidelines for industry. In these guidelines, NAPs are classified according to their degradability, organicity, function, approvals and how they have been synthesized. Accordingly, the safety and efficacy data requirements are described.

This document may serve as useful guidelines for manufacturers, importers of NAPs and other stakeholders involved in research and development of NAPs. These guidelines are aligned with the provisions of REACH, OECD and FAO/WHO with certain specific aspects of quality, safety and efficacy applicable to nano-agriproducts. Specific scientific evidence is required for approval as per approaches for evaluation of such products that has been covered under this guideline. Each application should be considered on its own merit of the data submitted using scientific evaluation and valid justification.

(ii) Nanofeed: Safety, evaluation and other quality studies of nanofeed should be conducted under Cattle Feed (Regulation of manufacture and sale) Order, 2009 with additional criteria for inclusion of nanofeed.

5. Definition and categorization

5.1 Definition of nano-agriinproducts (NAIPs)

A NAIP is defined as an agricultural input preparation containing nanomaterials intended for external and internal applications (through soil, seed, foliar, and drip in crops as well as by other means) on crop for the purpose of agricultural farming.

NAIPs consist of materials with any of the three dimensions, that is, zero, one or two, on the nanoscale or with an internal or surface structure in the nanoscale. The nanomaterial is defined as a material that ranges in size from 1 to 100 nm at least in one dimension. However, if the particle size is >100 nm and <1000 nm, it may also fall within the definition, provided it has altered the agri-input product characteristics associated with the application of nanotechnology compared with active ingredient. The variations in definition of nanomaterials with respect to size in different countries and respective regulatory bodies are presented in Annexure 3**.**

5.2 Definition of nano-agriproducts (NAPs)

A NAP is defined as an agricultural preparation containing nanomaterials intended for consumption or applications in food/feed and their supplements as well as nutraceutical delivery. These are the products that contain materials with any of the dimensions (zero, one or two) falling under the size range of >100 nm and <1000 nm, provided the particle size has altered the agriproduct characteristics associated with the application of nanotechnology compared with the active ingredient.

5.3 Categorization of NAIPs

NAIPs could be categorized depending on the properties and functionalities of NMs and the existing products containing synthesized and engineered NMs. Complete categorization scheme of nanomaterials is given in Annexure 4. NAIPs could be categorized as follows:

(i) According to degradation nature of nanomaterial

- **Biodegradable:** Biodegradable NMs are used frequently as nanocarrier systems and other agri-inputs due to their unique and useful properties. A few examples of biodegradable NMs are alginate, polyhydroxybutyrate, carrageenan, dextran, silk protein, micelles and emulsions (based on biodegradable surfactants/emulsifiers), PEG, albumin, PLA, PLGA, chitosan, gelatin, polycaprolactone, poly(alkyl cyanoacrylates) and nanoparticles of bioactives and nanoclay.
- **Nonbiodegradable:** Nonbiodegradable NMs are also used in NAPs (more commonly used in controlled and slow released fertilizers). Some examples of non-biodegradable NMs include metal oxides, metal nanoparticles, nanocarbon allotropes, synthetic polymers (for seed and fertilizer coatings), quantum dots, boron and carbon nitrides.

(ii) According to chemical nature of NMs: NMs could also be categorized based in their chemical nature. They broadly fall under organic or inorganic categories. Besides their chemical nature, their properties at nanoscale also differ widely depending upon their method of synthesis and interaction with other atoms.

- **Organic:** These are the NMs composed of organic compounds such as lipids, proteins and carbohydrates. They are primarily used in agriculture due to their low toxicity. Examples of organic NMs used in agriculture include synthetic nano-biochar, liposome, albumin, polymer–protein or polymer conjugates. The precursor materials used for synthesis of organic materials are generally considered to be non-toxic and biodegradable.
- **Inorganic:** Inorganic NMs, owing to their high stability, simple synthesis methods using bottom-up approaches, and a wide range of tunable physicochemical properties such as shape, size, surface charge, surface area, crystallinity and composition, are a versatile choice for agri-inputs compared to organic NMs. The properties such as optical (absorption and fluorescence), electrical (conductivity and surface charge), magnetic and thermal can be easily tailored for a specific application requirement.
- **Composite NMs:** These are the materials that contain mixture of several different categories of materials. They include all types of materials mentioned in the material categories list.

(iii) According to nano form of the ingredient

- **Nanocarriers loaded with active ingredient (AI):** A nanocarrier is a soft and hard nanomaterial used as a carrier system for targeted agri-input NMs. Common examples include polymer conjugates, polymeric nanoparticles, carbon-based materials (carbon nanotubes, graphene, carbon NPs, nano-biochar), lipid-based carriers (liposomes, micelles), dendrimers, metallic nanoparticles, nanozeolites, metal oxide and so on. These also have the advantage of controlled and slow released delivery of agri-inputs.
- **Active ingredient converted to nano form:** Active molecules/compounds could be converted into nano forms, thereby increasing their potential for improved stability and efficacy.

(iv) According to the synthesis

- **Biologically synthesized NMs:** Nanomaterials that are synthesized using bio-agents and their bio-actives. Examples include metallic NPs, bimetallic NPs, metal oxide NPs, quantum dots, nanoclusters and reduced graphene.

- **Chemically synthesized NMs:** Nanomaterials that are synthesized using synthetic chemicals as reducing, oxidizing and template. Examples include metallic NPs, bi-metallic NPs, metal oxide NPs, quantum dots, nanoclusters, reduced graphene and molybdenum disulphide.
- **Physically synthesized NMs:** Nanomaterials that are synthesized using physical processes such as ball milling, laser ablation, temperature and microwave assisted, ultrasonication, glow discharge, plasma, pulsed laser deposition and UV assisted. Examples include carbon nanotubes, graphene, metallic NPs, bi-metallic NPs, metal oxide NPs, quantum dots, nanoclusters, reduced graphene, molybdenum disulphide and nitrides**.**

6. Scientific rationale for manufacturing of NAIPs and NAPs

The rationale underlying manufacturing of NAIPs and NAPs should be demonstrated and specified with reference to their claimed advantage in comparison to conventional products. The NMs and their transformed waste disposal may have an adverse impact on the ecosystem. Therefore, the known and supposed adverse impacts on ecosystem should also be taken into consideration. The following aspects should be specifically addressed for justification of the use of NAIPs and NAPs:

- The claim should be made on the basis of parameters that must include safety, efficacy, application modes and frequency, improved crop yield and productivity or any other benefit over conventional products.
- Addressing any issue arising out of a significantly different mode of action and assimilation than that of the conventional products.
- Addressing the issue of specific adverse effect/property associated with the conventional products, if any, such as soil and plant toxic effects.

7. Specific considerations for evaluation of NAIPs and NAPs in the context of Insecticide Act, FCO, BIS, and FSSAI.

These guidelines are developed in provisions of Insecticides Act, FCO, BIS and FSSAI, with specific requirements and adaptations for NAIPs and NAPs wherever considered necessary. While these provisions specify the general requirements and guidelines to manufacture or import new fertilizers (FCO), pesticides (Insecticide Act), food additives and preservatives (FSSAI) or to undertake quality checks, this document provides guidance on specific requirements for agri-input and agriproducts developed based on nanotechnology. General requirements as specified in these provisions will be applicable for any new products whether nanotechnology based or not. However, because of the involvement of interdisciplinary sciences and the complex nature of NAPs, a ‘case-by-case basis’ approach should be adopted for their evaluation with respect to enhanced efficacy and safety.

Considering the unique process conditions of nanoformulations compared to the conventional agri-input products and agriproducts, the product description should include detailed description methods of manufacturing process (excluding critical intellectual property information) and process controls to be included. The method of nanoparticle waste disposal and environmental impact may be declared.

‘Nanocomponents’ incorporated into some specific materials such as plastic, ceramic and regenerated cellulose films are subject to different kinds of regulations. The policy 2002/72/EC (14) implemented in Great Britain may be followed to regulate plastic and other food contact articles to deal with food

contamination issues due to migration of lead and cadmium. European Regulation No. (EC) 1935/2004 may be followed to evaluate quality and safety of foodstuffs. The 12 principles of green chemistry proposed by EPA in 1991 may also provide guidance for engineering safe NAIP and NAPs. These include prevention, atom economy, less hazardous chemical syntheses, designing safer chemicals, safer solvents and auxiliaries, design for energy efficiency, use of renewable feedstocks, reduce derivatives, catalysis, design for degradation, real-time analysis for pollution prevention and inherently safer chemistry for accident prevention.

8. Excipients used in NAIPs and NAPs

Excipients help in the manufacture of formulations of NAIP and NAPs and improve performance and stability of the product. Examples of excipients in NAIPs and NAPs include stabilizers to prevent agglomeration and aggregation, preservatives to prevent microbial growth, surfactants and coupling agents to modify surface characteristics of nanomaterial.

9. Stability testing of NAIPs and NAPs

The general storage stability requirements and procedures for agricultural chemical products may also be applied on NAIPs and NAPs to ensure stability. The following four principal types of storage stability studies as per OECD TG 318, FAO/WHO, AVMPA may be adopted:

(a) Accelerated storage stability tests

(b) Ambient storage stability tests

(c) Low temperature storage stability tests

(d) Testing for reactivity towards container materials

The test parameters for stability testing of NAIPs and NAPs are also considered. The following test parameters (whichever is applicable) may be considered for each product. Relevant scientific argument should be provided to explain why to exclude any one of the following test parameters:

(a) Selection of containers

(b) Shelf-life statement

(c) Batch (laboratory-, pilot- or production-scale) and size of products

(d) Duration of storage stability

(e) Validation of analytical methods

(f) Technical characteristics (colour, odour, acidity or alkalinity and pH, wettability, suspensibility, dispersion stability, dilution stability, particle size distribution, emulsifiability, re-emulsifiability, emulsion stability, viscosity, flowability, crystalline state, release kinetics and leakage).

(g) Microbial stability

10. Safety of manufactured NAIPs and NAPs

Depending upon the product type, application and exposure to humans and environment, the suitable *in vitro* methods for hazard assessment and effective regulation of NAIPs and NAPs should be adopted from the listed items. Each of the *in vitro* assays mentioned is based on existing OECD Testing Guidelines (TGs) for application to testing manufactured NMs (OECD, 2019):

Dermal exposure/toxicity: OECD TG 428 (*in vitro*); OECD TG 427 *in vitro* kin corrosion: OECD TG 431
Eye irritation: OECD TG 437
Genotoxicity: OECD TG 471, 473, 476, 482 and 487
Inhalation exposure (toxicity): OECD TG 403

Cytotoxicity assays used in the OECD testing programme: ATP CellTiter-Glo, neutral red uptake, LDH release, MTT, XTT, cell impedance, trypan blue, BrdU, Alamar Blue, WST-1, live/dead cell counting, colony forming efficiency, genotoxicity assays used in the OECD Testing Programme: Comet assay and DNA double-strands breaks

Ecotoxicity:
(A) Aquatic test: OECD TG 201 (freshwater algae, cyanobacteria and growth inhibition test), OECD TG 202 (*Daphnia* sp. acute immobilization test), OECD TG 211 (*Daphnia magna* reproduction test)
(B) Soil and sediment test: OECD TG 222 (Earthworm reproduction test)
(C) Effect on soil microbiota OECD guidelines: OECD Method No. 216 and 217

11. Residue analysis (nanoactive ingredients, additives, and nanocarrier materials)

Information on the persistence of NAIPs and NAPs should be provided in the registration dossier and residues analysis of used nano active ingredients, additives, and nanocarrier materials needs to be performed during the life cycle of the product. Data on nanomaterial residue, presence of any nanosized degradation products in food/feed, excipients or surface coating used on food contact material need to be declared by the manufacturer during product registration. The report must mention the following details:

- Method for determination (detection and quantitation limits) of residues from the used active ingredient, additive and nanocarrier
- Quantities of generated residues and summary of anticipated risks of generated residues: The requirement for toxicological data, there is no migration of elements from food contact materials or the migrating species are not in the nanomaterial form (in which case standard risk assessment should apply)

12. Information required for evaluation of NAIPs

Nanopesticides (chemical and biological sources) and growth regulators are required to be registered under the existing Insecticides Act, 1968 and Rules 1972 through nodal agencies, namely, Central Insecticides Board and Registration Committee. Likewise major fertilizers (chemical and biological sources) and micronutrients are required to be registered under the existing FCO, 1985 through the nodal agency Department of Fertilizers, Government of India and

agriculture department of state governments. The food and feed products developed using nanotechnology interventions are required to be registered as per the guidelines by FSSAI. The nanotechnology intervention used in these products must be registered under the existing regulations. The additional data sets required for registration of nano-agriproducts based on the active ingredient and nanocarrier formulated products are as follows:

(A) Overview

- A brief description of NAIPs
- Intended use
- Category
  - Nanofertilizers (e.g., major, secondary and micronutrient)
  - Nanopesticides (insecticide, fungicide, nematicides, acaricide and rodenticide)
- Are there relevant source particles of NM analogues available of the similar chemical and physical structure?
- Justification for developing nanoproducts (claims)
- Draft of label

(B) Detailed Information

a. Information on the ingredients

- Information on nanomaterials used (active ingredient/nanocarrier)
- Used nanomaterials based on the method of production and composition
- Nanomaterials property characterization
- Hydrodynamic particle size and distribution (polydispersion index)
- Surface charge (using zeta potential)
- Crystallinity (XRD)
- Transmission electron microscopy (for shape, size and actual average particle size)
- Aspect ratio (only for 1D and 2D nanomaterials using TEM, SEM and FE-SEM)
- Hydrophilicity/lipophilicity using contact angle measurement
- pH using pH meter
- Viscosity (in case of liquid formulation using viscometer)
- Electrical conductivity (in case of liquid formulation using conductivity meter)
- Organic (HPLC and GC data); inorganic (XRF and ICP-MS data)
- FTIR spectrum
- X-ray diffraction chromatogram

b. Stability data (as per OECD 318 TG)

c. Impurities detail

d. Quality control checks parameters and test protocols
Sampling procedure and preparation for specific analysis
Testing protocol/s
Certificate of analysis

e. Preliminary toxicity analysis data (confirmatory toxicity analysis would be performed by CIB)

- Cytotoxicity: ATP Cell Titer-Glo, neutral red uptake, LDH release, MTT, XTT, cell impedance, trypan blue, BrdU, Alamar Blue, WST-1, live/dead cell counting, colony forming efficiency
- Genotoxicity: OECD TG 471, 473, 476, 482 and 487

f. Comparative field efficacy data (bulk versus NAIPs) and residue report (as per section 9(3) of the Insecticides Act, 1968; for herbicide, data to be generated for two seasons and three locations and for others, data to be generated for one season and four locations).

g. Occupational hazard, exposure and fate assessment

Decision framework (OECD, ENV/JM/MONO(2019)12) for inclusion of physico-chemical parameters for exposure and fate assessment of nanofertilizers and nanopesticides may be followed.

13. Nano agriproducts: nanofood and nanofeed products (suggested for inclusion in the existing regulatory act by FSSAI)

In addition to the information mentioned in Section 12, the information discussed next will also be required for NAPs.

13.1 Exposure risk

NM exposure measurement is essential for hazard characterization and risk assessment. Migration of NMs or its degraded products in non-nano form (its type and quantity) from agri produce or via animals for food production or from food contact materials (like packaging) should be considered in exposure measurement and hazard characterization and ADME studies are required. Specific testing protocols for analysis of migrated products are required. Food sampling, variability in composite sampling and concentration variations between samples are critical sampling issues in exposure evaluation. The decision framework defined by OECD (ENV/JM/MONO (2019)12) may be followed for inclusion of physico-chemical parameters for exposure and fate assessment of nanofertilizer, nanopesticide and NMs in food. Guidance on risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain (EFSA, 2018) may also be followed.

13.2 Hazard characterization

Hazard identification and characterization require appropriate *in vitro* and *in vivo* studies to determine the fate of NMs. Toxicity testing should be customized for case of exposure.

1. **In case of the non-stable NMs in food preparation/formulation:** For example, when NMs are completely degraded/solubilized/transformed to their non-nano form in food matrices, general protocol for toxicity measurement of non-nano form in the intended application can be considered. But strong scientific evidence should be produced demonstrating its solubility. This criterion applies to non-persistent NMs in marketed foods and foods where nano form transforms to non-nano form before injection.

2. **NMs that get transformed during digestion:** For NMs that get completely degraded/dissolved in gastrointestinal tract and where there is no possibility of their absorption in nano state, the hazard characterization can be relaxed and can rely only on data for non-nano form. This scenario should be strongly supported by *in vitro* genotoxicity and *in vivo* testing for local effects and other *in vivo* tests. When regulations on non-nano form are not available, FSSAI has to come up with regulations.

3. **Stable nano materials:** For the nanomaterials that are stable in food formulations/agri produces and in gastrointestinal tract, two scenarios considered:

a. When characteristics and toxicity of non-nano form of NMs used are known through toxicity testing and ADME (repeated-dose 90-day oral toxicity) and genotoxicity studies of two forms can identify the major difference between them. If the difference is identified, more stringent toxicity testing and ADME testing should be considered. In case of less hazard NMs, further testing can be relaxed upon strong scientific evidence.

b. When hazard characteristic of its non-nano form is NOT available through toxicity testing and ADME studies are required for nanomaterial hazard characterization and regulations.

4. **Migration of food contact materials:** When there is no migration from FCM, toxicological concerns are negligible. If not, stringent toxicity studies need to be enforced.

The following types of toxicity testings are required for NMs (EFSA Scientific Committee, 2011):

I. ***In vitro* studies:** They help to understand biological responses of NMs and underlying mechanism for toxicity screening. However, suitability of test system and possible structural and functional changes arising from interaction of NMs with culture medium should be considered.

a. ***In vitro* digestion studies:** Physiochemical and mechanical conditions of the human gastrointestinal tract can be simulated to understand dissolution and degradation of NMs during digestion. This leads to limited or no further studies for hazard analyses. There are many *in vitro* digestion models available, notably dynamic gastrointestinal digestion system (present in Indian Institute of Food Processing Technology (IIFPT) under MoFPI) (Parthasarathi et al., 2018; The Hindu science, 2018), Dynamic Gastric Model Institute of Food Research (Norwich, UK) (Thuenemann et al., 2015), Model Stomach system (Kong & Singh, 2008), Human Gastric simulator (UC Davis, Food Science and Technology) (Kong and Singh, 2010), TIM-1 (Netherlands) (Minekus, 2015), SHIME (ProDigest and Ghent University, Belgium) (Van de Wiele et al., 2015) and Dynamic in-vitro human stomach, China (Wang et al., 2019). They help to understand the digestibility and release behaviour of ingested food components and thus fate of added NMs.

b. ***In vitro* genotoxicity testing:** Use of bacterial reverse mutation assay cannot be considered for detection of genotoxicity of NMs due to the fact that bacterial cells do not phagocytose particles like mammalian cells and NMs cannot penetrate bacterial cell wall (Landsiedel et al., 2009). Studies such as OECD TG 476 for induction of gene mutations in mammalian cells (preferably the mouse lymphoma TK assay with colony sizing) and OECD test guideline 487 for an *in vitro* micronucleus assay should be considered for evaluating NMs in food.

c. **Other *in vitro* studies:** This includes various *in vitro* models to assess the effects of NM on permeability/integrity of the gastrointestinal barrier, inflammatory responses to assess gut maintenance, immune cells and immune responses etc. Cells, like differentiated CaCo-2 cells, primary human oesophageal epithelial cells and M-cells (modified enterocytes present throughout the epithelial lining) are used to simulate the *in vivo* conditions.

II. ***In vivo* studies:** *In vivo* studies are essential to identify ADME profile, adverse responses and dose-dependent toxicity. Forms of administration of NMs (e.g., adding to feed, water or by gavage) for *in vivo* studies also influence the toxicity profiling. For example, NMs that interact with food and form complex matrices, simulant cannot be used and it should be homogeneously blended in food.

a. **ADME studies:** Absorption, distribution, metabolism and excretion (ADME) studies are essential for toxicity evaluation of nanomaterials. Appropriate measuring systems should be adopted to detect nanomaterials in organs, tissues or biological fluids. Labelling with radioactive isotopes, fluorescent dyes and comprehensive mass balance studies are to deal with nanomaterial polydispersity, and toxicokinetic changes upon repeated administration should be considered while designing ADME studies. Simple ICP-Ms cannot determine the presence of nanomaterials.

b. ***In vivo* repeated-dose 90-day oral toxicity study:** Repeated-dose 90-day oral toxicity study in rodents as per the OECD TG 408 is required to access orally ingested NMs. Emphasis on assessment of cardiovascular and inflammatory parameters, endocrine-related endpoints and oestrous cycles is required during oral toxicity studies.

c. ***In vivo* genotoxicity testing:** If genotoxicity is observed in any of the *in vitro* studies, or when it is impossible to conduct *in vitro* studies for selected NMs, any of the following *in vivo* tests may be adopted: *in vivo* micronucleus test (OECD TG 474), *in vivo* comet assay and transgenic rodent gene mutation assay.

d. **Other *in vivo* toxicity tests:** If there is evidence of toxic effects and accumulation of NMs (or degradation of products/metabolites) in organs and tissues, chronic toxicity by following OECD TG 453 may be appropriate in order to reveal progressive toxic effects or delayed toxicity and developmental toxicity and to identify a BMDL or a NOAEL.

OECD test guidelines 414, 415 and 416 may be adopted for study design of reproduction and developmental studies.

13.4. Uncertainty analysis

Analysing possibility of uncertainty in assessing the above-mentioned assessments. Some of the possible reasons for uncertainty in assessing ENM are as follows:

- Non-availability of standard methods for physico-chemical characterisation of various ENM structures and associated properties
- Sample preparation procedures and calibration of the analytical equipment dictate characterisation accuracy
- Differences in the physical principles applied by various measurement techniques
- Aggregation/agglomeration behaviour of ENM and other factors such as dilutions or dispersions vary with their interaction with various environmental factors

Reduced information can be provided when no exposure to NMs is confirmed by data indicating no migration from food contact materials or when complete degradation/dissolution is demonstrated with no absorption of engineered nanomaterials as such (EFSA Scientific Committee, 2011; Leena et al., 2019).

14. Conclusions

Hundreds of NAIPs and NAPs using nanotechnology are already on the market even though there are no specific policies regulations for their control. Therefore, there is a need to develop the policy and regulations in place.

15. References

1. AVMPA (Australian Pesticides & Veterinary Medicines Authority) 2005, "Guidelines for the Generation of Storage Stability Data of Agricultural Chemical Products," Kingston ACT Australia 2604, December 2005.
2. Commission Directive 2002/72/EC relating to plastic materials and articles intended to come into contact with foodstuffs.
3. ECHA's Guidance on information requirements: Guidance on regulation (EU) 528/2012 concerning the making available on the market and use of biocidal products (BPR)Version 1.0 July 2013:(http://www.echa.europa.eu/documents/10162/15623299/biocides_guidance_information_requirements_en.pdf)
4. EFSA (European Food safety Authority). 2009. Scientific opinion of the Scientific Committee on a request from the European Commission on the potential risks arising from nanoscience and nanotechnologies on food and feed safety. EFSAJournal, 958: 1–39 (http://www.efsa.europa.eu/de/scdocs/doc/958.pdf, accessed 25 October 2011).
5. EFSA Scientific Committee. (2011). Scientific opinion on guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain. EFSA Journal, 9(5), 2140. https://doi.org/10.2903/j.efsa.2011.2140
6. FDA. 2012. Guidance for industry. Assessing the effects of significant manufacturing process changes, including emerging technologies, on the safety and regulatory status of food ingredients and food contact substances, including food ingredients that are color additives. Draft guidance for industry. (http://www.fda.gov/downloads/ Cosmetics/GuidanceComplianceRegulatoryInfo>rma>tion/GuidanceDocuments/ UCM300927.pdf, accessed 1 February 2013).
7. FDA. (2014a). Guidance for Industry Considering Whether an FDA-Regulated Product Involves the Application of Nanotechnology. Retrieved from https://www.fda.gov/downloads/RegulatoryInformation/Guidances/UCM401695.pdf
8. FDA. (2014b). Guidance on the Safety and Regulatory Status of Food Ingredients and Food Contact Substances Including Food Ingredients that are Color Additives. Retrieved from https://www.fda.gov/downloads/Cosmetics/GuidanceRegulation/GuidanceDocuments/UCM300927.pdf
9. FDA. (2015). Guidance for Industry Use of Nanomaterials in Food for Animals. Retrieved from https://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM401508.pdf
10. FSANZ (Food Standards Australia New Zealand). 2011. Nanotechnology and food. Canberra and Wellington: Food Standards Australia New Zealand. (http://www. foodstandards.gov.au/consumerinformation/nanotechnologyandfoo4542.cfm, accessed 16 November 2011).
11. Insecticides Act, 1968 (Act No.46 of 1968).
12. Kong F, Singh RP (2010) A human gastric simulator (HGS) to study food digestion in human stomach. J Food Sci 75(9):E627–E635

13. Leena, M., Moses, J. A., & Anandharamakrsishnan, C. (2019). Ethical and Regulatory Issues in Applications of Nanotechnology in Food. In C. Parthasarathi, S., Anandharamakrishnan (Ed.), *Food Nanotechnology: Principles and Applications* (pp. 67–94)
14. OECD (2015) Guidance Manual Towards The Integration Of Risk Assessment Into Life Cycle Assessment Of Nano-Enabled Applications Series On The Safety Of Manufactured Nanomaterials No. 57.
15. OECD (2009a) Preliminary review of OECD Test Guidelines for their applicability to manufactured nanomaterials.
16. OECD (2014) Report of the OECD expert meeting on the physical chemical properties of manufactured nanomaterials and test guidelines.
17. OECD Guidelines for the Testing of Chemicals, Section 3: Test No. 318: Dispersion Stability of Nanomaterials in Simulated Environmental Media.
18. Minekus, M. (2015). The TNO gastro-intestinal model (TIM). In The impact of food bioactives on health (pp. 37-46). Springer, Cham.
19. Parthasarathi, S., Bhushani, J. A., & Anandharamakrishnan, C. (2018). Engineered small intestinal system as an alternative to in-situ intestinal permeability model. Journal of Food Engineering, 222, 110–114. https://doi.org/10.1016/j.jfoodeng.2017.11.019
20. Pesticide Specifications, Manual on the development and use of FAO and WHO specifications for Pesticides, second revision of the first edition, World Health Organization and Food and Agriculture Organization of the United Nations, Rome 2010.
21. REACH Regulation - Registration, Evaluation, Authorisation and Restriction of Chemicals Regulation (EC) No. 1907/2006.
22. Subrahmanian K S, Rajkishore S K (2018). Regulatory framework for Nanomaterials in Agri Food Systems. In: M Rai, J K Biswas (eds) Nanomaterials: Ecotoxicity, Safety and Public Perception, https://doil.org/10.1007/978-3-030-05144-0_16
23. Thuenemann E.C., Mandalari G., Rich G.T., Faulks R.M. (2015) Dynamic Gastric Model (DGM). In: Verhoeckx K. et al. (eds) The Impact of Food Bioactives on Health. Springer, Cham
24. TSCA § 3(9), 15 U.S.C. § 2602(9). EPA's regulatory definition of a "new chemical substance" tracks the statutory definition. https://www.epa.gov/tsca-inventory
25. Van de Wiele T., Van den Abbeele P., Ossieur W., Possemiers S., Marzorati M. (2015) The Simulator of the Human Intestinal Microbial Ecosystem (SHIME®). In: Verhoeckx K. et al. (eds) The Impact of Food Bioactives on Health. Springer, Cham
26. Wang, J., Wu, P., Liu, M., Liao, Z., Wang, Y., Dong, Z., & Chen, X. D. (2019). An advanced near real dynamic in vitro human stomach system to study gastric digestion and emptying of beef stew and cooked rice. Food & function, 10(5), 2914-2925.

Annexure 1: Nanotechnology Stakeholders in India

SECTORS

Research & Education

- Colleges & Universities
- Research Institutes
- Social Science Policy Research
- Environment Health & Safety Research

Finance

- Venture Capital
- Public Funds
- Private Funds
- International Funds

Policy Makers

- Department of Biotechnology (DBT), Ministry of Science & Technology
- Department of Science & Technology (DST), Ministry of Science & Technology
- Ministry of Agriculture
- Ministry of Chemicals & Fertilizer
- Ministry of Food Processing Industries
- Ministry of Animal Husbandry, Dairying and Fisheries
- Ministry of Environment, Forests and Climate Change
- Ministry of Earth Sciences
- Council for Industrial Research (CSIR)

Regulators

- Fertilizer Control Order (FCO)
- Central Insecticide Board (CIB)
- Food Safety and Standards Authority of India (FSSAI)
- State agriculture departments
- BIS

Industries

- Fertilizers
- Pesticides
- Seeds
- Plant Growth Regulators
- Food Processing

Annexure 2: Global status for regulation of nanoproducts in agri-food systems (Subrahmanian & Rajkishore 2018)

Country	Regulatory Body/Responsible Organization	Legislation	Provisions in the available legislation
USA	Food and Drug Administration (FDA) & US-Environmental Protection Agency (US-EPA)	Federal Food, Drug and Cosmetic Act (FFDCA), Federal Insecticide, Fungicide and Rodenticide Act (FIFRA)	No specifications on nanoproducts in FFDCA of FIFRA
Canada	Canadian Food Inspection Agency (CFIA) and Public Health Agency of Canada (PHAC)	Food and Drugs Act (7)	Nanoproducts are regulated under existing legislative
EU	European Commission (EC), European Parliament and Council	Regulation (EU) No 2015/2283 and Regulation (EU) No 1169/2011 (8)	States that material that meets the criteria for an engineered nanomaterial in Novel Food on the Provision of Food Information to Consumers, i.e. nanomaterials that, amongst other criteria, have particle sizes in the defined nanoscale (1–100 nm). Provides guidance as how to perform risk assessment of nanomaterials in the food and feed area (e.g. novel food, FCMs, food/feed additives and pesticides).
		Regulation (EC) No 1333/2008	States that a food additive already authorized but obtained using nanotechnology requires a re-evaluation before marketing
		Regulation (EC) No 1332/2008 on food enzyme	States that a food enzyme already included in the Community list but prepared by different methods or using starting materials significantly different (It is specified that

			"Significantly different" could mean a change in particle size) from those included in the risk assessment of the Authority, should be submitted for re-evaluation
		Regulation (EC) No 1334/2008	Flavourings must undergo a common (EU-wide) assessment and authorization prior marketing and lays down a Union list of flavourings and source materials approved for use in and on foods and their conditions of use.
		Directive 2002/46/EC	Food supplements Stated that the food supplements (minerals or vitamin) can be used which are listed by EC. The use of nanoforms of minerals and vitamin requires a safety evaluation prior marketing which will be done under Novel Food Regulation, due to the differences in production, potential differences in nutritional value and bioavailability when compared to macro-scale counterparts
		Regulation (EC) No 450/2009a	Although nanomaterials are not directly mentioned, there is a reference to "substances deliberately engineered to particle size which exhibit functional physical and chemical properties that significantly differ from those at a larger scale"; therefore, a case-by-case analysis has to be followed for active and intelligent materials and

			articles containing nanomaterials
		Regulation (EU) No 10/2011a	States that the substances in nanoform should be used only if listed in the Annex I of the regulation
		(EU) No 528/2012 (9)	As of today, nanomaterials based biocidal products are not eligible for a simplified authorisation procedure. For subsequent nanomaterials based product authorisation and approving nanomaterials as active substances, the test methods applied to the nanomaterials shall be accompanied and standardized by an explanation addressing their exact appropriateness considering the specific characteristics of each nanomaterials
		Registration, Evaluation,Authorization and Restriction of Chemicals (REACH) regulation (1907/2006)	Legislation has nanospecific provision but no exclusive NM legislation.
Switzerland	Federal Office for the Environment (FOEN) Swiss Federal office of public health (FOPH)		Existing regulations are ensured for safety of NMs
Australia and New Zealand	Food standards Australia New Zealand (FSANZ)	Australian New Zealand Food Standards Code	Amended FSANZ Application Handbook to support new food regulations to manage risks from nanoproducts
China	Ministry of Agriculture, Ministry of Health	Food Safety Law of China	National Centre for Nanoscience and Technology (NCNST) and the commission on nanotechnology standardization
South Korea	Ministry of Food and Drug Safety (MFDS)	National Nano-Safety Strategies Plan	No NM specification

	Korean Food and Drug Administration (KFDA) Korean Agency for Technology and Science (KATS)	(2012/2016) and Food Sanitation Act	
Japan	Ministry of Health, Labour and Wlfare	Food Sanitation Law	No NM Specific regulation
Iran	Iran Nanotechnology Initiative Council (INIC) Nanotechnology Committee of Food and Drug Organization		Food and Drug Organization (FDO) constituted guidelines for nanoproducts in food, beverages, pharmaceutical, medical equipment but agriculture is not yet included.
Taiwan	Taiwan Nanotechnology Industry Development Association (TANIDA)	Nanoproducts are certified	TANIDA established nanoMark system to certify the nanoproducts
Thailand	Food & Drug Administration of the Ministry of Public Health		NanoQ label has been introduced for nanoproducts (but not for agri/food) that are certified by the Nanotechnology Association of Thailand
India			
Food novel, food or novel food ingredients or processed with the use of novel technology New additive New processing aids including enzymes Articles of food and food ingredients consisting of, or isolated from microorganisms, bacteria, yeast, fungi or algae.	Food Safety Authority of India under the Ministry of Health and Family Welfare, Government of India	Food Safety and Standards Act (2006) Food Safety and Standards (Approval of Non-Specified Food and Food Ingredients) Regulations, 2017	DST Nano mission has come up with safe handling of NMs in workplace and industry. But no specific legislation/regulation for agriculture.

Enzymes, flavouring and additives	Food Safety Authority of India under the Ministry of Health and Family Welfare, Government of India	Food Safety and Standards Act, 2006 (Food Product Standards and Food Additives) Regulations, 2011	But no specific legislation/regulation for agriculture.
Health supplements, nutraceuticals, food for special dietary use, food for Special Medical Purpose, functional food, novel food	Food Safety Authority of India under the Ministry of Health and Family Welfare, Government of India	Food Safety and Standards Act,2006 Food Safety and Standards (Health Supplements, Nutraceuticals, Food for Special Dietary Use, Food for Special Medical Purpose, Functional Food, and Novel Food) Regulations, 2016	But no specific legislation/regulation for agriculture.
Food contact materiel	Food Safety Authority of India under the Ministry of Health and Family Welfare, Government of India	Food Safety and Standards Act, 2006 Food Safety and Standards (Packaging) Regulations, 2018	But no specific legislation/regulation for agriculture
Insecticides	Central Insecticide Board and Registration Committee (CIB&RC) under the Directorate of Plant Protection, Quarantine & Storage, Department of Agriculture & Cooperation set up by the Ministry of Agriculture	Section 9(3) &9(3b) of the Insecticides Act, 1968 Insecticides (Amendment) Rules.	But no specific legislation/regulation for agriculture
Fertilisers	Fertilizer (Control) Order, 1985 administered by Dept. of Agriculture	Under section 3 of Essential Commodities Act, 1955	But no specific legislation/regulation for agriculture

	Cooperation, Govt. of India		
Biocides	Central Insecticide Board and Registration Committee (CIB&RC)	Guidelines for the Registration of Biocide and Biocide Products (Manufacturing Use Products)	But no specific legislation/regulation for agriculture

Annexure 3: Comparison of NM definition in current regulatory frameworks in selected countries available for food sector (Subrahmanian & Rajkishore 2018)

Country and regulation	Size	Solubility	Aggregates and agglomerates	Distribution Threshold	Intentionally manufactured /engineered	Novel properties
European Commission Recommendation for a Definition	1-100nm	no	yes	50% by number	no	no
European Parliament and the Council of the European Union on the Provision of Food Information to Consumers	1-100nm and larger	no	yes	No specific mention	Yes	yes
European Commission Cosmetics Directive	1-100nm	yes	yes	No specific mention	yes	no
European Commission Biocides Directive	1-100nm	no	yes	50% by number	no	no
United States Food and Drug Administration	1-100nm and larger	no	No specific mention	No specific mention	yes	yes
United States Environmental Protection Agency	1-100nm	no	yes	10% by weight	yes	yes
Australian Government Department of Health and Ageing	1-100nm	no	yes	10% by number	yes	yes
Health Canada	1-100nm and larger	no	yes	No specific mention	yes	yes
Taiwan Council of Labor Affairs	1-100nm	no	yes	50% by number	no	no
Swiss Federal Office of Public Health and Federal Office for the Environment	1-100nm	no	yes	50% by number	no	no

Annexure 4: Nano materials categorization scheme