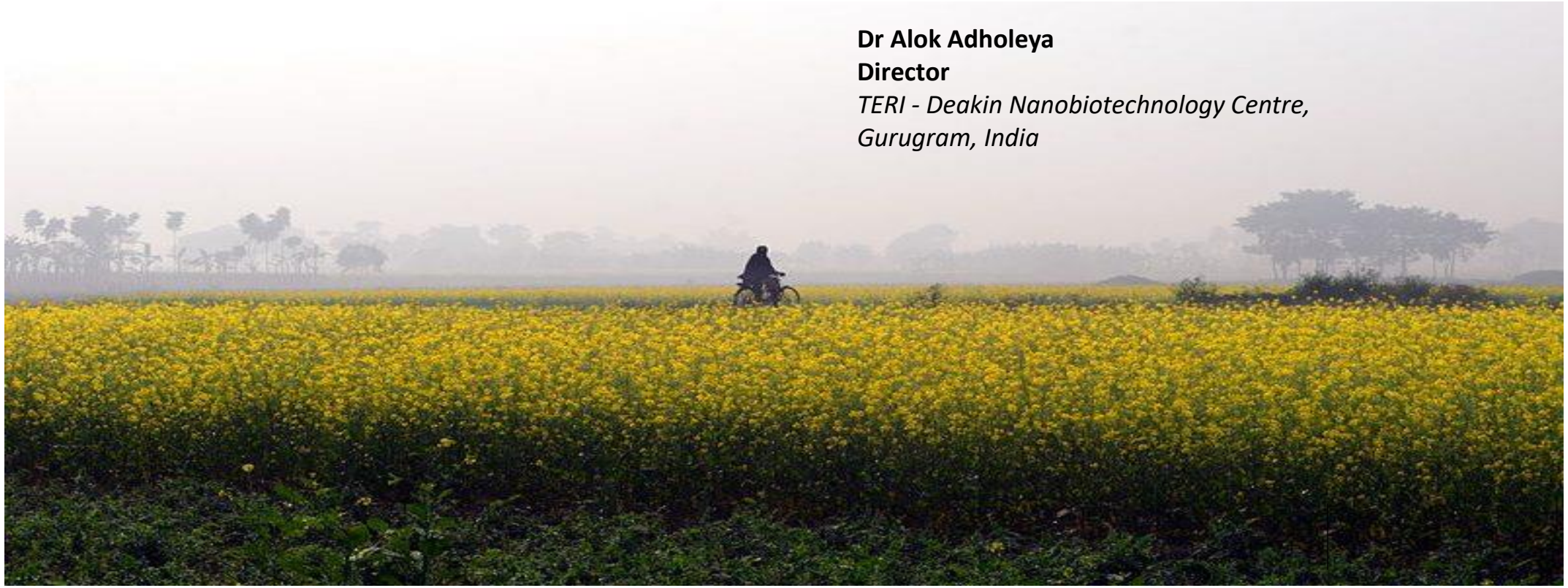
GUIDELINE FOR EVALUATION OF NANO-BASED AGRI-INPUT AND FOOD PRODUCTS IN INDIA



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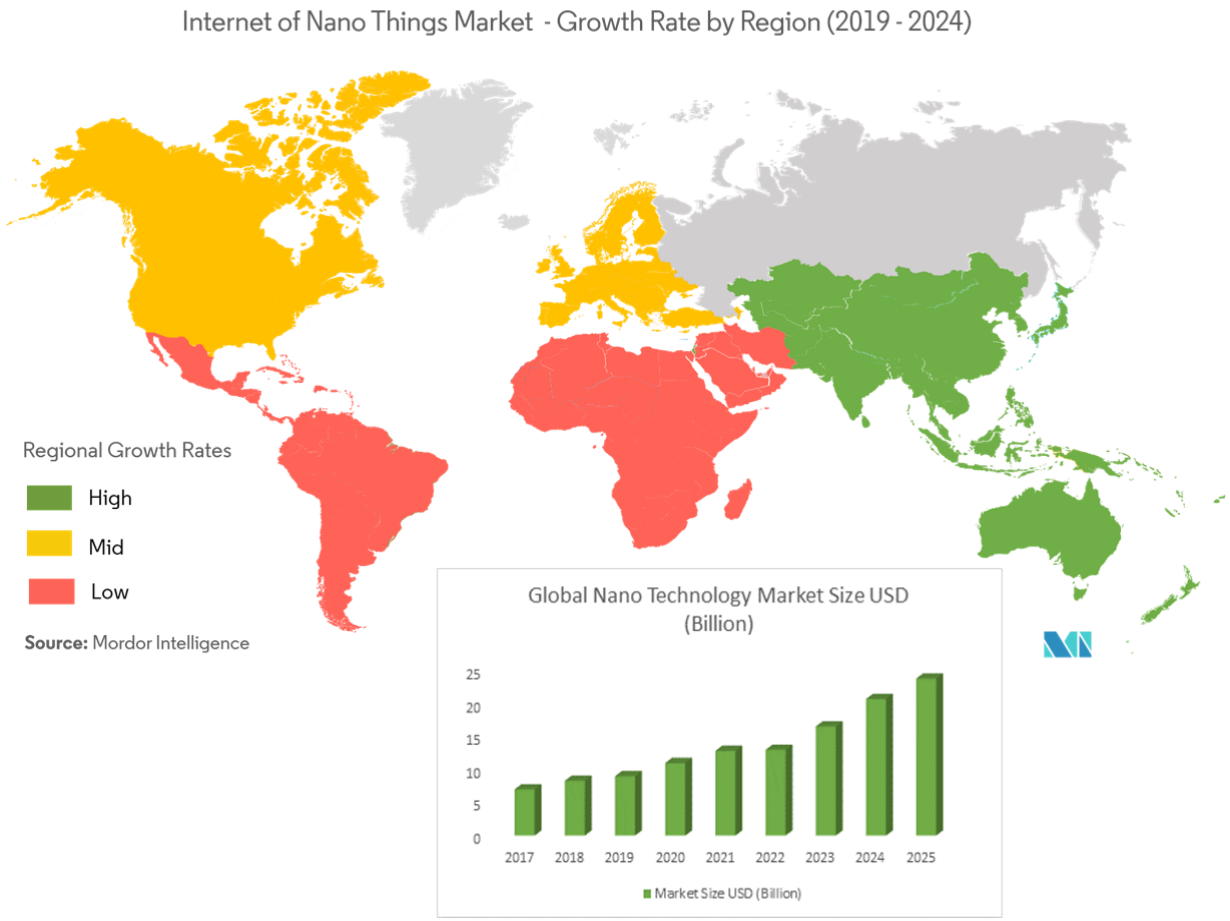
4th EU-Asia Dialogue on Nanosafety
7th October, 2020

Nanotechnology in Agriculture

- Interventions of nanotechnology applications holds enormous potential for improvement in agriculture.
  - **for crop production, protection, management, post-harvest and packaging**
- Controlled release of these products can increase the nutrients-use efficiency.
- Nanotechnology strategies would improve performance on terms of reduced load of chemicals in agriculture at lesser treatment cost while increasing the yield potential of food crops and reducing carbon foot prints.



India: Progress in Nanotechnology Sector

2007: Plan for Nano-infrastructure

- First phase for its five year plan
- The Government of India made significant investments worth Rs.1000 crore (US$250 million) in 2007
- To provide rich infrastructure to academic institutions, R&D institutes & national laboratories.

2007-2012. Funding from venture capitalists

- Additional $100 million funding was attained from venture capitalists.

2015. Capacity Development

- In 2015 a survey showed that, India had over 1.000 scientists working on nanotechnology and 17 universities offered nanotechnology curriculum.

2015-2020. Publications and Translations

- Increasing number of peer-reviewed scientific literature, where in **India stand second only to USA** in the distribution of publications among the most productive countries
- large number of translation research projects

The Hurdle; market size remains small

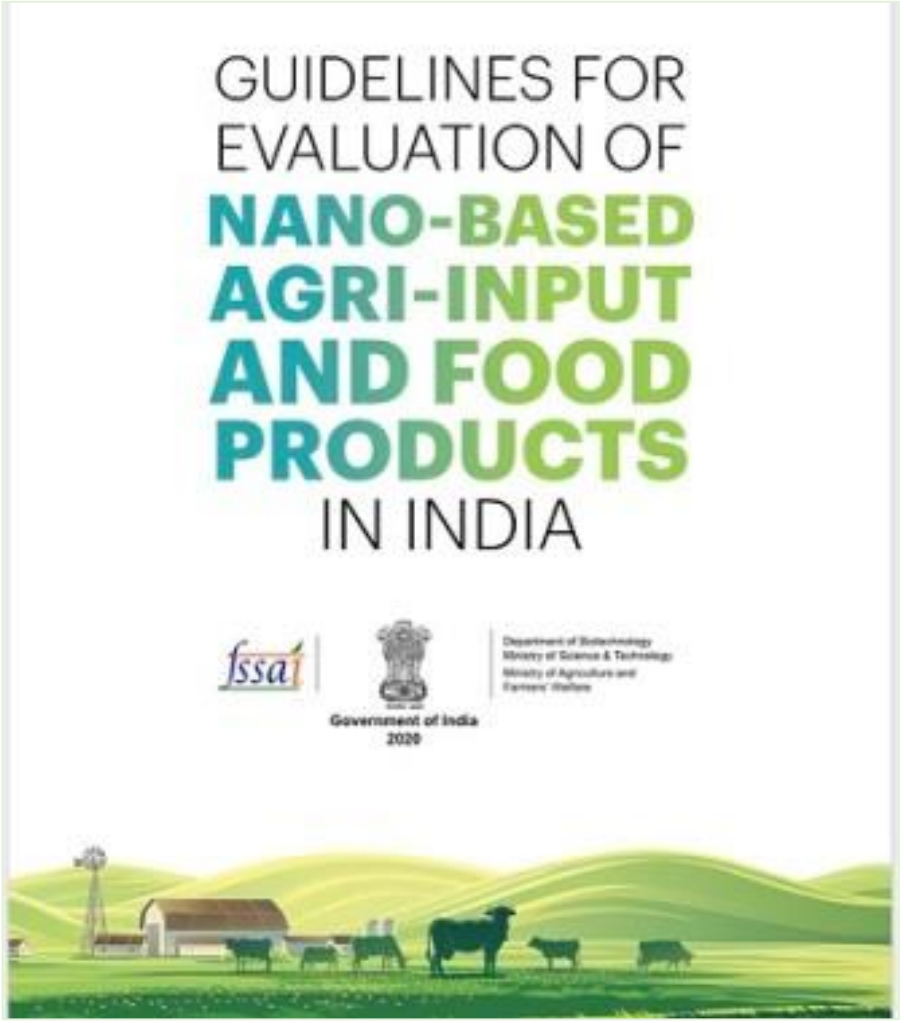
- **This is mainly because clarity with respect to regulation for registration and commercialization of nanoagri products in India is largely lacking among the stakeholders**

Regulatory aspects of the nano-based products

- **New consideration of application of nanotechnology in agriculture necessitate defining regulatory aspects of the nano-based products for agriculture and food.**
- **Guidelines for evaluation in India and across the world therefore are highly desired.**
- **The guidelines will encourage development of *safe, next generation nano-based agri-input and food products to benefit farmers and other stakeholders***

GUIDELINE FOR EVALUATION OF NANO-BASED AGRI-INPUT AND FOOD PRODUCTS IN INDIA

Department of Biotechnology Govt. of India



- **India is forging ahead at the global level and has developed a comprehensive and inclusive guidelines with the intent to support appropriate regulation landscape in India.**

- **These guidelines will empower the researchers, innovators and regulators in agriculture and food industry to achieve a greater social and economic impact.**

http://dbtindia.gov.in/sites/default/files/Guidlines%20Document.pdf

GUIDELINE FOR EVALUATION OF NANO-BASED AGRI-INPUT AND FOOD PRODUCTS IN INDIA

- **In India, there are different Government Agencies and different provisions that regulate different agriinput and food products; however, no specific provisions are available**

- **Department of biotechnology (DBT), Govt. of India conducted extensive inter-ministerial consultations with active participation of domain experts and developed the guidelines**

- **Representation from Govt. agencies (DST, CSIR, ICAR, CIB&RC, FCO, FSSAI, MoFPI, MoAHD&F, MoA&FW), industry, Industry Associations and other stakeholders.**

GUIDELINE FOR EVALUATION OF NANO-BASED AGRI-INPUT AND FOOD PRODUCTS IN INDIA

- **The multidisciplinary nature of nanotechnology poses 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.**
- **Moreover, different issues and activities concerned with nanotechnology and nanoproducts are dealt by different Departments and different Ministries in the Government,**
- **Thus, inter-departmental and inter-ministerial convergence is also required, along with different concerned stakeholders**
- **The present guidelines are developed to support the existing national regulatory provisions with specific requirements and adaptations for NAIPs and NAPs of**
  - Central insecticide board and registration Committee (CIB&RC),
  - Food control order (FCO) ,
  - Food Safety and Standards Authority of India (FSSAI)
  - Bureau of Indian Standards (BIS),
  - Ministry of Food Processing Industries (MoFPI)
  - Department of Animal Husbandry & Dairying (DAHD)
- **These guidelines are also harmonized with the applicable provisions for NAIPs and NAPs** as per the international guidelines of REACH, OECD, US EPA, TSCA, APVMA, FAO/ WHO, US FDA, EFSA, FSANZ and Codex; and the principles of ICH.

SCOPE OF THE GUIDELINES

1. Agri-input products **(NAIPs)** in the nano form

- Nanotechnology application for crop production, protection, management, harvesting, post-harvesting and packaging
- Pesticides, fertilizers, agrochemicals, biofertilizers and pheromones delivery, plant nutrients, anti-transpiration agents, plant growth regulators biostimulants, and genetic manipulation

2. Agri-products **(NAPs)** in the nano form of finished food formulations

- finished food, feed, dairy formulations, nano processing aids,
- nano composites for food packaging
- nano composites and sensors made from NMs
- and those that require direct contact with crops, food and feed for data acquisitions.

General Considerations: Definition and Categories of NMs

- **Nanomaterial (NM):** A material that ranges in size from 1 to 100 nm at least in one dimension

OR

- any materials that possess improved properties or phenomena because of the effect of dimension(s), even if these dimension(s) fall outside the nanoscale range, up to 1000 nm.

CATEGORIES

1. According to nanoform of the ingredient

- Nanocarriers loaded with AI
- AI converted to nanoform

2. According to the synthesis

- Biological
- Physical
- Chemical

3. According to chemical nature of NMs

- Organic
- Inorganic
- Composite

4. According to degradation nature of NMs

- Biodegradable
- Non-biodegradable

Physicochemical Characterization

Detailed information on Physicochemical properties

1. Information on the ingredients
2. Information on NMs used (AI/nanocarrier)
3. Used NMs based on the method of production and composition
4. NMs property characterization
5. Hydrodynamic particle size and distribution (poly-dispersion index)
6. Surface charge (using zeta potential)
7. Crystallinity (XRD)
8. Hydrophilicity/ lipophilicity using contact angle measurement
9. pH using pH meter
10. Static viscosity (in case of liquid formulation using conductivity meter)
11. Electrical conductivity (in case of liquid formulation using conductivity meter)
12. BET surface area and porosity
13. Organic (HPLC, GC, and mass spectroscopy data); inorganic (XRF and ICP-MS data)
14. FTIR spectroscopy
15. Any other information relevant to the specific product
16. Stability data (as per OECD 318 TG)
17. Impurities detail
18. Quality control checks parameters and test protocols
19. Sampling procedure and preparation for specific analysis

SAFETY ASSESSMENT: Human Health

1. 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**

2. Genotoxicity:

- OECD TG 473 - In vitro mammalian chromosomal aberration test
- OECD TG 476 - In vitro mammalian cell gene mutation tests using the hprt and xprt genes
- OECD TG 487 - In vitro mammalian cell micronucleus test

3. Dermal exposure/toxicity:

- IATA for skin corrosion and irritation (ENV/JM/MONO (2014) 19)
- OECD TG 428 - Skin absorption: In vitro method
- OECD TG 431 - In vitro skin corrosion: Reconstructed human epidermis (RHE) test method

4. Eye irritation

- OECD TG 437 - Bovine corneal opacity and permeability test method for evaluation of chemicals likely to cause serious eye damage

5. Inhalation Exposure

- OECD TG 433 – Acute inhalation toxicity for evaluation of health hazard from short-term exposure to a test chemical by inhalation.
- OECD 436 - Acute inhalation toxicity: acute toxic class method

SAFETY ASSESSMENT: **Environmental safety**

Environmental safety

OECD TG 201 - Aquatic test: fresh water algae, cyanobacteria and growth inhibition test

OECD TG 202 - *Daphnia sp. acute immobilization test*

OECD TG 203 - Fish acute toxicity test

OECD TG 211 - *Daphnia magna reproduction test*

OECD TG 236 - Fish embryo acute toxicity (FET) test on embryonic stages of fish (*Danlorerio*)

OECD TG 208 - Terrestrial plant test: seedling emergence and seedling growth test

U.S. Environmental Protection Agency (US EPA) - Six bioassays: *Allium* and *Vicia* root tip chromosome breaks, *Tradescantia* chromosome break, *Tradescantia* micronucleus, *Tradescantia* stamen-hair mutation, and *Arabidopsis* mutation bioassays - for detecting the genotoxicity of environmental agents (Ma et al., 2005)

Effects on soil microbiota and macrobiota:

9.2.8.1 OECD TG 222 – Earthworm reproduction test for assessing the effects of chemicals in soil on the reproductive output (and other sub-lethal end points) of the earthworm species *Eisenia fetida* or *Eisenia andrei*

9.2.8.2 OECD TG 216 - for the long-term effects after a single exposure of chemicals, on nitrogen transformation activity of soil microbiota

9.2.8.3 OECD 217 – to investigate long-term effects after a single exposure of agrochemicals/ non-agrochemicals on carbon transformation activity of soil microorganisms

9.2.8.4 OECD TG 471 - Bacterial Reverse Mutation Test. Additionally, Clinical and Laboratory Standards Institute, methods 02, 07, and 11 may be adopted for *in vitro* evaluation of anti-microbial susceptibility of NAPs and NAIPs.

9.2.8.5 Suitable standard guidelines to evaluate risk to macrobiota may also be developed and provided by regulators.

Suitable evaluation method may be included to assess effect of NAIPs on the alteration of biotic and abiotic soil properties. The outcome of toxicity tests may be used to establish thresholds or safe levels of chemicals in soil. Methods for estimation of the half-life of the NMs in soil may also be included to predict (potential) risk.

OECD TG 307 may be adopted with necessary adaptations for aerobic-anaerobic transformation of NAIPs or NAPs in soil.

OECD section 3; Test 318 - In order to assess the environmental risk of particular chemicals, information allowing the estimation of its likely concentrations in the environment is necessary.

Additional assessments for Nanopesticides

OECD TG 404 - Acute dermal irritation/corrosion in rabbit

OECD TG 429/406 - Skin sensitization: local lymph node assay

OECD TG 405 - Acute eye irritation/corrosion in rabbit

OECD TG 403 - Acute inhalation toxicity

OECD TG 443/416 - An extended one-generation reproductive toxicity

OECD TG 414 - Prenatal developmental toxicity study in rats and rabbits

OECD TG 451 - Carcinogenicity (two-year for rat and seventy-eight weeks for mice)

OECD TGs 424, 426 & 419 - Neurotoxicity study in rodents

OECD TG 407 - Repeated dose 28-day oral toxicity study in rodents (immunotoxicity)

OECD TG 417 - Toxicokinetics (in rat)

OECD TG 505 - Residues in livestock

OECD TG 503 - Metabolism in livestock

OECD TG 207 - Earthworm acute toxicity test

OECD TG 223 - Avian acute oral toxicity test

OECD TG 205 - Avian dietary toxicity test

OECD TG 206 - Avian reproduction toxicity test

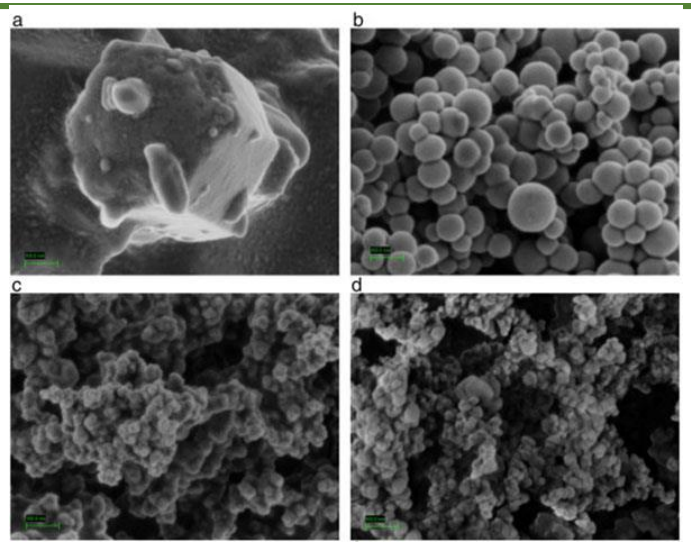
OECD TGs 213 & 214 - Acute oral and contact toxicity to honey bees

Stability assessment 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 storage stability tests as per OECD Test Guidelines (TG) 318, FAO/WHO and APVMA may be adopted:**

1. Accelerated storage stability
2. Ambient storage stability
3. Low temperature storage stability
4. Photostability
5. Container compatibility test or any other requirement of the packaging data requirement
6. Stability and transformation dynamics in soil system
7. Shelf-life
8. Batch (laboratory-, pilot- or production-scale) and size of products
9. Duration of storage stability
10. Validation of analytical methods
11. Technical characteristics (colour, pH, wettability, suspensibility, dispersion stability, dilution stability, particle size distribution, emulsifiability, re-emulsifiability, emulsion stability, viscosity, flow ability, crystalline state, release kinetics, and leakage).
12. Microbial stability (oxygenic, anoxygenic, and light)
13. pH stability and reactivity

NAIPs DEVELOPED BY TERI DEAKIN NANOBIOTECHNOLOGY CENTRE

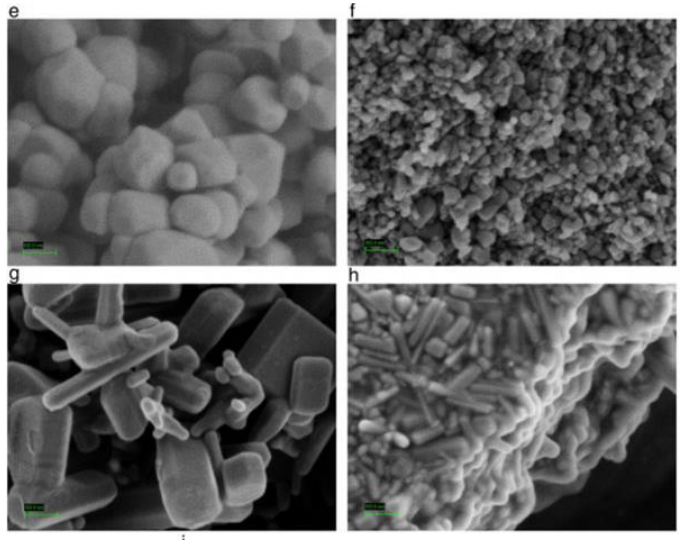


BIOLOGICALLY PRODUCED NANOPHOSPHORUS

It is a unique product developed to deliver phosphorous directly to the plant through foliar fertilization approach and/or seed priming. It is bioengineered to contain a high concentration of optimally balanced phosphorus in stable nanostructured form having biological capping of proteins and other biomolecules. Its application in crops plays an important role in photosynthesis, respiration, energy storage and transfer, cell division, cell enlargement and several other processes in plants.

BIOLOGICALLY PRODUCED ZINC IRON NANOCOMPOSITE

Zinc-iron nanocomposite (nanoZn-Fe) is an innovative product that contains biologically produced agriculturally important nanostructured Zn & Fe micronutrients. These nanostructured Zn & Fe micronutrients have been functionalized with biological macromolecules to enhance efficacy and consequently improve the use efficiency. The Zn & Fe nanofertilizer is able to provide value added solutions to maximize the natural potential of seed and crop in the field under biotic and abiotic stress conditions.

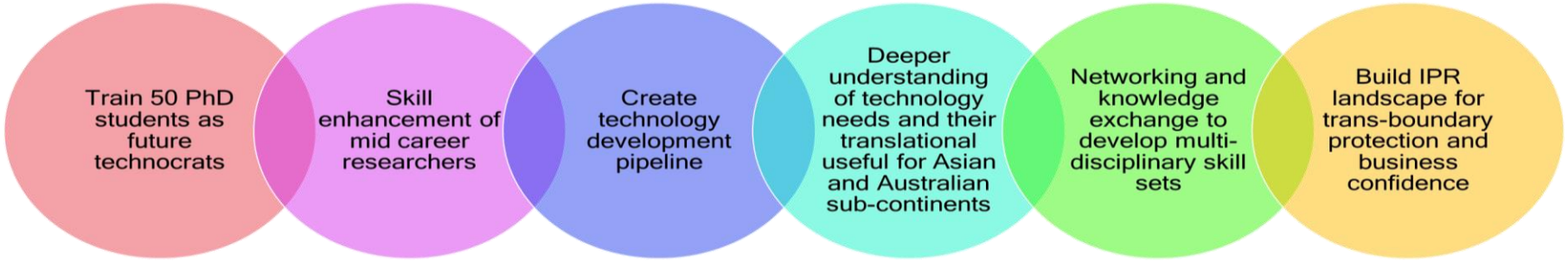


CHALLENGES & SCOPE

- The challenge that remains in front of policymakers is that of regulating a field where vast areas of knowledge are still being investigated and are unknown.
- In this situation, over-regulation may end up stifling further development while under-regulation could expose the public to adverse health effects.
- **Alignment of different regulatory bodies:**
  - Amendments in existing regulations
  - Identification of specific regulators is needed who should be dealing with specific guidelines for specific categories of NanoAgi products
  - **Identification of similar/counter-part International agencies for achieving harmonized and internationally acceptable guidelines for NanoAgriproducts**

DBT-TDNBC-Deakin Research Network Across continents for learning & innovation

Developing intercontinental research network in nanobiotechnology with hub in India & Australia



Mission of DTD-RNA

- **To reach** out to potential beneficiary research centre's across the globe
- **To organize** regular interaction with all stake holders (TDNBC, Deakin University, other Institutions across the globe and Indian Network partners) to discuss and define common and mutually beneficial Research platforms for attaining excellence by exchanges and knowledge sharing.
- **To convert** product prototypes to desired products for reaching out to common man together with Indian and Global industries.
- **To impart** specific knowledge and skill development/enhancement via short and long duration training modules.
- **To organize** winter and summer schools besides forays for attaining attenuation in ongoing research platforms or flagship projects by institutions across continents.
- **To organize** research modules and platforms to enhance skills and knowledge across institutions.

THANK YOU!