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

Department of Biotechnology
Ministry of Science & Technology

Ministry of Agriculture and
Farmers' Welfare

Government of India
2020

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Farmers' Welfare

Government of India
2020

Coordination:

Department of Biotechnology (DBT)

Dr. Suchita Ninawe, Adviser & Head, NanoBiotechnology
Dr. A. Vamsi Krishna, Scientist 'E' & Program Officer, NanoBiotechnology

Editors:

Dr. Alok Adholeya, TERI-Deakin Nanobiotechnology Centre, Gurugram
Dr. Amit Dinda, AIIMS, New Delhi
Prof. K. S. Subramanian, TNAU, Coimbatore
Dr. C. Anandharamakrishnan, IIFPT, Thanjavur
Dr. Suchita Ninawe, DBT, New Delhi

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सबका साथ, सबका विकास, सबका विश्वास
Sabka Saath, Sabka Vikas, Sabka Vishwas

सत्यमेव जयते

डॉ हर्ष वर्धन
Dr Harsh Vardhan

स्वास्थ्य एवं परिवार कल्याण, विज्ञान और प्रौद्योगिकी व पृथ्वी विज्ञान मंत्री, भारत सरकार

Union Minister for Health & Family Welfare, Science & Technology and Earth Sciences Government of India

MESSAGE

Agriculture holds the key to the overall development of a country's economy. Science & Technology are the drivers of change and Nanotechnology is an alternative solution to address sustainable agriculture concerns across the globe. India has been ranked number three in the nanotechnology field and has been making significant strides in developing nanofertilizers, nanopesticides, nanofood, nanocarriers and nanoformulations for agriculture and food. Nanobiotechnology has the potential to improve agricultural output through increase in plant productivity and better crop protection, thereby meeting not only the changing needs due to climate change but also the necessity of providing food to the growing population and thus impacting social development, economies and businesses in India.

2. I am pleased to know that, keeping in view the importance of nano-based products in agriculture and food, the Department of Biotechnology, Ministry of Science and Technology; Indian Council of Agriculture Research, Food Safety and Standards Authority of India and Central Insecticides Board and Registration Committee have successfully coordinated with all the relevant stakeholders to bring out this important document on "Guidelines for evaluation of nano-based agri-input and food products in India". These guidelines will facilitate translational research towards the development of nano-based novel products, processes and solutions for agriculture and food sector.

3. I am confident that these guidelines will provide the much needed impetus to develop Nano-based Agri Products for boosting the Agriculture Sector.

(Dr. Harsh Vardhan)

कार्यालय: 348, ए–स्कंध, निर्माण भवन, नई दिल्ली–110011 • **Office:** 348, A-Wing, Nirman Bhawan, New Delhi - 110011
Tele: (O) : +91-11-23061661, 23063513 • **Telefax:** 23062358 • **E-mail:** hfwminister@gov.in
निवास: 8, तीस जनवरी मार्ग, नई दिल्ली–110011 • **Residence:** 8, Tees January Marg, New Delhi - 110011
Tele: (R) : +91-11-23794649 • **Telefax:** 23794640

नरेन्द्र सिंह तोमर
NARENDRA SINGH TOMAR
D.O. No. 595 /AM

सत्यमेव जयते

कृषि एवं किसान कल्याण,
ग्रामीण विकास और पंचायती राज मंत्री
भारत सरकार
कृषि भवन, नई दिल्ली
MINISTER OF AGRICULTURE & FARMERS WELFARE,
RURAL DEVELOPMENT AND PANCHAYATI RAJ
GOVERNMENT OF INDIA
KRISHI BHAWAN, NEW DELHI

27th January, 2020

MESSAGE

Agriculture sector of the country employs more than 50 per cent of the total workforce in India and contributes around 17-18 percent to the country's GDP. We also focus on soil health, food and water safety, food and nutritional security and crop productivity with "doubling farmer's income". The backbone of any agricultural revolution is assess of modern and advanced agricultural inputs to farmers. The ability of nanotechnology to provide efficient and cost-effective solutions to various critical needs of people across economies is serving as the major driver for advanced research in the field of agriculture. On the other side, there has been reservations worldwide from the risk assessment and risk management point of view for nano agri-inputs, which are also required to be critically examined for safety to the human, animals and environment.

This important document on "Guidelines for evaluation of nano-based agri-input and food products in India" is the timely initiative by the GoI through Department of Biotechnology, Ministry of Science and Technology; involving all the stakeholders. These guidelines will prove as guiding document on agriculture innovation and R&D in the field of nano-based agri-inputs and food products.

I congratulate all those who have been involved and contributed to this important document

27/1/20

(Narendra Singh Tomar)

Office : Room No. 120, Krishi Bhawan, New Delhi-110 001 Tel.: 23383370, 23782691 Fax : 23384129
Resi. : 3, Krishna Menon Marg, New Delhi-110001, Ph. : 011-23794697 / 98, Fax : 011-23794696

सत्यमेव जयते

डॉ. रेणु स्वरूप
DR. RENU SWARUP

सचिव
भारत सरकार
विज्ञान और प्रौद्योगिकी मंत्रालय
जैव प्रौद्योगिकी विभाग
ब्लॉक-2, 7वां तल, सी० जी० ओ० काम्पलेक्स
लोधी रोड, नई दिल्ली-110003
SECRETARY
GOVERNMENT OF INDIA
MINISTRY OF SCIENCE & TECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY
Block-2, 7th Floor, C.G.O. Complex
Lodhi Road, New Delhi-110003

Foreword

Nanotechnology has gained significant recognition in terms of its applications to newer scientific disciplines. Department of Biotechnology, Ministry of Science & Technology has played an important role in supporting nanobiotechnology related flagship programs, Centres of Excellence, State of art laboratory facilities and also focussed in developing human resources and forging international collaborations. Nanobiotechnology has potential application in agriculture and food sectors, with indirect benefits to the environment. Apart from Department of Biotechnology, other Government Ministries/ Departments and agencies have been supporting various programmes/missions on Nanotechnology. However, at present, we do not have explicit guidelines that facilitate the regulatory process for the application and usage of these products in agriculture and food. These guidelines are aimed at providing science based information to help the regulators to evaluate quality, safety and efficacy of the targeted products.

I thank all the domain experts, inter-ministerial committee members, regulators, representatives from industry and industry associations, government organizations, researchers, and the public at large who have contributed to the development of the "Guidelines for evaluation of nano-based agri-input and food products in India". These guidelines will help to build the regulatory provisions for nano-based agriculture and food products in India and would pave the way for promoting innovations and commercialization of the novel nano-products for the benefit of farmers and society at large.

(Dr. Renu Swarup)

Tele : 24362950 / 24362881 Fax : 011-24360747 Email : secy.dbt@nic.in

SANJAY AGARWAL
SECRETARY

भारत सरकार
कृषि एवं किसान कल्याण मंत्रालय
कृषि, सहकारिता एवं किसान कल्याण विभाग
Government of India
Ministry of Agriculture & Farmers Welfare
Department of Agriculture, Cooperation
& Farmers Welfare

FOREWORD

Nano-biotechnology has the potential for categorical shift to the concerning issues of sustainable food and agriculture which is of utmost importance to the mankind. The era of green revolution, high yielding varieties and input intensive technologies is leading way to smart and precision agriculture for better input and delivery management ensuring safety and security of food systems of present and future generation. In this context, nanotechnology seems to be relevant.

In broad sense, these guidelines accommodate various sectors of agriculture inputs covering general multidisciplinary aspects for evaluation of nano-based agri-inputs. With relation to nano-based agri-inputs, the risk assessment and risk management aspects are most complicated and underexplored, which are required to be seriously looked into for safety to the human, animals and environment.

The guidelines will ensure the quality, safety and efficacy as well as encourage the commercialisation of nanotechnology based innovations, translational research and industries in the field of agri-nanotechnology and food technology with high benefit and low-risk ratio. Since the nanotechnology and nano-products are currently dealt by different ministries and departments, these guidelines will further lead to inter-departmental and inter-ministerial cooperation.

I congratulate Department of Biotechnology for taking the lead in developing this guidance document.

Sanjay

(Sanjay Agarwal)

February 11, 2020

Office : Krishi Bhawan, New Delhi-110001, दूरभाष / Phone : 23382651, 23388444 फैक्स सं० / Fax No. : 23386004
E-mail : secy-agri@gov.in

त्रिलोचन महापात्र, पीएच.डी.
एफ एन ए, एफ एन ए एस सी, एफ एन ए ए एस
सचिव एवं महानिदेशक

TRILOCHAN MOHAPATRA, Ph.D.
FNA, FNASc, FNAAS
SECRETARY & DIRECTOR GENERAL

भारत सरकार
कृषि अनुसंधान और शिक्षा विभाग एवं
भारतीय कृषि अनुसंधान परिषद
कृषि एवं किसान कल्याण मंत्रालय, कृषि भवन, नई दिल्ली 110 001

GOVERNMENT OF INDIA
DEPARTMENT OF AGRICULTURAL RESEARCH & EDUCATION
AND
INDIAN COUNCIL OF AGRICULTURAL RESEARCH
MINISTRY OF AGRICULTURE AND FARMERS WELFARE
KRISHI BHAVAN, NEW DELHI 110 001
Tel.: 23382629; 23386711 Fax: 91-11-23384773
E-mail: dg.icar@nic.in

Foreword

Agriculture is one of the major sectors which has been benefited through the interventions of nanotechnology applications. There is an increasing interest among researchers and end users to explore the applicative potentials of different nano-agri-input products. The application of nanotechnology in agriculture and allied sectors for crop production, protection, management, post-harvest and packaging is significant. These products can reduce nutrient losses during application and quantity of agro-chemicals to attain higher crop production. Controlled release of these products can increase the nutrient-use efficiency and also provide stress-tolerating ability to different crops. It is highly expected that the future nanotechnology strategies would improve performance in terms of reduced load of chemicals in agriculture at lesser treatment cost while increasing the yield potential of food crops. New considerations of application of nanotechnology in agriculture also necessitated the initiation of regulatory aspects of the nano-based products for agriculture and food. This document on ***"Guidelines for evaluation of nano-based agri-input and food products in India"*** is very important and desirable step. These guidelines will encourage designing of next generation nano-based agri-input and food products to benefit farmers and other stakeholders.

I am pleased that Indian Council of Agricultural Research has played an important role in formulation of these guidelines. I equally appreciate the efforts of all Agencies involved in developing these guidelines. I thank all the academic experts, industry representatives, regulators, government institutes for their valuable comments and suggestions to develop these guidelines.

(Trilochan Mohapatra)

Dated the 15th January, 2020
New Delhi

अध्यक्ष
Chairperson

Telefax : 011-23220991
Email : chairperson@fssai.gov.in

भारत सरकार
भारतीय खाद्य संरक्षा एवं मानक प्राधिकरण
स्वास्थ्य एवं परिवार कल्याण मंत्रालय
एफडीए भवन, कोटला रोड़, नई दिल्ली-110002

Government of India
Food Safety and Standards Authority of India
Ministry of Health and Family Welfare
FDA Bhawan, Kotla Road, New Delhi-110002
Website : www.fssai.gov.in

FOREWORD

Food nanotechnology has the potential to meet the needs of high nutrition levels, efficient nutritional delivery, as well as achieving longer shelf life and durability of food-products through effective food packaging materials. Smart and efficient delivery of active food components, nano encapsulation of nutraceuticals are gaining recognition in food and agriculture nanotechnology. It is being recognized that there needs to be a better understanding of nanoparticle based products for their technological development and acceptance in the commercial world and the society. The present document on "Guidelines for evaluation of nano-based agri-input and food products in India" is a very encouraging step, for reporting and testing of nano food and nano feed products before commercialization.

These guidelines will help researchers in the development of products for agriculture and human consumption that ensure both safety and quality. The guidelines will also support the regulators in assessing the quality and safety of nano-based agriculture and food products.

I appreciate the leadership role of Department of Biotechnology in developing these guidelines along with the Food Safety and Standards Authority of India (FSSAI) and other concerned agencies. I thank all the stakeholders who have contributed to this document.

(Rita Teaotia)

Dated the 15th January, 2020

INDEX

PREFACE

The nanotechnology intervention has opened a new horizon for agriculture productivity, pest/disease prevention, control and management, fertilizers, agrochemicals, biofertilizer and pheromones delivery, plant nutrients, anti-transpiration agents, plant growth regulators biostimulants, genetic manipulation of crop using nanomaterial as a carrier system for crop benefits, nanocarriers for nutraceuticals delivery, nano processing aids, nanocomposites for food packaging and nanosensors for food/feed packaging, food/feed safety applications and for dairy products safety applications. Every year several new nano-based agri-input and food products are being introduced into the market globally.

Nanomaterials display unique properties due to their large surface area to volume ratio. The innovative nano-intervention in agriculture and food sector could generate low-cost, high-efficacy solutions in terms of products and processes. The present guidelines are compiled with an aim of evaluation of agri-input and food preparations containing nanomaterial(s) imparting the significant advantages over the existing active agri-input and food ingredients in terms of altered beneficial properties, dimensions or phenomenon associated with the application of nanotechnology that is intended to be used in agriculture, food and allied sectors for crop production, protection, management, harvesting, post-harvesting, food/feed and packaging.

The present guidelines apply to nano-agri-input products (NAIPs) and nano-agri products (NAPs). These guidelines also apply to nano composites and sensors made from NMs and those that require direct contact with crops, food and feed for data acquisitions. These guidelines do not apply to the conventional products or formulations with incidental presence of natural NMs.

In India, there are different Government Agencies and different provisions that regulate different agri-input and food products; however, no specific provisions are available to deal with nano based agri-input and food products. The present guidelines are developed to support the existing national regulatory provisions of CIB&RC, FCO, FSSAI and BIS, with specific requirements and adaptations for NAIPs and NAPs, wherever considered necessary. 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.

These guidelines would help policy makers and regulators to frame effective provisions for future novel nano-based products in the agri-input and food sectors of India. These guidelines will encourage the Indian innovators and industries to develop and commercialize new nano-based agri-input and food products.

With rapid advances in basic sciences, our understanding about nanomaterials is continuously updated. The novel multifunctional nanomaterials may need additional new tests for quality, safety and efficacy assessment in future. So this document may need modification with new edition from time to time. We sincerely hope that these guidelines will empower the Indian agriculture and food industry to achieve a greater social and economic impact through application of Nanobiotechnology – the cutting edge technology.

India is forging ahead at the global level by developing such comprehensive and inclusive guidelines with the intent to support appropriate regulation landscape in India.

New Delhi, March 2020 Editors

ACKNOWLEDGEMENT

Department of Biotechnology (DBT) and other government agencies have funded many projects to promote fundamental and applied research in the area of Nanobiotechnology in last couple of years. The success of the initial phase of DBT's Nanobiotechnology program laid a strong foundation to accelerate and advance interventions of this cutting edge technology in different sectors of Biosciences. Some of the funded projects were translated into patentable technologies. Now, the emphasis is to create technologies that have high commercial and societal impact. Keeping this in view, DBT has always emphasized on formulation of required set of procedures for evaluation of nanoproducts for the use by the funding agencies, regulators, researchers, industry and other stakeholders.

The development of "Guidelines for Evaluation of nano-based agri-input and food products in India" was quite challenging and exciting as different ministries and departments deal with nano-agri-input and nano-food products and there are such limited documents in public domain. In this endeavour, TERI-Deakin Nanobiotechnology Centre (TDNBC) with support of DBT had prepared the concept document of such guidelines. This document was circulated during 'International Conference on Nanobiotechnology for Agriculture: Research to Innovation 2017'. Since then, as directed by DBT, TDNBC through a series of meetings and brain storming sessions involving the industries, academia and policy makers; and along with the Working Group constituted by DBT, framed the 'Zero Draft' of the guidelines. We sincerely acknowledge the efforts put in by TDNBC towards framing the initial draft. Special thanks are due to Dr. Alok Adholeya, Director, TDNBC. We also acknowledge timely interventions of the Working Group members towards modifications in the draft guidelines.

Recognizing the importance of such guidelines to promote commercialization with necessary safety aspects of new nano-innovations in the field of agriculture and food sector, DBT conducted extensive inter-ministerial consultations with active participation of domain experts, representatives from Govt. agencies (DST, CSIR, MoA&FW, ICAR, MoC&F, CIB&RC, MoFPI, MoAHD&F, FSSAI, MoEFCC, MoES), industry, Industry Associations and other stakeholders. We profusely thank contributions by each and every participant. The help of TDNBC Team in preparing Action Taken Report on the Public Opinion is highly appreciated. After necessary modifications in the document through initial inter-ministerial meeting, public consultation process was undertaken by DBT. More than 150 comments were received and considered. We are grateful for the constructive criticism and suggestions by each respondent which helped us to modify the document further.

Incorporation of suggestions and comments through following consultations and meeting of Inter-ministerial Expert Committee chaired by Dr. Renu Swarup, Secretary, DBT and Co-chaired by Dr. Alok Adholeya, Director, TDNBC and Dr. B Sesikeran, Former Director, National Institute of Nutrition in the said document resulted in the final version. We are extremely grateful to Chair, Co-chairs and Members of the Inter-ministerial Expert Committee who contributed profusely.

Special thanks to the Editors for their persevering and fervent efforts that led the Zero Draft to Final Version of the Guidelines. Sincere efforts of TDNBC Researchers Team – Dr. Reena Singh, Dr. Braj Raj Singh, Dr. Mukul Kumar Dubey, Dr. Pushplata Singh, Dr. Ratul Kumar Das and Dr. Sapna Johnson are highly appreciable. The efforts of Dr. A. Vamsi Krishna, Scientist 'E' and Dr. Balendra Singh, Scientist 'C', Nanobiotechnology, DBT in the final stages of preparation of this document are appreciated. The hard work of Ms. Reema Saxena and other supporting staff in DBT is acknowledged.

New Delhi, March 2020

Suchita Ninawe

ABBREVIATIONS

ADME	Adsorption, Distribution, Metabolism and Excretion
AI	Active Ingredient
AOPs	Adverse Outcome Pathways
APVMA	Australian Pesticides and Veterinary Medicines Authority
BET	Brunauer-Emmett-Teller
BIS	Bureau of Indian Standards
BMDL	Benchmark Dose Level
BrDU	Bromodeoxyuridine / 5-Bromo-2'-Deoxyuridine
CIB&RC	Central Insecticide Board and Registration Committee
DPPQS	Directorate of Plant Protection, Quarantine and Storage
EFSA	European Food Safety Authority
EPA	Environmental Protection Agency
EPAA	European Partnership for Alternative Approaches to animal testing
EU	European Commission
FAO	Food and Agricultural Organization
FCO	Fertilizer (Control) Order
FDA	Food and Drug Administration
FE-SEM	Field Emission Scanning Electron Microscopy
FSSAI	Food Safety and Standards Authority of India
FSANZ	Food Standards Australia New Zealand
FTIR	Fourier-Transform InfraRed Spectroscopy
GC	Gas Chromatography
HPLC	High Performance Liquid Chromatography
LDH	Lactate Dehydrogenase
IATA	Integrated Approaches for Testing and Assessment

ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
MTT	[3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyl Tetrazolium Bromide]
NAIP	Nano-Agri-Input Product
NAP	Nano-Agri Product
NMs	Nanomaterials
NOAEL	No-Observed-Adverse-Effect-Level
OECD	Organization for Economic Co-operation and Development
QSAR	Quantitative Structure Activity Relationship
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
SEM	Scanning Electron Microscopy
TEM	Transmission Electron Microscopy
TG	Test Guidelines
TSCA	Toxic Substances Control Act
US FDA	United States Food and Drug Administration
US EPA	United States 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

1. INTRODUCTION

Nanomaterials (NMs) display unique properties due to their large surface area to volume ratio. They thus support in development of novel products and processes. Existing products are also improved from such interventions. Nanotechnology has recently been employed to develop agriculture input products such as fertilizers and pesticides for improvement of crop yields. Agriculture and food products have also been improved with desirable characteristics. The innovative nano-interventions 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 nano-based products (such as any new product) are needed to be evaluated for adverse effects, if any, in humans and for environment. The activity, efficacy and impact of NMs depend upon interaction of their physico-chemical parameters with diverse environmental factors; hence require a multidisciplinary approach for development of new alternative strategies and methods for their evaluation.

To supplement existing policies and regulations related to agriculture and food, developing certain new guidelines for evaluation of novel nano-based products on the basis of current scientific understanding is a need of the hour. 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. 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 (Annexure 1).

As on date, there are no unanimously acceptable international guidelines for nano-based agri-input and food products. A few provisions are in place globally for NMs with certain specific guidelines for quality, safety and efficacy. However, continued innovation with alteration of functionality of NMs makes it difficult to apply a universal set of evaluation parameters for emerging nanoproducts. Many a time, the case-by-case basis evaluation approach is needed for nano-based agri-input or food products.

Nanobiotechnology research initiative by the Government of India through Department of Biotechnology (DBT) complementing to the NanoMission of the Department of Science & Technology (DST), along with other such programs of the Government undertaken by other concerned agencies has laid a strong foundation to accelerate and to advance cutting edge research at the frontier of nano-biotechnology for agriculture and food. However, it has been recognized that there are no specific provisions and guidelines in place for evaluating nano-based agri-input and food products in India. Such provisions are required not only to accelerate the commercialization but also to ensure quality, safety, efficacy and performance attributes of the products. The current guidelines developed by DBT, in consultation with the concerned Government Agencies and the relevant stakeholders provide a brief overview of existing global legislative

and regulatory provisions for nano-based agri-input and food products. The existing regulatory provisions of India may further add desired provisions based on these guidelines. These guidelines are aimed to help researchers, manufacturers, importers and other stakeholders involved in research and development of nano-based agri-input and food products and to encourage commercialization of these products. These guidelines also provide suggestions to ensure human, animal and environmental safety considerations for these upcoming novel products.

2. SCOPE OF THE GUIDELINES

These guidelines apply to the following two categories of products:

i) Agri-input products in the nano form of finished formulation as well as active ingredient(s) (AI) 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, agrochemicals, biofertilizers and pheromones delivery, plant nutrients, anti-transpiration agents, plant growth regulators biostimulants, and genetic manipulation of crop using NM as a carrier system for crop benefits. These products have been termed as nano-agri-input products (NAIPs) in the guidelines (refer 4.1.2 section for definition).

ii) Agri-products in the nano form of finished food formulations, finished feed formulations, finished dairy formulations, food/feed formulations from marine resources, nano carriers for nutraceuticals delivery, nano processing aids, nano composites for food packaging and nano sensors for food/feed packaging, food/feed safety applications and for dairy products safety applications. These products have been termed as nano-agri products (NAPs) in the guidelines (refer 4.1.3 section for definition).

These guidelines also apply to nano composites and sensors made from NMs and those that require direct contact with crops, food and feed for data acquisitions.

These guidelines do not apply to the conventional products or formulations with incidental presence of natural NMs.

3. GENERAL CONSIDERATIONS OF THE GUIDELINES

In India, there are different Government Agencies and different provisions that regulate different agri-input and food products; however, no specific provisions are available to deal with such products.

The global status for regulation of nanoproducts in agri-food systems is given in Annexure 2. The European Union (EU) along with Switzerland has particular provisions in the legislation to deal with nanoproducts. In some countries, the existing legislative and regulatory frameworks (with necessary adaptations for NMs) deal with nanoproducts.

The present guidelines are aligned with the national regulatory provisions of Central Insecticide Board and Registration Committee (CIB&RC), The Fertilizer (Control) Order (FCO), Food Safety and Standards Authority of India (FSSAI) and Bureau of Indian Standards (BIS). These guidelines are also harmonized with the provisions of international guidelines of Registration, Evaluation, Authorization and Restriction of Chemicals (REACH), Organization for Economic Cooperation and Development (OECD), U. S. Environmental Protection Agency (US EPA), Toxic Substances Control Act of 1976 (TSCA), Australian Pesticides and Veterinary Medicines Authority (APVMA), Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO), U.S. Food and Drug Administration (US FDA), European Food Safety Authority (EFSA), Food Standards Australia New Zealand (FSANZ) and Codex. Their applicable provisions for NAIPs and NAPs should be referred. Since different NAIPs and NAPs are considered in the guidelines, their evaluation should be conducted as per their type and regulatory framework. In case, any specific study is not included in the suggested regulatory framework, the principles of The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) may be followed. Each application should be considered on its own merit of the data submitted using scientific evaluation and valid justification.

These guidelines have been developed to support provisions of the CIB&RC, FCO, FSSAI and BIS, with specific requirements and adaptations for NAIPs and NAPs wherever considered necessary. These existing provisions specify the general requirements and guidelines to manufacture or import of insecticides (the Insecticides Act, 1968 (Act 46 of 1968)) (CIB&RC), fertilizers (FCO, 1985), food additives and preservatives (FSSAI) or to undertake quality checks and necessary certification (BIS).

3.1 NAIPs are proposed to be regulated as follows:

3.1.1 Nanofertilizers (with or without carriers [nano or non-nano]): Safety, efficacy, functionality and other quality data for proposed nanofertilizers should be conducted under FCO, 1985 with additional criteria for inclusion of nanofertilizers. FCO is issued under the Essential Commodities Act, 1955, which lays down registration requirement for fertilizers.

3.1.2 Nanopesticides (with or without carriers [nano or non-nano]): Studies on chemistry, bio-efficacy and residues, toxicity, packaging and processing of nanopesticide products for registration (for manufacture or import) should be conducted as per the regulatory aspect provisions under Section 9 specified in the Insecticides Act, 1968 (Act 46 of 1968) with additional criteria for inclusion of nanopesticides as per the requirement of CIB&RC of Directorate of Plant Protection, Quarantine & Storage (DPPQS).

FCO and CIB&RC, DPPQS are administered under the Department of Agriculture Cooperation, Ministry of Agriculture and Farmers Welfare, Government of India.

3.2 NAPs are proposed to be regulated as follows:

3.2.1 Nanofood: The FDA guidelines (FDA, 2014a), (FDA, 2014b), (FDA, 2015) and Food Safety and Standards Act, 2006 with additional criteria for inclusion of nanofood may be adopted by FSSAI.

3.2.2 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 may be adopted by FSSAI.

FSSAI is an autonomous body of the Ministry of Health & Family Welfare, Government of India.

Implementation of standards should be conducted as per BIS with additional criteria for inclusion of NAIPs and NAPs. BIS is a national Standards Body working under the aegis of Ministry of Consumer Affairs, Food & Public Distribution, Government of India engaged in the preparation and implementation of standards, operation of certification schemes both for products and systems, organisation and management of testing laboratories, creating consumer awareness and maintaining close liaison with international standards.

4. DEFINITIONS AND CATEGORIZATION

4.1 Definition of NMs, NAIPs and NAPs

4.1.1 Nanomaterial (NM) is defined as 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. The variations in definition of NMs with respect to size in different countries and respective regulatory bodies are presented in Annexure 3.

4.1.2 Nano-agri-input product (NAIP) is defined as an agricultural input preparation containing NMs (as defined in section 4.1.1) intended for applications (through soil, seed, foliar and drip in crops as well as by other means) on crop for the purpose of farming. NAIPs consist of materials with any of the three dimensions i.e. zero, one or two, on the nanoscale or with an internal or surface structure in the nanoscale.

4.1.3 Nano-agri product (NAP) is defined as an agricultural preparation containing NMs (as defined in section 4.1.1) intended for consumption or application in food/feed and their supplements as well as nutraceutical delivery. NAPs consist of materials with any of the three dimensions i.e. zero, one or two, on the nanoscale or with an internal or surface structure in the nanoscale.

4.2 Categorization of NAIPs and NAPs

NAIPs and NAPs can be categorized depending on the properties and functionalities of NMs and the existing products containing synthesized and engineered NMs, as follows.

4.2.1 According to nanoform of the ingredient -

4.2.1.1 Nanocarriers loaded with AI: A nanocarrier is a soft and hard NM used as a carrier system for targeted agri-input NMs. These also have the advantage of controlled and slow released delivery of agri-inputs.

4.2.1.2 AI converted to nano form: Active molecules/compounds could be converted into nano forms, thereby increasing their potential for improved stability and efficacy.

4.2.2 According to the synthesis -

4.2.2.1 Biologically synthesized NMs: NMs that are synthesized using bio-agents and their bio-actives.

4.2.2.2 Chemically synthesized NMs: NMs that are synthesized using synthetic chemicals as reducing, oxidizing agent and template.

4.2.2.3 Physically synthesized NMs: NMs 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.

4.2.3 According to chemical nature of NMs -

4.2.3.1 Organic: These are the NMs composed of organic compounds such as lipids, proteins and carbohydrates. They are used in agriculture primarily due to their low toxicity.

4.2.3.2 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.

4.2.3.3 Composite NMs: These are the materials that contain a mixture of several different categories of materials.

4.2.4 According to degradation nature of NM –

4.2.4.1 Biodegradable: They are used frequently as nanocarrier systems and other agri-inputs due to their unique and useful properties.

4.2.4.2 Non-biodegradable: They are used in NAIPs and NAPs more commonly in controlled and slow released fertilizers.

5. 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(s) in comparison to conventional products. The NMs and their transformed waste disposal 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:

i) The claim should be made on the basis of parameters that must include efficacy, safety, application modes and frequency, improved crop yield and productivity or any other benefit over conventional products.

ii) Addressing any issue arising out of a significantly different mode of action and assimilation than that of the conventional products.

iii) Addressing the issue of specific effect/property associated with the conventional products, if any, such as soil and plant toxic effects.

6. SPECIFIC CONSIDERATIONS FOR VALUATION OF NAIPS AND NAPS IN THE CONTEXT OF CIB&RC, FCO, FSSAI AND BIS

These guidelines provide assistance on specific requirements for NAIPs and NAPs. General requirements as specified in the provisions of CIB&RC, FCO, FSSAI and BIS will be applicable for any new product whether nanotechnology based or not. However, a 'case-by-case basis' approach should be adopted for evaluation of NAIPs and NAPs with respect to enhanced efficacy and safety because of the involvement of interdisciplinary sciences and their complex nature.

According to the current status of approval of AI of NAIPs or NAPs and carrier NMs as follows, requirement for further evaluation and approval of NAIPs or NAPs shall be decided on the following criteria:

(i) The AI is not yet registered as per the existing specific provisions and the nanocarrier is also new and not approved in the country.

i) The AI is not yet registered as per the existing specific provisions, but the nanocarrier is already approved for other NAIPs or NAPs.

ii) Conventional/traditional form of the NAIPs or NAPs formulation approved for use in the country as per the existing specific provisions but the nanocarrier system is new and not approved for use in the country.

iii) Conventional/ traditional form of the NAIPs or NAPs and the nanocarrier system, both are approved under the existing specific provisions for use in the country.

Considering the unique process conditions of nanoformulations compared to the conventional agri-input products and agriproducts, the process controls to be included. The method of NMs waste disposal and environmental impact may be declared.

NMs 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 US 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, reducing derivatives, catalysis, design for degradation, real-time analysis for pollution prevention and inherently safer chemistry for accident prevention. Exposure via agricultural runoff or agricultural wastewater needs to be evaluated by using US EPA tools. Also, if NAIPs and NAPs products show any effect after interaction with environmental or biological systems, then it should be described. Life- cycle analysis of NAIPs and NAPs is to be carried out.

The requirement as per the provisions under the CIB&RC, FCO, FSSAI and BIS may vary with need based data requirements than what has been mentioned in the guidelines for evaluation for nano-based agri-input products.

7. EXCIPIENTS USED IN NAIPS AND NAPS

Excipients help in the manufacturing 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 NM. The information regarding the excipient used in NAIP and NAPs is to be provided.

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

8.1 Accelerated storage stability

8.2 Ambient storage stability

8.3 Low temperature storage stability

8.4 Photostability

8.5 Container compatibility test or any other requirement of the packaging data requirement

8.6 Stability and transformation dynamics in soil system

The test parameters for stability testing of NAIPs and NAPs may also be 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:

8.7 Selection of containers and its testing for transport worthiness

8.8 Shelf-life

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

8.10 Duration of storage stability

8.11 Validation of analytical methods

8.12 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).

8.13 Microbial stability (oxygenic, anoxygenic, and light)

8.14 pH stability and reactivity

9. SAFETY ASSESSMENT OF MANUFACTURED NAIPS AND NAPS

The mammalian toxicity (health effects) and ecotoxicology test guidelines for conventional chemicals may also apply to NMs with necessary adaptations (OECD, 2009a; European Chemicals Agency (ECHA), 2014; other necessary provisions). Depending upon the product type, application and exposure to humans and environment, the suitable in vitro and in vivo methods for hazard assessment and effective regulation of NAIPs and NAPs should be adopted from the listed items. Data from these in vitro and in vivo methods, as well as in silico models, can be combined with existing information and physico-chemical properties of the test material, exposure conditions (e.g. route, dose, etc.), and mechanism of action, to design Integrated Approaches for Testing and Assessment (IATA) and IATA's Adverse Outcome Pathways (AOPs) (ENV/JM/MONO(2016)67 and 12).

Further, consideration should be given to reduce the number of animals once ample data is available in public domain on animal studies on nanofertilizers and nanopesticides for assessment of safety of NAIPs and NAPs. It is proposed that appropriate test guidelines as recommended by OECD or national regulatory authorities or any other international authority be followed to generate sound scientific data on the product. Before considering any new animal testing, all available information on the test NM including the identity, chemical structure, and physico-chemical properties of the test NM; results of any in vitro or in vivo toxicity tests; anticipated use(s) and potential for human exposure; available QSAR data and toxicological data on structurally related substances, should be considered.

Most of the in vitro assays suggested here are based on existing OECD TGs for testing manufactured NMs (ENV/JM/MONO (2018) 4). In addition, the U.S. National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and guidelines from several other international and national agencies may also be used for testing (NICEATM; US EPA under good laboratory practices (GLP) conditions).

9.1 Human health safety (ENV/JM/MONO (2018) 4)

9.1.1 Dermal exposure/toxicity:

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

9.1.2 Eye irritation:

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

9.1.3 Inhalation exposure:

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

9.1.4 Genotoxicity:

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

9.1.5 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

In addition, Test No. 489 - In vitro mammalian alkaline comet assay for measuring DNA strand breaks in eukaryotic cells may also be included in the test panel.

9.2 Environmental safety

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

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

9.2.3 OECD TG 203 - Fish acute toxicity test

9.2.4 OECD TG 211 - *Daphnia magna reproduction test*

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

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

9.2.7 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)

9.2.8 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.

9.2.9 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.

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

9.2.11 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.

Such an estimate should initially be based on knowledge of the likely use and disposal patterns of the chemical, its physical-chemical properties and the characteristics of the receiving environment. OECD guidelines for the testing of chemicals, Section 3: Test No. 318 may be adopted with modifications for NAIPs and NAPs.

9.3 Additional safety assessment of nanopesticides

In addition to the above mentioned standard tests, safety assessment of nanopesticides should also involve the following OECD TG driven tests:

9.3.1 OECD TG 404 - Acute dermal irritation/corrosion in rabbit

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

9.3.3 OECD TG 405 - Acute eye irritation/corrosion in rabbit

9.3.4 OECD TG 403 - Acute inhalation toxicity

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

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

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

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

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

9.3.10 OECD TG 417 - Toxicokinetics (in rat)

9.3.11 OECD TG 505 - Residues in livestock

9.3.12 OECD TG 503 - Metabolism in livestock

9.3.13 OECD TG 207 - Earthworm acute toxicity test

9.3.14 OECD TG 223 - Avian acute oral toxicity test

9.3.15 OECD TG 205 - Avian dietary toxicity test

9.3.16 OECD TG 206 - Avian reproduction toxicity test

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

9.4 Occupational health safety and waste disposal

Exposure control strategies, waste disposal and best practices should be followed while handling NMs in accordance with 'Guidelines and Best Practices for Safe Handling of NMs in Research Laboratories and Industries', developed by NanoMission, DST, Government of India.

The toxicity evaluations should be done following the norms of Good Laboratory Practices (GLP). These should be conducted only by trained and qualified staff using calibrated and standardized equipment. Written protocols should be absolutely followed. Standard-operating procedures (SOPs) should be followed for all laboratory and management work for toxicity evaluation. New perspectives and advances in the use and development of in vitro and in silico (AOP) methods for predicting the in vivo toxicity induced by NMs may be considered in future revisions of the present guideline, when more scientific evidence become available.

10. RESIDUE ANALYSIS

Information on the persistence of NAIPs and NAPs should be provided in the registration dossier and residues analysis of used nano AI, additives, nanocarrier materials of NAIPs and NAPs needs to be performed during the life-cycle analysis of the product. Data on NM 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:

10.1 Method for determination (detection and quantitation limits) of residues from the used AI, additive, and nanocarrier.

10.2 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 NM form (in which case standard risk assessment should apply).

10.3 Bioaccumulation behavior of NAIPs and NAPs should be performed according to the test guideline given below or any other.

10.3.1 OECD TG 201 - Aquatic test: freshwater algae, cyanobacteria and growth inhibition test

10.3.2 OECD TG 202 - Daphnia sp. acute immobilization test

10.3.3 OECD TG 211 - Daphnia magna reproduction test

10.3.4 OECD TG 305 - Bioaccumulation in Fish: Aqueous and Dietary Exposure

10.3.5 OECD TG 317 - Soil and sediment test

10.3.6 Bioaccumulation in Terrestrial Oligochaetes

10.3.7 OECD TG 222 - Earthworm reproduction test

11. INFORMATION REQUIRED FOR EVALUATION OF NAIPs

Nanopesticides are required to be registered under the existing Insecticides Act, 1968 and Rules 1972, and major nanofertilizers and nano-micronutrients are required to be registered under the existing FCO, 1985, through the nodal agency of Government of India and Agriculture Department of State Governments. These regulatory frameworks have already defined dataset requirements for registration of fertilizers and pesticides. Additional data sets have been proposed to be included for registration of NAIPs, as follows.

11.1 Overview

11.1.1 A brief description of NAIPs

11.1.2 Formulation detail (liquid, powder, pellet, tablet, gel, aerosol, or any other)

11.1.3 Intended use

11.1.4 Methods of application (soil, foliar, seed, drip, drenching, fertigation and drones)

11.1.5 Category

- 11.1.5.1 Nanofertilizers (major, secondary and micro nutrient)
- 11.1.5.2 Nanopesticides (insecticide, fungicide, herbicides, nematicides, acaricide, rodenticide, plant growth regulator)

11.1.6 Are there relevant source particles of NM analogues available of the similar chemical and physical structure?

11.1.7 Preliminary interaction analysis data of nanoproducts (AI or nanocarrier, excipient, preservatives, stabilizer, antioxidant or any other additives)

11.1.8 Justification for developing NAIPs (claims)

11.1.9 Draft of label: Every NAIP that has NMs in it should have labels indicating the presence of NMs in it and its characterization based information.

11.2 Detailed information

11.2.1 Information on the ingredients

- 11.2.1.1 Information on NMs used (AI/nanocarrier)
- 11.2.1.2 Used NMs based on the method of production and composition
- 11.2.1.3 NMs property characterization
- 11.2.1.4 Hydrodynamic particle size and distribution (poly-dispersion index)
- 11.2.1.5 Surface charge (using zeta potential)
- 11.2.1.6 Crystallinity (XRD)

11.2.1.7 Shape, size and average particle size should be determined by measuring a minimum of 100 NMs from a single grid (TERM) aspect ratio (only for 1D and 2D NMs using TEM, SEM and FE-SEM)

11.2.1.8 Hydrophilicity/ lipophilicity using contact angle measurement

11.2.1.9 pH using pH meter

11.2.1.10 Static viscosity (in case of liquid formulation using conductivity meter)

11.2.1.11 Electrical conductivity (in case of liquid formulation using conductivity meter)

11.2.1.12 BET surface area and porosity

11.2.1.13 Organic (HPLC, GC, and mass spectroscopy data); inorganic (XRF and ICP-MS data)

11.2.1.14 FTIR spectroscopy

11.2.1.15 Any other information relevant to the specific product

11.2.2 Stability data (as per OECD 318 TG)

11.2.3 Impurities detail

11.2.4 Quality control checks parameters and test protocols

11.2.5 Sampling procedure and preparation for specific analysis

11.2.6 Testing protocol/s

11.2.7 Identification and quantification of nanocarriers and AIs

11.2.8 Certificate of analysis: preliminary analysis data of nano products (AI or nanocarrier), certified reference material for identifying and quantifying of nanocarriers and AIs for nanopesticides. Minimum five-batch analysis is required to be performed by the manufactures to confirm the consistency and submit to the regulators (confirmatory analysis to be performed by regulators).

11.2.9 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, reactive oxygen species (ROS) generation colony-forming efficiency

11.2.10 Genotoxicity: OECD TG 471, 473, 476, 482 and 487

11.2.11 Comparative efficacy data of the conventional vs NAIPs and other data viz. residue, environment fate, etc. and as per the guidelines of the concerned regulators to be submitted.

For nanopesticides, bio-efficacy data requirements are i) Bio-effectiveness, ii) Phytotoxicity, iii) Residue – Plant, Soil and Water, iv) Persistence - Plant, Soil and Water, v) Effect on natural enemies, vi) Effect on succeeding crops (in case of herbicide), vii) Effect on soil micro-organisms, viii) Effect on honey bees and ix) Effect on earthworms.

11.2.12 Occupational hazard, exposure and fate assessment

11.2.13 Packaging compatibility data

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

12. INFORMATION REQUIRED FOR EVALUATION OF NAPS

The nanofood and nanofeed products are required to be registered as per the Food Safety and Standards regulations (FSSAI, 2016). The information requirement for evaluation of NAIPs as mentioned in Section 11 is applicable for NAPs also. In addition, the following information is required for registration of NAPs.

12.1 Exposure Risk

Migration of NMs or its degraded products in non-nanoform (its type and quantity) from agriproduce 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 samplings, variability in composite sampling and concentration variations between samples are critical sampling issues in exposure evaluation. Guidance on risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain (EFSA, 2018) may also be followed.

12.2 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 as per the exposure.

12.2.1 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. In case of unstable intermediates and impurities degradation pathways, levels of natural or unavoidable defects in foods that present no health hazards for humans may be suggested in detail according to US FDA guideline (FDA Food Defect Levels Handbook, 2018). Defect levels and subsequent action should be addressed to meet quality with specification limits/criteria mentioned with characteristics of each and every intermediate.

12.2.2 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 may not be carried out in stringent manner and data for non-nano form can be considered. However, 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, relevant regulations need to be considered.

12.2.3 Stable nano materials: For the NMs that are stable in food formulations/ agriproducts and in gastrointestinal tract, two scenarios are considered:

12.2.3.1 When characteristics and toxicity of non-nano form of NMs used are known through toxicity testing and ADME (repeated-dose 90-day oral toxicity): If the genotoxicity studies of two forms can identify the major difference then more stringent toxicity testing and ADME testing should be considered for toxic form. In case of less hazard NMs, further stringent testing may not be required depending upon strong scientific evidence.

12.2.3.2 When hazard characteristic of its non-nano form is not available through toxicity testing and ADME studies, then hazard characterization and regulations are required for NMs.

12.2.4 Migration of food contact materials: When there is no migration from food contact materials, toxicological concerns are negligible. Stringent toxicity studies need to be enforced if there is migration.

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

12.2.5 *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.

12.2.5.1 *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 to understand the digestibility and release behaviour of ingested food components and thus fate of added NMs. (Partha sarathi et al., 2018, Thuenemann et al., 2015, Kong & Singh, 2008, Kong and Singh, 2010, Vande Wiele et al., 2015, Wang et al., 2019).

12.2.5.2 *In vitro* genotoxicity testing: 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.

12.2.5.3 Other i*n vitro* studies:

i) 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 vitro* studies to access microbial membrane disorganization and generation of reactive oxygen species adopted to assess the ecotoxicological assessment like impact on microorganisms and/ or microbiome of soils/plants/animals.

iii) Humane Society International (HIS) recommends inclusion of a general animal welfare/3R section that accepts all pesticide assessments and individual study protocols and that includes acceptance of all validated *in vitro* methods data for safety assessment of NM.

iv) *In vitro* ADME studies as a precursor to in vivo studies are used and recommended by various European Partnership for Alternative Approaches to animal testing (EPAA) industry sectors. Following HSI recommendations can be considered:

- Physiologically based biokinetic (PBBK) modeling for assessing ADME.
- Appropriate *in vitro* models for identifying ADME and specific organ toxicity before consideration of any in vivo; waive *in vivo* toxicity testing when the combination of more than one *in vitro* tests gives negative results.
- Prior to any *in vivo* testing *via* the dermal route, it should be compulsory to conduct an in vitro dermal absorption study (OECD TG 428) to determine the degree of dermal bioavailability and rate of percutaneous penetration. This information can contribute to a weight-of-evidence assessment of the need, or lack thereof, for ADME or other types of systemic testing via the dermal route.
- HSI/India would recommend to clearly state that applicants have to carefully define their testing strategy according to the exposure route, and to the human population under evaluation (e.g. workers, bystanders, consumers), in order to avoid unnecessary animal testing. Especially for workers, inhalation may be the most likely route of exposure to NM.
- Possible, sacrifice of large animals like cattle should be avoided in feeding study using livestock.

12.2.6 *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, stimulant cannot be used and it should be homogeneously blended in food.

12.2.6.1 ADME studies: These studies are essential for toxicity evaluation of NMs. Appropriate measuring systems should be adopted to detect NMs in organs, tissues or biological fluids. Labeling with radioactive isotopes, fluorescent dyes and comprehensive mass balance studies are to deal with NM polydispersity, and toxicokinetic changes upon repeated administration should be considered while designing ADME studies. Simple ICP-MS cannot determine the presence of NMs.

12.2.6.2 *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. Acute single-dose toxicity test would be appropriate for testing consumption of large quantity of NAPs.

According to OECD TG 408, a repeated-dose 90-day toxicity study in rat does not need to be conducted if (i) a reliable short-term toxicity study 28 days, as specified in OECD TG 402 is available showing severe toxicity effects according to the criteria for classification, for which the observed 28-day NOAEL with the application of an appropriate uncertainty factor allows the extrapolation towards the 90-day NOAEL for the same route of exposure;

and the substance is non-reactive, insoluble, not bio-accumulative and not inhalable and there is no evidence of absorption and no evidence of toxicity in a 28-day limit test, particularly if such a pattern is coupled with limited human exposure.

12.2.6.3 *In vivo* genotoxicity testing: Prior to conducting an in vivo genotoxicity study, toxicokinetic studies should be carried out to determine if the NM reaches the target tissue, where the target tissue is not the site of contact. In the absence of data to the contrary, the test is not applicable for detecting primary genotoxicity if the NM does not reach the target tissue. In the absence of toxico-kinetic information demonstrating systemic availability and/or exposure of target tissue(s), it is recommended to investigate the genotoxic effects in the site of contact tissue(s) (ECHA, 2017, R7-1).

12.2.6.4 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.

In-vivo neuro developmental studies: NMs should be tested for neuro developmental toxicities following their administration during gestation period. Evaluation until 2-month postnatal period is very critical. Critical differences in oxidative stress levels and neurotransmitter levels in the fetal brain between NM-treated and untreated group should be studied.

12.2.6.5 Other i*n 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.

12.2.6.6 *In vivo* studies to access synthetic NM's toxicological impact on microorganisms and/or microbiome of soils/plants/animals

Decisions on whether tests are necessary for reproductive and developmental toxicity need to be considered in light of the toxicity data and toxicokinetics information available. If the toxicokinetic study shows that the NM is systemically available in the test species (normally rodents) or suspected to be systemically available in humans, or if there are indications of effects on reproductive organs or parameters in the 90-day repeated-dose toxicity study, testing for reproductive and developmental toxicity is required (EFSA 2018).

Consideration may be given to avoid unnecessary animal testing with the provision of more detailed test guidance to applicants. In the case that in vivo testing is deemed necessary, extended one-generation in vivo test design can be adopted (OECD TG 443). This flexible study design allows for optional subdivision of F1 offspring in up to three different cohorts to assess reproductive and developmental endpoints including optional production of an F2 generation, impacts on the developing nervous system, and/or impacts on the developing immune system.

12.3 Food safety labeling

Every food product that has NMs in it should be labeled as per the Food Safety and Standards (Labeling) regulations, and include information on chemical/elemental composition of NMs.

12.4 Uncertainty analysis

Some of the possible reasons for uncertainty in assessing NMs are as follows:

12.4.1 There is no single standard method for physio-chemical characterization of all the various NMs and associated properties. Possibility of additional toxic effects caused by NMs that are not readily detectable by standard testing protocols. There are no specific assays for testing of allergy of NMs in food components.

12.4.2 Sample preparation/ handling procedures and calibration of the analytical equipment state characterization accuracy.

12.4.3 Possibility of difference between the form of NMs present in the test system and its actual form in food/feed increases the uncertainty in risk characterization.

12.4.4 Differences in the physical principles applied by various measurement techniques

12.4.5 Aggregation/agglomeration behavior of NMs and other factors such as dilutions or dispersions vary according to their interaction with various environmental factors, and also depends on storage period.

Exhaustive information may not be required in case of no exposure risk of NMs as confirmed by data (EFSA Scientific Committee, 2011; Leena et al., 2019).

The information requirement for evaluation of NAIPs and NAPs as mentioned in Section 11 and Section 12 are dynamic in nature and may be subject to any further additional requirements as per the decision of the concerned regulatory agency.

13. CONCLUSION

These guidelines would help policy makers and regulators to frame effective provisions for future novel nano-based products in the agri-input and food sectors of India. These guidelines would be useful to the researchers, manufacturers, importers and other stakeholders involved in research and development of these products. These guidelines would provide directions to the funding agencies to plan the road map to promote nanotechnology interventions in agri and food sectors. The guidelines will facilitate research and development activities while maintaining desired safety practices towards product or process development or else basic research. India is forging ahead at the global level by developing such comprehensive and inclusive guidelines with the intent to support appropriate regulation landscape in India.

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Annexure 1:
NANOTECHNOLOGY STAKEHOLDERS IN VARIOUS SECTORS IN INDIA

1. Policy Makers and Funders

- Department of Biotechnology (DBT), Ministry of Science and Technology
- Department of Science and Technology (DST), Ministry of Science and Technology
- Ministry of Agriculture and Farmers Welfare
- Ministry of Chemicals and Fertilizers
- Ministry of Food Processing Industries
- Ministry of Animal Husbandry, Dairying and Fisheries
- Ministry of Environment, Forests and Climate Change
- Ministry of Earth Sciences
- Ministry of Labour and Employment
- Ministry of Jal Shakti
- Council of Scientific and Industrial Research (CSIR)

2. Regulators

- Fertilizer control order (FCO)
- Central Insecticides Board and Registration Committee (CIB&RC)
- Food Safety and Standards Authority of India (FSSAI)
- State agriculture departments
- Bureau of Indian Standard (BIS)

3. Research and Education

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

4. Industries, other Stakeholders and Consumers

- Fertilizers
- Pesticides
- Seeds
- Plant Growth Regulators
- Food Processing
- Planting material
- Farmers
- Farmer cooperatives
- Consumers of nanofoods, traders, importers and manufacturers

5. Finance

- Venture Capital
- Public Funds
- Private Funds
- International Funds
- Insurance Companies

Annexure 2:

GLOBAL STATUS FOR REGULATION OF NANOPRODUCTS IN AGRI-FOOD SYSTEMS (SOURCE: SUBRAMANIAN AND RAJKISHORE 2018)

Country	Regulatory Body/ Responsible Organization	Legislation/ Concerned Documents	Available Provisions and Remarks
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 nano-products in FFDCA of FIFRA
Canada	Canadian Food Inspection Agency (CFIA) and Public Health Agency of Canada (PHAC)	Food and Drugs Act (7)	Nano-products 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 engineered NMs in Novel Food on the Provision of Food Information to Consumers, i.e. NMs that, amongst other criteria, have particle sizes in the defined nanoscale (1–100 nm). Provides guidance as how to perform risk assessment of NMs 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

Country	Regulatory Body/ Responsible Organization	Legislation/ Concerned Documents	Available Provisions and Remarks
		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
		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 450/2009 a (EC)	Although NMs 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 NMs
		Food Contact material regulation 1935/2004	Regulates the migration of nanocomponents into food from food contact material and articles and requires that these materials may not transfer their constituents into food under normal and foreseeable conditions of use in quantities that could endanger human health or bring unacceptable change in composition of food.
		Regulation (EU) No. 10/2011a	States that the substances in nanoform should be used only if listed in this regulation

Country	Regulatory Body/ Responsible Organization	Legislation/ Concerned Documents	Available Provisions and Remarks
		(EU) No 528/2012 (9)	As of today, NMs based biocidal products are not eligible for a simplified authorisation procedure. For subsequent NMs based product authorisation and approving NMs as active substances, the test methods applied to the NMs shall be accompanied and standardized by an explanation addressing their exact appropriateness considering the specific characteristics of each NMs
		Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) regulation (1907/2006)	Legislation has nanospecific provision but no exclusive NM legislation.
		EFSA's Novel Food Regulation 258/97	Pre-market approval for food or ingredients (other than food additives) and processes which are not consumed within EU. It emphasizes on details of composition, nutritional value metabolism, level of microbiological and chemical component studies on toxicology and allergen city and intended use (does not consider particle size).
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. Nano-products in agri-food systems is not yet approved by the authority

Country	Regulatory Body/ Responsible Organization	Legislation/ Concerned Documents	Available Provisions and Remarks
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	No NM specification
Japan	Ministry of Health, Labour and Welfare	Food Sanitation Law	No NM specific regulation
Iran	Iran Nanotechnology Initiative Council (INIC), Nanotechnology Committee of Food and Drug Organization (FDO)		FDO guidelines for nanoproducts in food, beverages, pharmaceutical, medical equipment. But agriculture is not yet included.
Taiwan	Taiwan Nanotechnology Industry Development Association(TANIDA)	Nano-Mark system to certify the nanoproducts	Nanoproducts are certified
Thailand	Food & Drug Administration of the Ministry of Public Health	Certification by Nanotechnology Association of Thailand	NanoQ label has been introduced for nanoproducts (but not for agri/food) that are certified by the Nanotechnology Association of Thailand

Annexure 3:

COMPARISON OF NM DEFINITION IN CURRENT REGULATORY FRAMEWORKS IN SELECTED COUNTRIES AVAILABLE FOR FOOD SECTOR (SOURCE: BOVERHOF ET AL 2015; SUBRAMANIAN & RAJKISHORE 2018)

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

Country and regulation	Size	Solubility	Aggregates and agglomerates	Distribution Threshold	Intentionally manufactured/ engineered	Novel properties
Health Canada	1-100 nm and larger[a]	No	Yes	No specific mention	Yes	Yes
Taiwan Council of Labor Affairs	1-100 nm and larger	No	No specific mention	No specific mention	Yes	Yes
Swiss Federal Office of Public Health and Federal Office for the Environment	1-100 nm and larger[c]	No	Yes	50% by number	No	No
Norwegian Environmental Agency	1-100 nm and larger	No	Yes	50% by number	No	No
Belgian Federal Public Service Health, Food Chain Safety and Environment (Belgium 2014)	1-100 nm	No	Yes	50% by number	Yes	No
Danish Ministry of the Environment (2014)	1-100 nm	No	Yes	50% by number	No	No

a Health Canada, the European Commission (for food and food contact materials) and the Taiwan Council of Labor Affairs have indicated the inclusion of materials larger than the nanoscale in all dimensions if they exhibit one or more nanoscale properties/ phenomena.

b The US FDA includes materials up to one micron if the material exhibits properties or phenomena that are attributable to its dimensions.

c The Swiss definition includes substances with primary particles, aggregates and agglomerates up to 500 nm, as well as respirable materials of up to 10 microns that may have nanoscale side branches.

Inter-ministerial Expert Committee constituted by Department of Biotechnology vide O.M. No. BT/Nano-Agri Guidelines/2019 dated 28th June 2019 for the finalization of "Guidelines for Evaluation of Nano Based Agri-input and Food Products in India

1.	Dr. Renu Swarup, Secretary, DBT	Chairperson
2.	Dr. Alok Adholeya, TERI, Gurugram	Co-Chair
3.	Dr. B. Sesikeran, Former Director, NIN, Hyderabad	Co-Chair
4.	Dr. Suchita Ninawe, Adviser, DBT	DBT Coordinator
5.	Representatives of DST Dr. Milind Kulkarni, Scientist 'G' Dr. Namrata Pathak, Scientist 'F'	Member
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14.	Representative of MoEFCC Dr. Rajesh Rastogi, Mrs. Madhumita Biswas	Member
15.	Representative of MoES Dr. Neloy Khare, Scientist 'G'	Member
16.	Dr. V. Ram Gopal Rao, Director, IIT, Delhi Dr. Kavya Dashora (Representative)	Member
17.	Dr. R. S. Sangwan, Director, AcSIR, Ghaziabad	Member
18.	Dr. C. Anandharamakrishnan, Director, IIFPT, Thanjavur	Member
19.	Dr. K. S. Subramanian, Director of Research, TNAU, Coimbatore	Member
20.	Dr. P. K. Patanjali, Chief – Formulation, IIPFT, Gurugram	Member
21.	Dr. Dinesh Mohan, Professor, JNU, New Delhi	Member
22.	Dr. Sumit Arora, NDRI, Karnal	Member
23.	Dr. Sadhan Bag, IVRI, Izatnagar	Member
24.	Dr. D K. Sharma, NDDB, Anand	Member
25.	Dr. Amit K. Dinda, AIIMS, New Delhi (Member of Working Group)	Special Invitee
26.	Dr. Vamsi Krishna, Scientist 'E', DBT	Member Secretary

These guidelines would help policy makers and regulators to frame effective provisions for future novel nano-based products in the agri-input and food sectors of India. These guidelines would be useful to the researchers, manufacturers, importers and other stakeholders involved in research and development of these products.These guidelines also provide suggestions to ensure human, animal and environmental safety considerations for these upcoming novel products.

Department of Biotechnology
Ministry of Science & Technology

Ministry of Agriculture and
Farmers' Welfare

Government of India
2020

For more details, please contact **sninawe.dbt@nic.in**, **vk.addanki@nic.in**