Government of India

Ministry of Science & Technology

Department of Biotechnology

In view of the recent developments in the field of Genome Editing Technologies a need was felt to bring out guidelines. After series of expert consultations, draft guidelines titled "Genome Edited Organisms: Regulatory Framework and Guidelines for Risk Assessment" has been prepared. The draft guidelines accommodates applicable laws, Acts, and procedures governing Genome Editing, general considerations and tiered approach for risk assessment of genome edited organisms and products derived thereof, regulatory approval road map, data requirement for risk assessment and institutional mechanism for governance and oversight.

The Department of Biotechnology invites comments on "**Draft document on Genome Edited Organisms: Regulatory Framework and Guidelines for Risk Assessment**" from the researchers/institutions and other stakeholders.

Click here to Download "Draft document on Genome Edited Organisms: Regulatory Framework and Guidelines for Risk Assessment"

It is requested that comments/ observations pertaining to the same may be conveyed, positively by **February 08th, 2020** to rcgm.dbt@nic.in or ibkp2019@dbt.nic.in or send through the IBKP portal at https://ibkp.dbtindia.gov.in/Content/PublicConsultation

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Proforma for submitting comments on 'Draft Genome Edited Organisms: Regulatory Framework and Guidelines for Risk Assessment '

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a) Mention "Comments on draft document on Genome Edited Organisms: Regulatory Framework and Guidelines for Risk Assessment" in the subject line.

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on

Genome Edited Organisms: Regulatory Framework and Guidelines for Risk Assessment

Department of Biotechnology
Ministry of Science & Technology,
Government of India

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Regulatory agencies shall ensure oversight mechanism for compliance of all genome editing related activities are properly abided by all concerned as per applicable national laws, Acts & Rules and international treaties to which India is a signatory. 36

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ABBREVIATIONS

bp	:	base pair
CRISPR	:	Clustered Regularly Interspaced Short Palindromic Repeat
Cas	:	CRISPR-associated system
DNA	:	Deoxyribonucleic Acid
DSB	:	Double-Strand Break
GE	:	Genetic Engineering
GEd	:	Genetically Edited
EPA	:	Environmental Protection Act
HDR	:	Homology-Directed Repair
HR	:	Homologous Recombination
Indel	:	Insertion/deletion
kbp	:	Kilo base pair
MN	:	Meganuclease
NHEJ	:	Non-homologous End-Joining
nt	:	Nucleotide
ODM	:	Oligonucleotide-directed mutagenesis
PN	:	Programmable Nuclease
rDNA	:	Recombinant DNA
RGENs	:	RNA-guided Engineered Nucleases
SSB	:	Single-strand Break
SDN	:	Site-directed Nuclease
TALEN	:	Transcription Activator-like Effector Nucleases
ZFN	:	Zinc-finger Nucleases
ZFP	:	Zinc-finger Protein

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1. Background

Biotechnology is one of the sunrise sectors propelling growth in biomedical, animal and agriculture sectors and contributing to the Indian economy. India has emerged as a scientifically and technically strong nation that can utilize advanced tools and technologies to derive the benefits of biotechnology for the public good, nationally and globally.

In a very short span of time, Genome Editing (GEd) Technology has demonstrated its potential applications in a wide range of sectors covering human and animal health, food, agriculture, microbial biotechnology, bio-economy, etc. These potential applications include, but are not limited to, improved crop protection and livestock breeding, improved animal welfare, modification of animal donors for xenotransplantation, products of microbial biotechnology, gene- and cell-based therapies to control diseases and prevent the inheritance of disease traits, control of vector-borne diseases such as Malaria, Dengue, Chikungunya, etc, biofuels, pharmaceuticals, and other high-value chemicals.

Like with all new technologies, GEd technologies have dual-use potential and therefore involve both safety & security issues.

Therefore, adoption of appropriate biosafety frameworks for research, development and application of Genome Editing Technologies in various sectors lays a roadmap for the development and sustainable use of Genome Editing Technologies in India. The judicious application of this technology in different areas will be a reflection of Government of India's long and underlying policies and commitment[1,2] towards securing and translating the benefit of scientific knowledge without compromising safety and security of the nation as well as the globe.

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[1]Science, Technology & Innovation Policy, 2013.

[2]Rao CNR. Science and technology policies: The case of India. Technology in Society 30 (2008) 242– 247.

2. Indian Biosafety Regulatory Frameworks

2.1 The process or product of genetic engineering technology in India is regulated under biosafety regulatory framework established under "Manufacture, use, import, export and storage of hazardous microorganisms/ genetically engineered organisms or cells, Rules 1989 (Rules 1989) under Environment (Protection) Act (EPA), 1986".

2.2 **Definitions in Rules 1989**

(i) "Biotechnology" means the application of scientific and engineering principles to the processing of materials by biological agents to produce goods and services;

(ii) "Cell hybridisation" means the formation of live cells with new combinations of genetic material through the fusion of two or more cells by means of methods which do not occur naturally;

(iii) "Gene Technology" means the application of the gene technique called genetic engineering, include self-cloning and deletion as well as cell hybridisation;

(iv) "Genetic engineering" means the technique by which heritable material, which does not usually occur or will not occur naturally in the organism or cell concerned, generated outside the organism or the cell is inserted into said cell or organism. It shall also mean the formation of new combinations of genetic material by incorporation of a cell into a host cell, where they occur naturally (self-cloning) as well as modification of an organism or in a cell by deletion and removal of parts of the heritable material;

(v) "Microorganisms" shall include all the bacteria, viruses, fungi, mycoplasma, cells lines, algae, protodones and nematodes indicated in the schedule and those that have not been presently known to exist in the country or not have been discovered so far.

2.3 Provisions applicable to new gene technologies under Rules 1989:

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(1) These Rules 1989 are applicable to the research, manufacture, import and storage of micro-organisms and Gene-Technological products.

(2) These rules shall apply to genetically engineered organisms/micro-organisms and cells and correspondingly to any substances and products and food stuffs, etc., of which such cells, organisms or tissues hereof form part.

(3) These rules shall also apply to new gene technologies apart from those referred to in clauses (ii) and (iv) of rule 3 and these rules shall apply to organisms /micro-organisms and cells generated by the utilisation of such other gene-technologies and to substances and products of which such organisms and cells form part.

(4) These rules shall be applicable in the following specific cases:

- sale, offers for sale, storage for the purpose of sale, offers and any kind of handling over with or without a consideration;
- exportation and importation of genetically engineered cells or organisms;
- production, manufacturing, processing, storage, import, drawing off, packaging and repackaging of the Genetically Engineered Products;
- production, manufacture etc. of drugs and pharmaceuticals and food stuffs distilleries and tanneries, etc. which make use of micro-organisms/ genetically engineered microorganisms one way or the other.

Living cells and/or organisms with targeted genetic change(s) in genomes are generally referred as "Genome Edited cells/organisms", "Gene Edited cells/organisms" or "Genetically Edited cells/organisms" (*hereinafter referred as **GEd organisms***).

The definition of gene technology under Rules 1989 covers genome editing, process and product.

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3. Other applicable Laws, Acts and Procedures Governing Genome Editing

The Genome Editing Technologies also have implications to International treaties/ agreements like Cartagena Protocol on Biosafety to the Convention on Biological Diversity, Biological Weapons Convention, Wassenaar Arrangement on Export Controls for Conventional Arms and

Dual-Use Goods and Technologies, Australia Group (AG). India being a party to these treaties/ agreements shall remain committed to the fulfilment of its obligations and shall take necessary steps to regulate genome editing whenever required.

The other applicable laws, acts & procedures related to biosafety and biosecurity are The Biological Diversity Act, 2002; Drugs and Cosmetic Act 1940; Seed Act, 1966; Protection of Plant Varieties and Farmers Rights, 2001; Food Safety and Standards Act, 2006; Plant Quarantine Order 2003; The Unlawful Activities (Prevention) Act, 1967; Disaster Management Act, 2005[3]; Weapons of Mass Destruction and Their Delivery System (Prohibition of Unlawful Activities) Act, 2005. Further, India is a signatory to The Convention on Biological Diversity (CBD)[4] and its subordinate protocols (Cartagena and Nagoya protocols)[5].

Export of hazardous microorganisms or toxins listed in SCOMET list and developed using genome editing technology shall require prior approval from DGFT as specified under Foreign Trade Policy of India. Biological Diversity Act, 2002 prohibits the acquisition of any biological resource[6] occurring in India or knowledge associated thereto for research or for commercial utilisation or for bio-survey and bio-utilisation without the approval of National Biodiversity Authority. FSSAI under Food Safety and Standards Acts, 2006 is responsible to assess the safety of food and its ingredients where food contains or consists of genome edited products.

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[3]"Disease, disability or death from natural (epidemics or pandemics), emerging or re-emerging diseases and man-made (intentional use) in Biological Warfare (BW) operations or incidents of Bioterrorism (BT)."

[4]The Convention on Biological Diversity (CBD) entered into force on 29 December 1993 (https://www.cbd.int/convention/).

[5]The Cartagena Protocol on Biosafety. The Cartagena Protocol on Biosafety to the Convention on Biological Diversity is an international agreement which aims to ensure the safe handling, transport and use of living modified organisms (LMOs) resulting from modern biotechnology that may have adverse effects on biological diversity, taking also into account risks to human health.(http://bch.cbd.int/protocol/).

[6] "Biological resources" means plants, animals and micro-organisms or parts thereof, their genetic material and by-products (excluding value added products) with actual or potential use or value, but does not include human genetic material.

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4. Application of Genome Editing Technologies in the Indian Context

Biotechnology offers safe and sustainable solutions to many environmental challenges. It is, therefore, envisioned that genome editing holds many promises to improve environmental quality as well as the quality of life and related services. The genome editing technologies offer solutions to address several issues related to Human & Animal Welfare and Protection of Environment.

Agriculture plays an important role to meet food and development needs of the Indian population and also as a source of increasing national economy through trade. New Technologies are anticipated to play a major role in meeting nation's food security and in achieving Sustainable Development Goals of UN[7] (for example- Goal 2: End Hunger, Achieve Food Security and Improve Nutrition, and Promote Sustainable Agriculture).

The Genome Editing Technology offers to increase yield and productivity of agricultural crops to meet constantly increasing demand for food and food security optimally by protecting them from various biotic and abiotic stresses and various other traits.

India is a fisheries giant with a total catch of about 3 million metric tons annually placing India among the world's top 10 fishing nations. India's livestock sector is also one of the largest in the world including broad spectrum of native breeds of cattle, buffalo, goats, sheep, swine, equine, camel and poultry with merits of adaptability to climate and nutrition, and resistance to diseases and stress.

The national targets for production of livestock and poultry products are 61% for milk, 76% for meat, 91% for fish, and 169% for eggs by the year 2020 over the base year TE 1999. The production potential in livestock is not realized fully because of constraints related to feeding, breeding, health, etc. Frequent outbreaks of diseases like FMD, BQ, PPR, Brucellosis, Swine fever, and Avian Influenza, etc. continue to reduce productivity and production.

[7]The Sustainable Development Goals 2015 – 2030. https://una-gp.org/the-sustainable-development-goals-2015-2030/

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With a minimal possibility of expansion of livestock population, the option available is to adopt systematic conservation, genetic improvement and sustainable utilization of indigenous breeds, where the role of genome editing is very promising[8,9].

In the healthcare sector, there are about 6000-8000 rare diseases known globally and out of which 450 of them have been reported in India. There are about 72-96 million people affected by such rare diseases. Some of them require treatment once in their lifetime whereas other diseases may require lifelong treatment and there are some diseases for which there is still no treatment available. About 95% of rare diseases have no approved treatment and where treatment is available they are very expensive and beyond the reach of the common man.

Genome editing tools offer new promise for protection of human health against various infectious and non-infectious diseases, prevention and treatment of rare diseases. A large number of efforts are going on at the international level to treat or cure fatal human diseases and rare genetic disorders using genome editing technologies. The somatic cell-based genome editing is considered as a better choice for treatment/cure of some of the rare genetic and other diseases and is currently being explored all over the world.

Research & experiments involving genome editing in germ-lines to understand basic biology under strict oversight and ethical monitoring is permitted but not beyond the two week stage in most of the countries. In India, as per the National Guidelines for Stem Cell Research (2017)[10] of Indian Council of Medical Research (ICMR), Department of Health Research (DHR) and Department of Biotechnology (DBT), Genome modification including gene editing (for example by CRISPR-Cas9 technology) of stem cells, germ-line stem cells or gamete and human embryos is restricted only to in vitro studies. It will require thorough review by the IC-SCR, IEC and IBSC, and finally by Review Committee on Genetic Manipulation (RCGM). More

[8]Agriculture Policy: Vision 2020.

[9]http://niti.gov.in/writereaddata/files/document_publication/DOUBLING%20FARMERS%20INCOME.pdf

[10]National Guidelines for Stem Cell Research. 2017. https://www.icmr.nic.in/sites/default/files/guidelines/Guidelines_for_stem_cell_research_2017.pdf

recently, ICMR, DBT and CDSCO issued National Guidelines for Gene Therapy Product Development & Clinical Trials, 2019[11].

5. General Considerations for Risk Analysis of GEd Organisms and Products Derived Thereof

For the safety assessment of genome edited organisms in general, the basic risk assessment framework published in "*Risk Assessment Framework and Guidelines for the Environmental Risk Assessment of Genetically Engineered Plants 2016*" (http://geacindia.gov.in/resource-documents/biosafety-regulations/guidelines-and-protocols/ERA_GuideforStakeholders.pdf) has been adopted. However, GEd organisms differ from GE organisms in many respects. Genome editing is a precise molecular method of mutation leading to deletion or addition or substitution of target base pair(s) in the native genes/ nucleic acid sequences. On the contrary, GE organisms (also known as GMOs/LMOs) typically contain foreign genes or DNA (with/ without prior knowledge of genome structure and function) derived from related or unrelated organisms to modify an existing trait or introduce a new trait. In addition, genome editing also facilitates the introduction of a foreign gene(s) to introduce a new trait(s), which is similar to GE organisms, but the site of integration is predetermined in GEd organisms unlike in GE organisms where site of foreign gene integration in the genome is random.

Within the GEd organisms, there are several differences depending on the type or nature of **S**ite **D**irected **N**uclease (SDN) or **O**ligo **D**irected **M**utagenesis (ODMs) used in genome editing process:

- The GEd organisms, may contain very specific modification of one or few base pairs within the existing genetic information of living organisms with known genome structure and function without involving foreign gene insertion.
- As a consequence of highly specific site of modification/integration, genome editing technologies may lead to products that might be undetectable and/or indistinguishable from

[11] National Guidelines for Gene Therapy Product Development & Clinical Trials (2019) https://www.icmr.nic.in/sites/default/files/guidelines/guidelines_GTP.pdf

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the naturally occurring mutants and from organisms produced from conventional breeding and/or artificial/induced mutagenesis (e.g., chemical, radiation) (Figure 1).

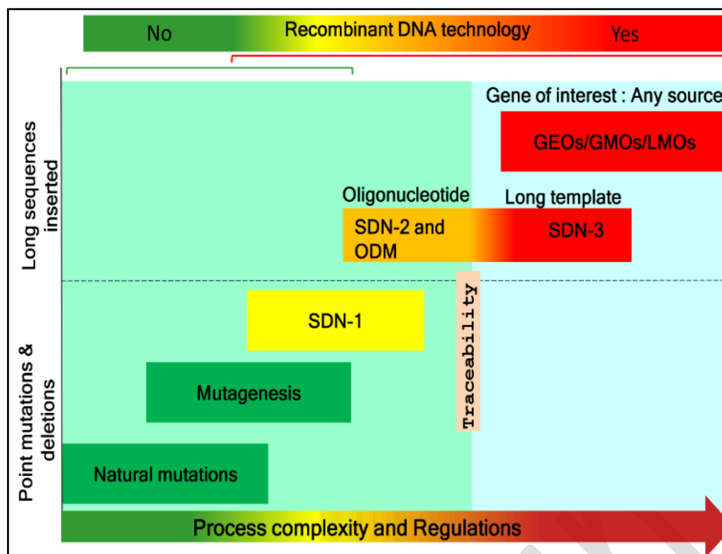

Figure 1. Process complexity, product features and traceability of Genome editing techniques relative to unregulated techniques (Conventional mutagenesis: natural mutations, chemical mutagenesis and radiation mutagenesis) and regulated techniques (transgenic technologies: inserting transgenes at random locus)

- Therefore, the genome editing tool can be used to create a wide range of genome modifications that includes production of 'nature-identical' traits, that is, traits that could also be derived by conventional methods, production of cisgenic and intragenic plants and animals, and introduction of exogenous genes with minimum change in the cell's/organism's genome.
- However, it is to be kept in mind that the currently available nucleases used for genome editing experiments are not completely error-free and therefore exhibit some off-target effect(s) /un-intentional genetic changes at other than the target location during the genome editing process.
- Therefore, biosafety assessment of GEd organisms/Cells takes into account both: 1) Modified/introduced trait efficacy as well as 2) The off-target effects leading to undesirable genetic changes in the genome and/or phenotype.

6. Tiered approach for the risk assessment of GEd products / organisms

Globally, there are varying views on how to regulate GEd products/process as per the law of the land. One view is that the GEd organisms need not be regulated as no foot print of genetic

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engineering is left in certain categories and they resemble organisms evolved through mutations or induced mutagenesis. The other view is that the GEd organisms need to be regulated but need not necessarily go through the same rigorous biosafety regulatory process that all GMOs/LMOs are being subjected. Such diverse views reflect the laws and acts under which each country regulates GEd organisms/products.

Keeping in view different processes followed and the resulting characteristics of GEd organisms, a broad risk assessment pathway that takes into account the nature of genome editing involved, complexity of modification created and the trait introduced in the organism/product, a systematic and structured approach for the risk analysis is adopted. Nevertheless, such technologies and market authorization of products shall be subject to all other laws which are in force now.

Based on the molecular and phenotypic characterization data, a wide range of potential pathways whereby unintended harm/safety to humans, animals, plants and the environment might occur are initially identified. Once the pathway(s) leading to harm are identified which in turn depends on the nature of identified hazard (consequences) and how likely harm could occur, the level of risk could be evaluated as per the "*Risk Assessment Framework and Guidelines for the Environmental Risk Assessment of Genetically Engineered Plants* 2016" and determine whether the projected risk is acceptable considering the benefits offered and prescribe a Risk Management Plan depending on the case.

Considering the uniqueness and the resulting products/organisms from genome editing technologies, a tiered approach has been proposed for risk assessment. In this approach, the degree of regulatory scrutiny will be determined by the kind of the genome editing tools/process used, extent of the resulting genetic change

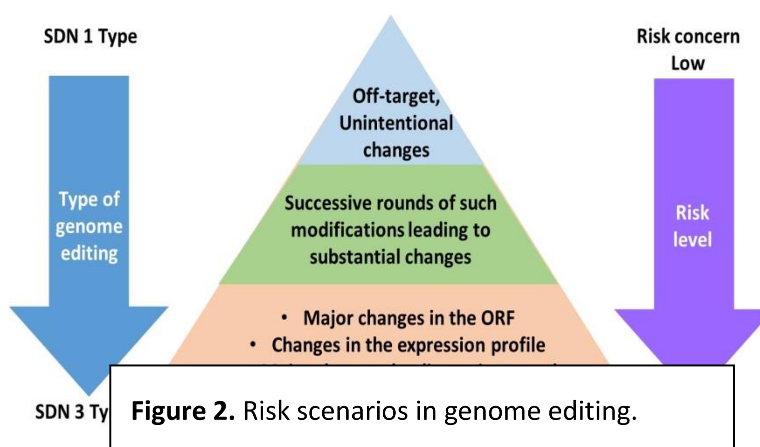

Figure 2. Risk scenarios in genome editing.

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(edit), the characteristics of GEd organism/cells, un-intended changes if any and intended use of the GEd product/organism. The attributes of each in conjunction with the overall process for insertion, targeting, modification, recovery and removal of transgenic elements may represent differing outcomes in terms of the way they will be assessed for regulation (Figure 2).

The risk evaluation matrix, in line with globally followed risk assessment for any new technology, has been developed to determine the overall risk levels. According to the Matrix, the risk level will be determined based on the extent of complexity/modification introduced and the risk category (Group I, II and III) in which an organism falls. The division of category is essentially based on the complexity of modification and prior knowledge / familiarity with the modification in natural/ existing population. Based on the category of modification the risk level could range from low, moderate or high risk groups. As the risk level increases, the data requirement and biosafety assessment level would increase.

Following explains about the Risk Categories:

Table 1: Grouping of the GEd organisms

GEd Group I	GEd Group II	GEd Group III
Single or few base pair edits/deletions/insertions leading to least complexity (Phenotype/ Genotype). Changes leading to knockdown/knock out of protein/ RNA that result in a new trait which may be familiar with prior knowledge. Chances of off-target effects.	Several base pair edits leading to certain degree of complexity in Phenotype/ Genotype (leading to improvement of an existing attribute or creation of a new attribute). Changes leading to gain of function with a new protein or RNA. May or may not be familiar with prior knowledge. Chances of off-target effects.	Insertion of foreign gene/DNA sequence leading to high degree of complexity in Phenotype/ Genotype (leading to creation of a new attribute, new metabolic pathways, etc.). Changes leading to gain of function with new protein or RNA. May not have prior knowledge. Chances of off-target effects.

Group I GEd organisms are similar to naturally occurring variants in the cultivated germplasm, wild species and mutants generated through chemical/radiation mutagenesis. The products derived from such organisms have a well-established history of safe use. The Group I GEd

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organisms mimic such naturally occurring mutants and are indistinguishable from such mutants and therefore, to be considered differently from other GEd organisms that fall under Group II and III. Some countries like Japan and Australia have recently amended their regulatory approval process for GEd organisms/products involving SDN-1 type modification.

GEd Group I: GEd cells/organism harbouring single or few base pair edits or small deletions like SDN-1, ODM, etc.

In contrast to conventional mutagenesis breeding (chemical and radiation) techniques, in GEd cells/organisms, the site of DNA breakage in the native genome is not random but designed in SDN-1 and ODM, that is, the DNA breakage and resulted edit occur precisely at a selected nucleotide sequence. This results in the genetic change from such targeted editing being much more predictable. However, SDN-1 and ODM may also lead to off-target genetic changes if the guide RNA sequence can have high complementarity to sequences of the genome other than the intended target sequence.

Although the position of the DNA DSBs by SDN-1 is precisely selected, the NHEJ DNA repair of the host cell could be random and results in small nucleotide deletions, insertion or substitutions. These change(s) can silence (knock out) or alter expression level of native gene(s), or modify the function of a protein by changing the amino acid sequence. Such changes may lead to acquisition of a new trait through knockdown of protein/RNA. In either case, screening and selection of the targeted change allows for identification and selection of the desired genomic mutational outcome. In general, biosafety concerns would be lesser for SDN1 and ODM genome edits as the target site is a single base pair or a few bases or a small deletion. Many newer and more improved nucleases are becoming available that are more specific to target site with lesser off-target effects. However, it may not be easy to detect single base pair edited plants without prior knowledge of the modification since they are genetically indistinguishable from naturally occurring alleles or mutations generated through chemicals/radiation regardless of the process from which they were derived.

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Considering these, following biosafety concerns should be addressed for GEd Group I organisms:

- Changes resulting in altered expression/ activity of native protein.
- Presence of vector/components used in the editing process.
- Confirmation of intended Trait efficacy
- Confirmation of Phenotypic equivalence
- Changes leading to protein with new/altered functions. In rare cases, single base pair mutation(s) might result in the introduction of novel trait (e.g., Herbicide tolerance) which might pose additional biosafety concerns.

A Novel trait is an entirely new trait not already present in the concerned variety or species itself and/or in a related species.

Successive or simultaneous rounds of modifications using targeted single base pair editing techniques could result in the accumulation of several related or unrelated edits and might lead to substantial phenotypic/compositional changes and may pose higher level of risks. Biosafety assessment will be at a higher level in such cases.

The GEd cells/organisms falling under Group I would be assessed mainly to confirm targeted edit(s) as well as absence of any biologically significant off-target genomic changes. Also, they would be subjected to phenotypic equivalence analysis on case-by-case basis.

GEd Group II: GEd cells/organisms harbouring targeted few/several base pair edits like SDN-2

Genome editing of few base/several bases generally employs a short homologous DNA repair template identical to the targeted DNA sequence except a few nucleotide changes. Such changes may lead to gain of function through formation of new protein/RNA. The outcome will be predetermined point mutations of few bases at the targeted site of the genome. Again, it may or may not be possible to detect/differentiate these genetically indistinguishable GEd cells/organisms from non-GEd cells/organisms without prior knowledge of the modification regardless of the process from which they were derived. Similar to SDN-1 and ODM, the SDN-2 are also specific and targeted and are prone to off-target effects.

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The cells/organisms produced from targeted editing of a few base pairs may also be genetically indistinguishable from those cells/organisms which could have occurred naturally or the products of techniques that involve chemical/ radiation mutations with a long history of safe use and are unregulated. Therefore, in general, it is unlikely that the resulting GEd organisms pose different risk(s) in comparison to mutated cells/organisms by conventional approaches. Reliable detection of such organisms presents a great challenge for enforcing compliance.

Although all the biosafety concerns of Group II/SDN-2 based GEd cells/organisms where few base pairs are edited are similar to single base pair GEd cells/organisms, the following additional biosafety concerns should be addressed considering increased potential of off-target effects:

- ➢ Insertion of exogenous DNA sequence leading to:
- • Altered expression of gene.
- • Deletion/knockout of gene expression.
- • Modification of amino acid sequence of a native protein.
- ➢ Insertion of allelic sequences having prior knowledge.
- ➢ Introduction of foreign gene with novel trait(s).

Further, depending on the extent of genetic modification created and the resulting complexity of species-trait combination, these cells/organisms may be subjected to additional biosafety assessment to address any safety concerns to plant/animal/human health and environment.

The GEd cells/organisms falling under Group II would be assessed to confirm targeted edit(s) as well as absence of any biologically significant off-target genomic changes. Also, they would be assessed for phenotypic equivalence and trait efficacy through appropriate contained and/or confined field trials.

GEd Group III: GEd cells/organisms harbouring targeted edit(s) synthetic/foreign DNA like in SDN-3

The DNA repair template used in SDN-3 contains a new DNA sequence which may comprise one or more genetic elements and the outcome of the technology would be the integration of

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large DNA sequences into the genome. The most likely application illustrating the use of SDN-3 would be the insertion of cisgenic/ intragenic transgenic expression cassettes or deletion of a large DNA segment at a selected genome location. These are relatively easy to detect using DNA- or Protein-based detection methods.

The GEd cells/organisms belonging to Group III type may containDNA sequences/transgene(s) and their gene products (including intragenic, intergenic DNA), derived from any source/organism including non-kingdom source. Potential hazards in such cases depend on nature and source of genes and sequences integrated into GEd cell/organism's genome. Nevertheless, the intergenic/transgenic SDN-3 GEd cells/organisms shall be considered as "new transformation GE events" with none or few disruptions of the existing genome. Such new transformations involving genome editing shall have to undergo complete step-wise evaluation and biosafety assessment including molecular characterization, food/ feed and environmental safety studies (if applicable for the recipient cells/organisms) to ensure that there are no unintended effects on the safety of humans, animals, plants, microbes or the environment. In this context, existing GE guidelines for specific cells/organisms {e g., In case of plants, Guidelines for the Environmental Risk Assessment of Genetically Engineered Plants, 2016; Guidelines for the Safety Assessment of Foods Derived from Genetically Engineered Plants, 2008} will be applicable.

GEd cells/organisms expressing novel trait(s) for the same species of non-GEd organism, will be subjected to case-by-case assessment to establish biosafety of novel trait/new or exogenous protein(s) taking account of the trait itself and the species into which it has been introduced.

The data requirement and biosafety assessment level would increase with the increase in the complexity of the modification. Nevertheless, regulatory approval for the GEd will depend on

Group III GEd cells/organisms harbouring large or foreign *DNA* in the recipient cell/organism genome, may represent similar biosafety concerns as that of genetically engineered (GE) cells/organisms with typical foreign gene insertion(s). Therefore, all the biosafety data requirements which are prescribed in existing food and environmental safety guidelines specific for GE cells/organisms on case-by-case basis where foreign genes are inserted, would be envisaged.

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biosafety assessment on a case-by-case basis. The next section will deal with the specific data requirements for each of GEd group organisms.

7. Regulatory Approval Road Map for Genome Edited (GEd) organisms/ Products derived thereof

The regulatory process and granting of approvals by IBSC/RCGM/GEAC for GEd products/organisms/processes will depend on the purpose for which approvals are sought and the extent of modification(s) introduced and Risk Levels of the resulting products/organisms/processes (**Table 2** and **Figure 3, Figure 4 & Figure 5**).

Table 2. Regulatory Approval Pathway for GEd Organisms/Products derived thereof.

Statutory Committee for Authorisation	**GEd Research & Product Development**	**Towards regulatory approvals** for release of GEd organism/cells/products		
		GEd Plants	**GEd Animals**: Laboratory animals and Livestock	**Human stem cells**: Gene therapy (Somatic stem cells),
IBSC	All research and product development experiments related to GEd Group I (Plants, Animals/ human stem cells).	GEd plants and products derived from Group I experiments (plants).	IBSC to recommend to RCGM after evaluation of molecular characterization data of Group I, Group II and Group III (Animals/human stem cells).	
RCGM	All research and product development experiments related to GEd Group II & III (Plants, Animals/ human stem cells).	RCGM to recommend to GEAC based on molecular characterization data and contained/confined trials data of GEd plants or product(s) of Group II and III experiments and GEd animals falling under Group I, II and III experiments.		RCGM to recommend to CDSCO based on PCT studies.
GEAC	-	GEd organisms and products derived from Group II and III experiments on Plants and Group I, II and III experiments on Animals/human stem cells for environmental release.		-

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Statutory market authorization agency	-	MoA&FW, GoI, FSSAI	CPCSEA, FSSAI, DAHR, MoA&FW, GoI	CDSCO, MoH&FW, GoI

The regulatory pathway for GEd plants, GEd animals & Human GEd stem cells and products derived thereof are given in Figure 3, 4, 5, respectively.

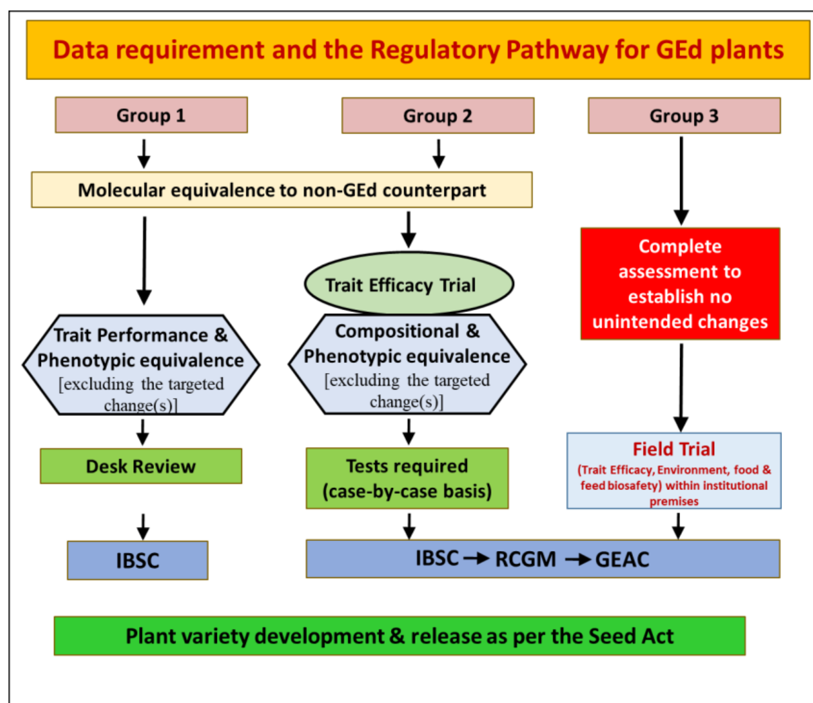

Figure 3. Regulatory pathway for GEd plants and products derived thereof.

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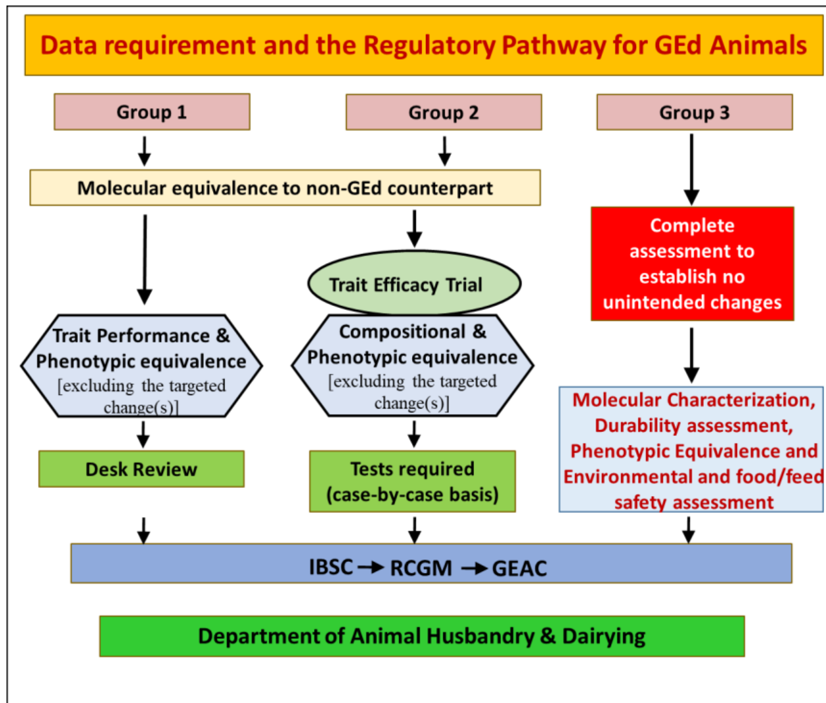

Figure 4. Regulatory pathway for GEd animals and products derived thereof.

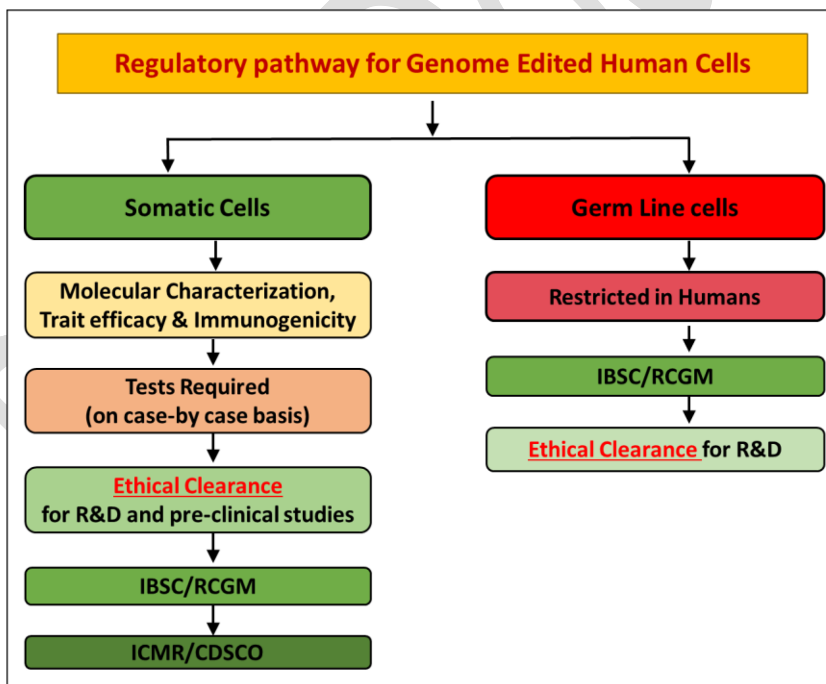

Figure 5. Regulatory pathway for Genome Edited Cells (Gene Therapy Product).

8. Data Requirement for Risk Assessment

Genome editing methods are fast evolving and vary vastly in composition of editing reagents (type of nucleases and rDNA), design of Programmable nucleases (specificity for DSB and target site), delivery method, target trait, and resulting organism phenotype. Off-target mutations if they occur, may lead to altered expression patterns of organism's gene(s), may affect composition, or may lead to phenotypic variation. Data requirement of GEd organisms depends on the process used and type of the product. Therefore, regulatory requirements for different categories of GEd organisms will vary depending on the potential biosafety concerns/risks posed by the extent of modification with maximum data requirements in case of GEd cells/organisms with large DNA fragment insertions and/or those with novel traits.

The data requirements for the biosafety assessment of GEd cells/organisms include, the editing reagents used, method of Programmable nuclease and recombinant nucleic acid delivery, the nature of the DNA-repair, insertion of rDNA, targeted change, untargeted alterations, edited loci, positional effects, and characteristics of the trait/phenotype that is developed should be considered. In general, following aspects are relevant for biosafety assessment of the GEd cells/organisms obtained by targeted genome editing:

- Biology of the organisms.
- Delivery method of editing reagents/programmable nucleases.
- The characteristics and molecular mechanisms of editing reagents/programmable nucleases/nickases (mode of action, double-strand breaks (DSB), nicks or double nicks).
- Group of the genome editing method.
- The different possible outcomes and resulting consequences.
- Molecular characterization of Intended change at the target site.
- Integration of complete/partial SDN-cassette or donor DNA at non-targeted loci and expression pattern of base editing enzyme.
- Off-target changes and resulting consequences.
- Phenotype change, Efficiency of the target trait, Compositional changes.
- Biosafety of new protein.

In case of genome editing in human somatic cell, following are the additional data requirement for biosafety assessment:

- Origin of somatic stem cells, Culture system and methods used

- Recovery and characterization of edited somatic cells
- Functional (*In vitro/In vivo/ Ex-vivo* assay based) characterization
- Immunogenicity, Sterility of GEd somatic cells for therapeutics
- Multiplication, storage of genome edited somatic cells for therapeutic use
- Biocontainment facilities used for conducting research

For the GEd cells/organisms having single or multiple base changes which are indistinguishable from naturally occurring mutations, posing low level of risk, data on: 1) molecular characterization, 2) phenotypic and/or substantial equivalence, and 3) trait efficacy should be provided. On the other hand, a complete risk assessment is required for all distinguishable GEd organisms. Data requirement encompass molecular characterization, stability assessment, Phenotypic and/or compositional equivalence, trait efficacy, and food/ feed safety and environmental safety assessment in case of plants and animals as applicable. The extent of food/feed safety and environmental safety assessment depends on the outcome of the assessment as stated above.

8.1 Molecular characterization of GEd organisms

Detailed molecular characterization data shall be provided for all types of genome editing. Molecular characterization is done for the various aspects of the genome editing process like the nucleases used in the process, method of delivery, the target site characterization and characterization of unintended off targets sites etc. The first step in the molecular characterization is to perform *in silico* analysis to check for the precise location of the intended target site and to check the possibility of unintended off-target site cuts.

8.1.1. Biology of parent organism

<u>In case of animals and plants</u>:

- **i.** Details of the common and scientific name of the parent organism.
- **ii.** Pedigree of the GEd organism if it is a hybrid, include relevant information.
- **iii.** Details of the origin of the species and/or the particular genotype/breed.
- **iv.** Natural habitat of the parent organism(s), and its range.
- **v.** Details of any known predators/parasites/pests of the parent organism in India.

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8.1.2. Cells and culture system of genome editing of human cells

In case of gene therapy for humans, the starting somatic cells shall be the human somatic or their iPSCs. Genome editing of somatic cells using CRISPR/Cas9, TALEN, ZF-TFs require well characterized cell culture systems for genetic manipulation. Such information is required to understand the role of each component while assessing safety and efficacy of edited cells.

i. Detailed information on generation of human somatic stem cells including the source and process followed for their isolation.
Attach all prior approvals obtained from competent authorities (eg. IBSC, ethical, consent letter from donor, etc.) for isolation and handling of somatic cells that are to be used for genome editing.

ii. Characterization data to ensure that the human somatic cells are free from any human pathogens.

iii. Information on culture system used for genome editing.

iv. Available alternate methods of the indication(s).

v. Justification for using genome edited human somatic cells for the treatment, especially in the context of Schedule J.

8.1.3 Design of Programmable nuclease/nickase

The genome editing is carried out using engineered nucleases, or "molecular scissors", which create site-specific double-strand breaks (DSBs) at desired locations in the genome. These induced double-strand breaks are then repaired through non homologous end-joining (NHEJ) or homologous recombination (HR), resulting in targeted mutations ('edits'). The nucleases differ in several respects such as specificities, mutation signatures, among other characteristics. Information on editing reagent/nuclease-specific features is essential for biosafety assessment. The specific data requirements are:

i. *Transformation vector*: Nature, source, detailed map (with base pair positions), origin of replication(s), selection method and annotated Nucleotide sequence of vector and DNA encoding the nuclease/nickases, sequence to be transferred to host cell, if any..

ii. *Details of the nuclease(s)/nickases(s) DNA, and recombinant nucleic acids for homologous recombination*: Donor organism, GenBank accession number (wherever available) and description including mode of action and all other genetic elements included.

iii. History of safe use of donor organism(s) of genetic elements (in recombinant nucleic acid; in case of category III organisms involving insertion of DNA sequence) present in GEd cells/organisms.

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With emphasis on allergens, antinutrients, toxicants, and/or pathogenic substances produced and pathogenicity of donor organism(s).

iv. Amino acid sequence of the programmable nuclease/nickases .

v. Features of the nuclease/nickases with emphasis on specificity of Target recognition site(s) and reducing off-target activity in comparison to the wild-type nuclease.

vi. Nucleotide sequence of the homologous rDNA to be transferred for homologous recombination/HDR.

8.1.4 Method of transformation/Mode of Delivery:

Site directed nucleases (SDNs) may be delivered to the cells in various ways, like transiently (without rDNA/exogenous DNA integration), or through co-integration/stable integration to the genome followed by segregation of the SDN from the recipient organism resulting in a final organism containing only the targeted edits, or could be stably integrated in an intermediate organism that serves as a delivery vehicle for the nuclease activity to a recipient organism.

Delivery methods in plant species include electroporation of protoplasts, biolistics, *Agrobacterium*-mediated transformation, and whisker-mediated transformation of cells, etc. GEd animals are developed through genome editing of somatic cells followed by somatic cell nuclear transfer (SCNT) cloning or injection of the gene editing reagents into zygotic cytoplasm of the next generation. SCNT has been a major technique for delivering nuclease-mediated genetic alterations in livestock. The GEd cell line obtained by SCNT can be genotyped and/or screened before transfer into the enucleated oocyte to ensure that the desired edits, and no off-target edits, have occurred. Once the targeted integration of the rDNA has been achieved, the introduced SDN gene and the rDNA at non-targeted loci can be removed by segregation to generate organisms containing only the targeted integration of the homologous rDNA but no other exogenous DNA. Genome editing in humans may be performed in Somatic cells as well as germ line cells. **However, the germ line editing has not been permitted for human use so far**. The following aspects are to be considered:

i. Category of the genome editing

ii. Details of the transformation /delivery methods.

Method of transformation. If a microbial transformation systems (other than the Agrobacterium-mediated) is used, information regarding whether it utilizes a pathogenic organism or pathogenesis related sequences.

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For in vivo gene editing of human somatic cells by viral vectors, chromosomal integration and duration of expression and possibility of viral shedding should be monitored in addition to immunogenicity.

iii. Information on SDN and vector DNA co-delivered/transformed into the host cells.

iv. Scientific Name and basic characteristics of the parent organism (non-GEd) genotype in case of animals and plants.

8.1.5 Selection & stability of Genome Edited cells/organism:

Another aspect to understand in the biosafety assessment is the process of selection of GEd cells/organisms and its stability across various generations. Following data is required:

i. Details of the selection method used to regenerate and generation advancement of the GEd line.

ii. Generation and Pedigree (starting from primary transformation/editing) of the GEd line and zygosity status of the edit in case of GEd animals and plants.

iii. Mendelian inheritance of the edit(s) in case of GEd plants and animals.

iv. Details on the stability of the genotype of the GEd plant and animal.

v. Confirmation of absence/ removal of nuclease/ vector DNA in the genome.

8.1.6 Characterization of the Site-directed mutation:

The most important data requirement is to understand the precise target edit(s) in terms of its location, precision, absence of any vector sequences, etc.

i. Confirmation of presence of site-directed genome editing by appropriate molecular analysis along with the protocol followed.
Methods such as PCR and sequencing, restriction enzyme digestion suppressed PCR (RE-PCR) which investigates the NHEJ introduced mutation, T7 endonuclease I assay, or Next Generation Sequencing (NGS), etc. may be followed as applicable.

ii. *In silico* analysis of the cleavage site/sequence at target location(s) in the genome.
Double-strand breaks site of the Site-directed nucleases, or single-strand break(s) site of Site-directed nickases/ paired nickases (nicking opposite strands of a target locus).

iii. Nucleotide sequence of the target locus (loci) highlighting the mutation/site-directed incorporation of rDNA/exogenous DNA.

iv. Nucleotide sequence of the edited locus (loci) as an evidence of editing.

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*The sequence(s) are required as data files in relevant editable format (e.g., *.txt, *.fasta, *.fsa, *.doc, *.docx).*

v. Organelle where edit(s) was introduced (nucleus, chloroplast in plants, or mitochondrial genome) of each edit.

vi. Chromosomal location of each nuclear edit, if available.

vii. ORF analysis of the edited locus in all six reading frames, including amino acid sequence to find creation of any unwanted new ORFs.

viii. Bioinformatics analysis of each expressed ORF(s) from edited locus (loci) of the GEd line to find homology with known protein allergens, toxins and antinutrients.

ix. Confirmation for absence/presenceof the programmed nuclease/nickases sequence(s) or/and transformation vector backbone sequence(s) in the host cell.

x. Transcript and/or Protein expression profile of each edited gene/ORF.

a) Details on expression: Spatial and Temporal; constitutive, inducible, tissue specific, development stage specific, etc.

b) Expression analysis of each edited gene/ORF and protein(s) in relevant tissue/organ at key developmental stages of the GEd organism.

   For inducible or development stage specific transgene(s), data generated on induction stimuli or at the particular developmental stage in addition to non-stimuli or growth conditions, respectively.

   Transcript expression analysis is essential for those gene edits where expressed RNA does not translate protein or expresses an intractable protein.

c) Mean and maximum level of the expression in edible portions of GE organisms of event(s) in case of GEd animals and plants. Appropriate statistical analysis of expression data (*Raw data also to be provided*).

d) If Partial or complete gene silencing is observed or predicted, confirmation of the same.

xi. Demonstration of phenotypic stability over multiple generations (in plants and animals) or vegetative cycles (in plants).

xii. In case of plants, expression data in multiple generations/growing seasons/years, and/or multiple environmental conditions to demonstrate the inheritance and stability of the edited/introduced trait(s).

Expression data is not required for those genome edits wherein transcript and/or protein is not expressed or the protein is intractable in nature.

xiii. Number of backcrosses of the genome edited line with parent line (if backcrossing was done; in plants and animals).

xiv. DNA fingerprint data of the GEd organism before and after the editing.

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8.1.7 Characterization of off-target mutation(s):

Although, SDN techniques are expected to be more precise in introducing changes in the genome of the target organism, changes elsewhere in the genome may occur as a result of off-target effects. Functional or biological significance of off-target changes are important for biosafety assessment. Off-target activity of the SDN depends on its specificity and the presence and accessibility of sequences similar to the SDN recognition site in the genome. For biosafety assessment of genome edited cells/organisms, it is critical to characterize these off-target mutations to understand if they have any biological significance in terms of affecting the expression of endogenous genes/ metabolites/phenotype, etc. Further, data should be generated on the expression/activity status of off-targeted genes, whether any protein coding sequences or 5' end of genes are affected, changes on non-coding DNA, epigenetic information of off-targets (H3K27Ac, H3K4me3 etc), changes on DNA sequences with purifying selection or positive selection, changes on evolutionarily constrained regions of genome, Status of non-coding DNA activity in cell type and Non coding mutations in the context of Topologically associated domains (TADs).

Hence following information is required to characterize the off-target mutations:

i. Details of selection of unique target site based on computer algorithm or web based bioinformatics analysis of whole-genome or whole-exome sequence analysis of the target organism (e.g., Cas-OFFinder- http://www.rgenome.net/cas-offinder/; CRISPR design tool- http:/crispr.mit.edu/; CasFinder- http://arep.med.harvard.edu/CasFinder/ etc. are available).

ii. List of potential off-targets detected by bioinformatics analysis.

iii. Sequence analysis of the potential off-target sites (e.g., *deep amplicon sequencing*) or the whole genome re-sequencing to detect off-target modifications present, or Unbiased *In vitro* Genome-wide Assays for detection of Off-targets if any on a case-by-case basis.

Whole-genome sequencing might seem to be an ideal method to assess genotoxicity in case of human cells.This approach will only work in a therapeutic process in which transplanted cells are derived from a single clone. Karyotyping, translocation capture for unbiased assessment for genome rearrangements, Unbiased DSB capture combined with sequencing, and/or "Onco-chip" to specifically assess mutations in genes associated with cancer may also be employed.

In case of genome editing in human somatic cells, sequencing-based methods of analysis should be complemented by functional methods of assessing genotoxicity. Functional genotoxicity assays would measure various aspects of cellular behaviour including apoptosis, proliferation,

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differentiated functionality (such as the ability to perform equivalently to unmodified cells), and potential to transform.

iv. Description of functional or biologically significant off-targets (e.g., creation or deletion of an open reading frame).

v. Backcross breeding for sufficient number of generations to remove any possible off-target changes in case of GEd plants.
In case of perennials or plants that reproduce mainly through vegetative propagation, additional molecular data may be required on a case-by-case basis.

vi. In case of GEd animals and plants, "omics" studies (transcriptomics, metabolomics, and/or proteomics or "epigenetic profiling" might be required on a case-by-case basis.

8.2 Trait efficacy

<u>In case of GEd plants</u>, trait performance data using greenhouse or nethouse for Group I organisms would be required. For Group II organisms, the trait efficacy data would be assessed on a case-by-case basis depending on the crop-trait combination. For Group III GEd plants having complexity of trait, besides green house/ net house data, trait efficacy data from limited confined field trials would be required.

<u>In case of GEd animals</u>,data on expressed trait (based on the intended use) and its consistency over multiple generations (for a minimum of F1 generation or for multiple generations depending on the type of species involved) is required.

<u>In case of human GEd somatic stem cells</u>, trait efficacy data based on *in vitro* and/or *in vivo* cell based or humanized animal models, if available, as supporting evidence before clinical applications would be required. Factors that should be considered in efficacy studies include, the selection of dose, establishment of relevant animal disease models (humanized or immunosuppressed mice etc.), efficiency of gene editing, and duration of efficacy. Function based assays would measure various aspects of trait efficacy of GEd cells. However, in principle, humanized animal models should be used for studying the on-target efficacy of genome-editing therapeutics. When non-human primates are used as large animal models, *in silico* based studies should be conducted to validate the similarity of target gene sequences between primates and humans.

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8.3 Phenotypic equivalence

Genome editing typically takes place at the target location(s) of the genome. However, functional off-target change(s), if occur, may lead to altered expression patterns of organism's gene(s), and/or may affect the phenotype. Following data is required to characterize the same:

i. Details of secondary effects that might be anticipated as a result of genome editing.

ii. Details of the genetic changes that limit or eliminate any capacity to reproduce or transfer genes to other organisms, if any.

iii. **<u>In case of GEd Plants</u>**:

- Data on phenotypic equivalence in comparison to closest non-GEd plants (comparator) to detect and measure agronomic and phenotypic differences (whether intended or unintended).

iv. **<u>In case of GEd Animals,</u>** phenotypic equivalence is established to ascertain whether there is any irrelevant biological change (e.g. physiology of the animal via the food/feed exposure pathway) by assessing:

- Data on the health of the genome edited animals, including veterinary and treatment records, growth rates, reproductive function, and behaviour.
- Data on the physiological status of the GEd animals, including clinical chemistry, hematology, histopathology, and post-mortem results.

Data should be collected from a generation of genome edited animals as close as possible to that intended for use in commerce.

v. **<u>In case of GEd human somatic cells:</u>**

- Comparative phenotypic analysis (cell phenotype/morphology) of somatic cells before and after genome editing.
- Comparative growth pattern of somatic cells before and after genome editing.
- Functional studies could include an examination of changes in the viability, proliferation, and cell-cycle behaviour of gene-edited cells.
- Data on Large-scale genomic changes such as chromosomal translocation, deletion, and inversion.

8.4. Compositional equivalence

In some cases, an off-target change could have an unintended effect on the nutrient composition. Therefore, the major considerations for biosafety assessment of GEd organisms includes compositional equivalence.

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In case of plants, data requirement for compositional assessment shall be according to existing biosafety practices and will be evaluated on a case-by-case basis depending on the plant-trait combination.

For GEd animals, compositional assessment of the edible tissues following existing biosafety practices is required. Similar to the GEd plant, the assessment would refer animal-trait combination. For complete food safety assessment, the Principles outlined in the Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Animals (http://www.codexalimentarius.org/download/standards/11023/CXG_068e.pdf) guideline should be followed.

The composition of tissues should be compared with the appropriate comparator such as conventional counterpart grown under the same conditions. The statistical significance of any observed differences should be assessed in the context of the range of natural variations for that parameter to determine its biological significance. The comparator(s) used in this assessment should ideally be matched in growth conditions (breed, age, sex, parity, lactation, or laying cycle (where appropriate) in case of animals). In cases where it is not feasible, conventional counterparts as close as possible should be chosen. The purpose of this comparison, in conjunction with an exposure assessment as necessary, is to establish that substances that are nutritionally important or that can affect the safety of the food have not been altered in a manner that would have an adverse impact on human, animal or plant health. Key components may be major constituents (fats, proteins, carbohydrates as nutrients or enzyme inhibitors as anti-nutrients) or minor compounds (minerals, vitamins). Key toxicants are those toxicologically significant compounds known to be inherently present in the organism, such as those compounds whose toxic potency and level may be significant to health and allergens. In animals, the presence of toxicants would be rare, whereas the presence of allergens would be common in some species.

8.5. Food and feed safety assessment

In case of Group I GEd plants, with thorough molecular characterization data of the intended target site and characterization of the unintended off target variations and based on the familiarity of the intended changeif it is already existing & being consumed, the complete

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food safety assessment would not be necessary. However, in cases of Group II and III GEd plants, the food/ feed safety assessment would be assessed as per the Guidelines for the Safety Assessment of Foods Derived from Genetically Engineered Plants (ICMR, 2008).

In case of GEd animals, following information would be required for food and feed safety assessment:

i. Details of whether food or feed derived from the GEd animal is safe for humans or animals consuming edible products from the animals.

ii. Details of whether there is any direct toxicity, including allergenicity, via food or feed consumption of the expression product of the article.

iii. Details of potential indirect toxicity associated with both - the article and its expressed product (e.g., whether location or expression of the article affects physiological processes in the resulting animal such that unintended food/feed consumption hazards are created, or whether existing food/feed consumption risks are increased).

iv. Details of potential adverse outcomes via the food/feed exposure pathway by determining whether there are any biologically relevant changes (1) to the physiology of the animal, and (2) in the composition of edible tissues from the GEd animal that suggest reason for toxicological concern compared with the appropriate non-GEd comparator.

8.6. Environmental safety assessment of GEd organisms

The environmental safety studies are important to ensure the safety of the GEd organisms to the environment. The cases which have very little knowledge, familiarity and are expected to produce traits that are not commonly available in nature, complete environmental safety assessment will be required. Irrespective of the nature of editing, if the edited trait is new or exotic to Indian agro-ecosystem, environmental safety assessment will be required to understand the behaviour of the GEd plant/ animal in different agro-climatic zones and its consequences to the non-target organisms present there. However, the degree and extent of data requirement shall be decided on a case-by-case basis considering the nature of editing and its possible interaction with environment both phenotypically and genotypically.

The environment safety studies for GEd plants/animals shall depend on the phenotypic and compositional study data in addition to molecular characterization data. Environmental studies

may be required to perform on a case-by-case basis for Group III modifications. Comparative assessment is recommended for environmental biosafety assessment.

The Environmental safety assessment of a GEd plant shall be conducted in a stepwise fashion through biosafety trials as defined in Guidelines for the conduct of Confined Field Trial of GE plant (Environmental risk Assessment (ERA) guidelines of MoEF&CC, 2016). Information on the general requirements of the data may be obtained from other guidelines issued for purpose of GE plant.

Genome editing of plant/animal cells is strictly performed inside contained laboratory environment and is not expected to be directly exposed or established to natural environment. As such, for laboratory studies on plant/animal genome editing, no separate environmental safety assessment will be required. However, conduct of such work must adhere to laboratory biosafety principles and practices to prevent any harm to laboratory workers as well as environment. The Regulations and Guidelines for Recombinant DNA Research and Biocontainment 2017 shall be applicable for this purpose and is legally binding pan India.

The following information will be required for safety assessment of Group III GEd animals:

1. <u>Information about the effects of the GEd animal (s) on the environment.</u>

a. Details of unintended effects, if any, on other animals resulting from the release of the GEd animals.
b. Details of any intended gains that are directly linked to changes in other characteristics of the subject species.

2. <u>Information about feral populations of subject species, if any, that exist in India or that may be established.</u>

a. Details of enhancing the ability of the species, if any, to establish feral populations by interbreeding with native population.
b. Details of the inherent property of natural cross breeding, if any.
c. Details of any agricultural, environmental or disease-control problems caused by feral populations of the subject species.
d. Details of any experimental work that has been done on expression of the novel genetic material in feral animals (such as cross-breeding of GEd animals with captive feral animals) and the results of such work.

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e. Details of the effect that the entry of the novel genetic material into a feral gene pool might have contribution in the spread of infectious disease.

f. Details of the management procedures and environmental factors, if any, that would be required for optimal expression of the introduced trait (s).

3. <u>Information about future dealings with the GEd animals(s)</u>

a. Details of whether an animal in the experiment is intended to be allowed to breed and, if not, whether breeding is planned in the future.

b. Details of whether the proposed arrangements for handling any offspring are the same as those for the experimental animal(s), and, if not, the proposed different arrangements

c. Has the proposed work been reviewed by the Institutional Animal Ethics Committee? Provide details.

d. Details on fulfilment of requirements of the proposed work with relevant state animal welfare legislation.

8.7. Specific data requirements for genome editing in human somatic cells

In case of genome editing in somatic cells of humans, RCGM would evaluate molecular characterization data before prescribing any pre-clinical studies. Following data requirement needs to be met in case of human GEd somatic cells:

A. Multiplication, sterility and storage of GEd somatic cells for therapeutics

i. For *ex vivo* GEd therapeutics, microbial contamination studies should be performed.

ii. In the case of *in vivo* GEd therapeutics, viral vectors should be tested and shown to be free of pathogenic microbial and mycoplasma contamination.

iii. Details on the multiplication of edited somatic cells and their storage conditions (personalized iPSC) should be provided along with viability data generated over a period time.

B. Biocontainment facilities to be used for conducting research

i. All the experiments are to be carried out in designated contained laboratory of BSL-1 or -2 only after obtaining the required approvals from the competent authorities.

ii. Handling of cells, equipment, decontamination and disposal of waste to be as per the DBT guidelines for Biosafety containment level (Regulations and Guidelines on Biosafety of Recombinant DNA Research and Biocontainment – 2017).

For human stem cells, the genetic materials for delivery of genome editing nucleases are not markedly different from those for conventional *ex-vivo*/*in-vivo* gene therapies. Therefore, quality control and efficacy evaluations of *in-vivo* genome editing therapeutics can be considered in the context of existing guidelines of gene therapy and genetically modified cells.

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Similarly, existing gene/cell therapy guidelines can be applied to *ex-vivo* genome editing therapeutics. However, in case of novel genome editing mechanisms using exogenous nucleases, complete assessment of the new protein needs to be carried out to ensure safety of the newly introduced protein and its effect in the receiving cells/ organism.

Further, for clinical studies, all the requirements to get approvals of relevant ministries/ Departments like CDSCO and ICMR need to be fulfilled. Some points to be considered at the time of assessment are: immunogenicity by any of the components (like nucleases, delivery vehicle etc) involved in modification, pharmacokinetics and bio-distribution, dosing, potential tumorigenicity of induced pluripotent cells etc.

9. Institutional Mechanisms for Governance and Oversight

The institutional mechanisms in genome editing shall have two layers i.e. Self-Governance and Institutional.

Self-Governance

i. Persons who are actively engaged in genome editing related activities including scientists, researchers, etc., shall be liable to adhere to the principles of self-governance:

- Must be aware of the applicable acts, rules, regulations and guidelines wherever applicable and should avoid conduct of prohibited/ restrictive research or activity.
- Ensure prior approval of competent authority and the adoption of appropriate safety and security measures before the commencement of the work
- Adhere to good laboratory practices and follow the rules of responsible research conduct[12,13].
- Restrict themselves from working in silos rather work cohesively in terms of risk assessment, risk management, and possible intellectual rights.

ii. For collaborative research, both parties shall remain informed and adhere to self-governance and must be familiarized with local, national and international rules and regulations duly supported by memorandums of understanding (MoU) and material transfer agreements (MTA) and necessary approval from collaborating institutes.

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[12]Code of Conduct for responsible Research, 2017. https://www.who.int/about/ethics/code-of-conduct-responsible-research.pdf

[13]Regulations & Guidelines for Recombinant DNA Research & Biocontainment, 2017.

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Institutional Governance & oversight

i. In India, stringent institutional oversight mechanisms exist to control activities within an Institution and act as an interface of communication between the Institution and the national governing bodies:

Institutional Committee/Agency	Competent authority under which Notified/Established under	Responsibilities of the institutional Functions
IBSC[14]	Competent authority notified under Rules 1989 of EPA 1986	IBSC is the nodal point for the implementation of the biosafety regulatory framework in India. The IBSC is responsible for preparation of an up-to-date on site emergency plan according to the manual (guidelines) of RCGM and to keep an oversight on r-DNA research work.
IAEC[15] Institutional Animals Ethics Committee"	Competent authority established under the Breeding of and Experiments on Animals (Control and Supervision) Rules 1998 under the Prevention of Cruelty to Animals Act, 1960	To supervise the trade of animals for the purpose of experiments and control and supervision of experiments on animal for the purpose of breeding animals.
IEC[16]	Committee to safeguard the dignity, rights, safety, and well-being of all research participants	Initial review of research proposals prior to their initiation, and regularly monitoring the approved research to ensure ethical compliance during the conduct of research.
IC-SER[17]	Committee established to comply with the NGSCR and existing regulatory framework.	Oversees all stem cell-related research activities and/or clinical trials in an

[14]For details refer Guidelines and Handbook for Institutional Biosafety Committee 2011.

[15]For details refer Compendium of CPCSEA 2018.

[16]For details refer National Ethical Guidelines for Biomedical and Health Research Involving Human Participants, Indian Council of Medical Research (ICMR), 2017.

[17]For details refer National Guidelines for Stem Cell Research, 2017.

		Institution through review, report, and training.
GTAEC[21]	Committee shall provide a hand holding for the investigators/ industry	To advise trial sponsors in designing and rigorously monitoring all first in-human or existing GTP trials in India and also give pre-IND consultations

Governance & oversight at national level

Regulatory agencies shall ensure oversight mechanism for compliance of all genome editing related activities are properly abided by all concerned as per applicable national laws, Acts & Rules and international treaties to which India is a signatory.

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[21]For details referNational Guidelines for Gene Therapy Product Development and Clinical Trials (2019)

OTHER APPLICABLE GUIDELINES and REFERRED DOCUMENTS

1.	Guidelines for the Safety Assessment of Foods Derived from Genetically Engineered Plants (ICMR, 2008)
2.	Protocols for Food and Feed Safety Assessment of GE crops (DBT, 2008)
3.	Regulations and Guidelines on Biosafety of Recombinant DNA Research and Biocontainment, 2017
4.	Risk Analysis Framework, 2016
5.	Guidelines for the Environmental Risk Assessment of Genetically Engineered Plants, 2016
6.	UNEP (Guidance On Risk Assessment Of Living Modified Organisms, 2012)
7.	Cartagena protocol (Risk Assessment of LMOs with Stacked Genes or Traits)
8.	OGTR (Discussion paper: Options for regulating new technologies, 2016)
9.	OECD (Report of the OECD Workshop on Environmental Risk Assessment of Products Derived from New Plant Breeding Techniques, 2016)

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GLOSSARY

Term	Definition
Agrobacterium tumefaciens	A naturally occurring soil bacterium (also known as *Rhizobium radiobacter*) that can transfer a part of its DNA (T-DNA region of plasmid/vector) into plant cells.
Artificial target locus	A locus that has been introduced via genetic engineering and can then be targeted by site-directed nucleases.
Backcross	A cross between a hybrid and one of its parents. Subsequent backcrosses of offspring to same (recurrent) parent produce offspring of increasing similarity to that parent.
Cisgenic	Transformation of recipient organism with a donor DNA/ rDNA from a crossable/ sexually compatible organism (same species or closely related species).
Clustered Regularly-Interspaced Short Palindromic Repeat (CRISPR)	A genomic locus in bacteria or archaea where protospacers and direct repeat sequences are arrayed in tandem. It is associated with adaptive immunity against invading phages and plasmids.
Donor DNA	DNA that is introduced into plant in order to serve as a template during genome editing or gene targeting.
Domain	A discrete portion with its own function. The combination of protein domains in a single protein determines overall function of a protein.
Double Strand Break (DSB)	Cleavage in both strands of double-stranded DNA where the two strands have not separated.
Edited genomic DNA	Refers to the portion of an organism's genome that has been intentionally altered by targeted genome editing.
Endonuclease	An enzyme that cleaves the phosphodiester bonds within nucleic acid molecules.
Engineered Meganuclease	Microbial derived meganucleases that are modified, fused, or rationally designed to cause site-directed DSB. Also referred to as LAGLIDADG endonucleases or homing nucleases.
***Fok*I**	A type IIS restriction enzyme found in *Flavobacteriumokeanokoites* that is composed of a separable DNA-binding domain and a nuclease domain that is used to construct ZFNs and TALENs.

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Genome Editing	Genetic engineering where one or more nucleotide(s) are intentionally substituted, inserted, or deleted from a genome, mostly using programmed nucleases/nickases.
Gene pool	Entire genes and alleles of a defined population of organisms at a given time.
Germplasm	Collection of genetic stocks (genotypes) of an organism.
Hazard	Any source that has the potential to cause an adverse effect on human health, animal health or the environment.
Homology-Directed Repair (HDR)	A mechanism for DSB repair using a DNA sequence homologous to the break site that serves as a template for homologous recombination.
Homologous Recombination (HR)	A genetic recombination process where two similar DNA strands exchange nucleotide sequences.
Insertions and deletions (Indels)	Small insertions or deletions of DNA sequences relative to a reference sequence.
Intragenesis	Transformation of a recipient organism that leads to a combination of different gene fragments from donor organism(s) of a sexually compatible species as the recipient.
Locus	The specific position of a gene or a DNA sequence on a chromosome.
Nickases	Enzymes that generate DNA single-strand breaks.
Non Homologous End Joining (NHEJ)	A means for repair of DNA double-strand breaks (DSBs) without the use of a homologous repair sequence. An error-prone process that often causes small substitutions, insertions, and/or deletions at the DSB site resulting in mutations.
Off-target changes	Changes in the genome made by programmable nuclease(s) at sites different from the target site in the genome due to limited specificity.
Oligonucleotide Directed Mutagenesis (ODM)	Site-specific mutation with chemically-synthesized oligonucleotide with homology to the target site.
Phenotype	The observable structural and functional properties of an organism, produced by the interaction between the organism's genetic potential (genotype) and the environment in which it finds itself.
Promoter	A DNA segment to which RNA polymerase binds to allow the initiation of the transcription of downstream (3') gene.
Recombination	The process of intermolecular exchange of DNA or chromosomes combining genetic information from

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	different sources. Site specific, homologous, transpositional and non-homologous (illegitimate) types of recombination are known.
RNA-guided engineered nucleases (RGENs)	Programmable nucleases composed of the nuclease/nickase protein and a guide RNA.
Rules, 1989	The rules for the manufacture, use/import/export and storage of hazardous microorganisms/ genetically engineered organisms or cells, 1989 notified under the Environment (Protection) Act, 1986.
Segregation	The separation of allele pairs from one another and their distribution to different cells, usually at meiosis and sometimes at mitosis.
Site Directed Nuclease (SDN)	Engineered DNA nucleases that are programmed to specific sites within the genome where they cleave a DNA chain by separating nucleotides.
T-DNA	DNA encoded on Ti plasmid of *Agrobacterium* that is transferred to the plant cell.
Targeted Genome Engineering	Modification of the genome at a precise, predetermined locus.
Transcriptional Activator-Like Effector Nuclease (TALEN)	Programmable nucleases comprised of the DNA binding domain of *Xanthomonas*-derived TAL effectors usually fused with *Fok*I restriction endonuclease.
Transgenesis	Introduction of genetic information into cells from non-sexually compatible species that leads to the transmission of the input genetic information (transgene) to successive generations.
Zinc Finger Nuclease (ZFN)	Programmable nucleases comprised of the DNA binding domain of a zinc-finger protein and the DNA-cleaving nuclease domain usually from the *Fok*I restriction endonuclease.

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