VOL 19 NO 10 OCTOBER 2022

South Asia Biosafety Program

NEWSLETTER FOR PRIVATE CIRCULATION ONLY - NOT FOR SALE



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INDIA

Standard Operating Procedures for Regulatory Review of Genome Edited Plants under SDN-1 and SDN-2 Categories, 2022

Dr. Arlene Asthana Ali, Biotech Consortium India Limited



These SOPs are targeted to meet the

threshold for exemption from the

provisions of the Rules, 1989, i.e., the

genome edited plant(s) must fall within

SDN-1 and SDN-2 categories and must

be free of exogenous introduced DNA.

Wheat field in India © Ram Kumar Verma | Dreamstime.com

In India, genome edited plants under categories SDN-1 and SDN-2 have been exempted from the provisions (Rules 7 to 11) under Rule 20

of the Rules for the Manufacture, Use/Import/ Export and Storage of Hazardous Micro Organisms/Genetically Engineered Organisms or Cells, 1989" (Rules, 1989) of the Environment Protection Act, 1986, as per the Ministry of Environment, Forest and Climate Change (MoEF&CC) Office Memorandum F. No. C-12013/3/2020-CS-

III, issued on March 30, 2022. Subsequently, the Department of Biotechnology (DBT) issued "Guidelines for the Safety Assessment of Genome Edited Plants, 2022" that provide for regulatory pathways and information/data requirements for the safety assessment of SDN-1, SDN-2, and SDN-3 categories of genome edited plants.

DBT has now notified "Standard Operating Procedures (SOPs) for Regulatory Review of Genome Edited Plants under SDN-1 and SDN-2, 2022" vide Office Memorandum F. No. PID-15011/1/2022-PPB-DBT

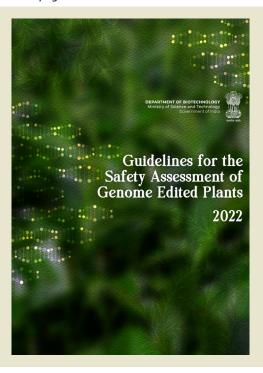
dated October 4, 2022. These SOPs are targeted to meet the threshold for exemption from the provisions of the Rules, 1989, i.e., the genome

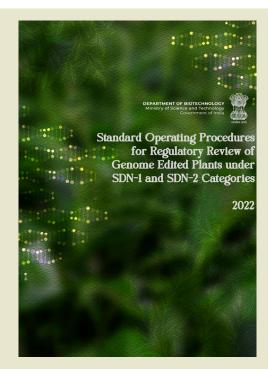
edited plant(s) must fall within SDN-1 and SDN-2 categories and must be free of exogenous introduced DNA. These SOPs are applicable only for research activities under containment and will be relevant for all organizations involved in research, development, and handling of SDN-1 and SDN-2 genome edited plants, from the date of notification.

The SOPs provide a step-wise guidance covering the following aspects:

- Initiating research and development of genome edited plants
- Suggested procedure for handling genome edited plants
- · Seeking exemption from Rules, 1989
- Import of SDN-1 and SDN-2 genome edited plants/seeds/propagules for research, testing, and product development
- · Record keeping

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Cover images of the "Guidelines for the Safety Assessment of Genome Edited Plants, 2022" and "Standard Operating Procedures for Regulatory Review of Genome Edited Plants under SDN-1 and SDN-2 Categories, 2022."

A flowchart indicating the steps in the process and the associated information requirements to be submitted to regulatory authorities *viz.* Institutional Biosafety Committee (IBSC) and Review Committee on Genetic Manipulation (RCGM), have been provided. Protocols to show that the gene edited plants are free from exogenous introduced DNA include demonstrating the absence of selection/scorable marker and absence of vector DNA using overlapping Polymerase Chain Reaction (PCR)/nested PCR. Other methods can be used only if these have the same level of stringency and with permission from IBSC. To ensure consistency among investigators, a method of nomenclature for genome edited lines that are free from exogenous introduced DNA has also been described.

A set of formats to ensure reporting to the regulatory authorities has been included:

 Format for information and review on SDN-1 and/or SDN-2 genome edited plants to IBSC.

- Checklist for information on SDN-1 and/or SDN-2 genome edited plants to IBSCs.
- Format for seeking permission to import SDN-1 and/or SDN-2 genome edited plants for research, testing, and development purposes.
- Format for communicating confirmation of the absence of exogenous introduced DNA from SDN-1 and/or SDN-2 genome edited plants by IBSCs.

An addendum clarifying certain requirements listed in the "Guidelines for the Safety Assessment of Genome Edited Plants, 2022" but not found necessary by the expert committee for SDN-1 and SDN-2 categories of genome edited plants has also been included. These SOPs are expected to support research and development of genome edited plants in the country.

A copy of the SOPs can be accessed at:

https://dbtindia.gov.in/sops-for-regulatory-review and https://ibkp.dbtindia.gov.in/Content/Rules

BANGLADESH

Genome Editing: Evolving Global Landscape

Hridi Prova Saha, Brac University

STUDENT SHOWCASE

To encourage written discourse on topics related to biosafety and biotechnology among the younger generation, the SABP Newsletter dedicates space in select issues to spotlight pieces written by students residing in South Asia. Since articles with the "Student Showcase" tag are meant to reflect the actual views and capabilities of the author(s), they are not revised for content and only lightly edited to conform with the newsletter's style guide.

According to the Cartagena Protocol on Biosafety, "living modified organism" means any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology. Since the beginning of the use of genome editing in research and product development, a broad public discourse on the approach to be taken for genome edited organisms for commercial release has been going on. The discussion has revolved around whether a genome edited organism should be treated as genetically modified organism (GMO) or

similar to an organism produced through natural or induced mutagenesis. To answer this, it is reasonable to understand the techniques used in the production of genome-edited organisms. Moreover, it is imperative for scientists, policymakers, and the public to understand genome editing in the context of traditional breeding so that an informed decision may be made, keeping in view the need or not for a pre-market safety assessment, which is a vital prerequisite for all LMOs/GMOs.

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Red grapefruit on tree. Many commercially significant grapefruit market cultivars can be traced back to a series of natural budsports or induced mutations of the original white grapefruit. © Karin De Mamiel | Dreamstime.com

The insertion of foreign genes is

avoided in most types of CRISPR/

Cas9 edits as through this technique,

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introduced (in SDN-1 and SDN-2).

Let us first understand what happens in nature. In nature, sometimes the chromosome breaks and through an inherent repair mechanism they join back. However, while doing so, changes in the genome sequence may happen. Such changes are known as point mutations. When scientists make similar changes in an organism's genome in a targeted manner, then it is known as genome editing. Genome editing can be achieved by a few techniques, including Zinc Finger Nucleases (ZFNs), Transcription Activator-Like Effector-based Nucleases (TALEN), and the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR/Cas) system. The most well-known system in the genome editing toolbox is CRISPR/Cas9, for which Emmanuelle Charpentier and Jennifer Doudna received the Nobel Prize in 2020.

Let us take a closer look into the CRISPR system. There are two components—a guide RNA (gRNA) targeting the gene of interest and an enzyme, CRISPR associated protein also known as Cas. The gRNA contains one portion called crRNA to target the sequence and another component,

tracer RNA, which helps to anchor with Cas. The Cas nuclease is directed to a specific region of the genome by the gRNA, where it creates a DNA double-strand break (DSB) upon target site recognition. Genome editing through site-directed nucleases (SDNs) encompasses different types, which include SDN-1, -2, and -3, etc. SDNs that induce small-sized, undirected alterations at the target site are called site-directed nuclease-1 (SDN-1). SDN-2 uses template-guided repair by homologous recombination to introduce a specific DNA sequence replacement in the genome, while SDN-3 inserts larger genetic elements (e.g., full genes) in a similar manner as transgenic research.

Originating from the bacterial immune system, the CRISPR/Cas9 system is a very precise and efficient tool for small base pair editing, thus introducing point mutations in desired genes. As this technique depends on precise editing of the desired gene sequence, it is necessary to ensure that the target sequence is efficiently recognized and cut accordingly. Scientists take the help of bioinformatics, thus genome sequencing the organism to identify the desired sequence for editing.

To cut the target sequence at that precise site, CRISPR nuclease plays an important role. Scientists have discovered Cas9 and Cas13, which cuts DNA and RNA, respectively, to achieve site-specific editing. Thus, the chance of off target genome editing is tackled. Still, for further confirmation, screening of the resulting product for the mutation is done. At this stage, whether editing has occurred or not, and whether it has occurred with precision or not, is checked. However, before these products are actually used, their traits (functional effect) are assessed and confirmed. Through these evaluations, finally the target point mutation products are selected for human benefit, answering all the concerns over the technology and the product.

Now, let us compare various processes of genetic/genome modification. Conventional breeding relies primarily on crossing and selecting offspring with the desired characteristics. Mutation breeding is the process of exposing seeds to mutagens to generate mutation in their genomes, producing desirable traits to be bred with

other cultivars. Both of these, after selection, readily go for commercial cultivation. In transgenic organisms, the desired gene(s) is introduced from an unrelated organism through recombinant DNA technology or modern biotechnology. In contrast, genome editing is a target-specific mutation resulting in changes in the genome, including point mutations, small insertions or deletions, allele replacement, and gene insertions.

While conventional breeding products are readily accepted, there is often major hindrances to the approval of products of modern biotechnology, especially GMOs. The major issue raised against GMOs is the insertion of foreign DNA (DNA from other organisms) that may have deleterious effects on the environment and human health. The insertion of foreign genes is avoided in most types of CRISPR/Cas9 edits as through this technique, mostly the endogenous genes are edited. No foreign genes would be introduced (in SDN-1 and SDN-2). The resulting offspring does not contain remnants of the scissors, only retaining the edited sequence. Thus, they are not fundamentally different from

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natural variants where such mutations might occur naturally. Therefore, compared to GMOs, the genome edited organisms using the CRISPR/ Cas9 system show genome modification, which is like naturally and conventionally bred organisms. Moreover, the CRISPR edited plants can produce transgene-free offspring, which should not have any issues for health safety, as traditionally mutated organisms are considered as safe.

CRISPR can be used for an array of benefits to agriculture, medicine, and human beings. To take advantage of the technique, before the edited lines can be introduced into breeding programs and especially, be used as a product, the country that intends to use the lines has to look into its regulatory framework. Different countries have adopted different regulation strategies based on their perception of whether they consider genome-edited organisms as equivalent to products of conventional breeding or GMOs. The USA, Canada, and four South

American countries have implemented regulations that categorize genome-edited crops (SDN-1 and SDN-2) as equivalent to products of conventional breeding. Russia has also categorized transgene-free edited crops as equivalent to those generated by conventional breeding. In the African continent, so far, two countries, Nigeria

and Kenya, have implemented regulations for a case-by-case review of genome-edited crops. Of these, Nigeria is the first country to adopt decision making through biosafety regulations on genome editing. If edited lines do not contain a new combination of genetic material, they can be considered as conventional varieties or products. However, the European Court of Justice categorized genome-edited plants with GMOs. The genome-edited organisms are regulated under the full provisions of Directive 2001/18/EC for the deliberate release of GMOs in 2018. Thus, in the EU, all genetically engineered organisms, including plants altered by SDNs, need to undergo an environmental, as well as food and feed risk assessment. But, this opinion is being revisited, with 20 out of 26 member states in favor of exempting genome edited plants from biosafety, as the technology has far greater precision and there is no need for marker genes. The Commission is currently working on rewriting the legal framework. The UK is also in favor of genome editing technology and is in the process of making rules regulating research on genetically engineered crops. In 2019, Japan became the first Asian country that did not distinguish between traditional breeding methods and genome editing in terms of safety. On March 30, 2022, the

Indian Government signed the Office Memorandum "Exemption of the Genome Edited plants falling under the categories of SDN1 and SDN2." The memorandum states that work with genome-edited plants must be carried out under strict safety precautions, until it can be ensured that exogenous introduced DNA is no longer present. The guidelines cover genome-edited plants produced by SDN-1 and SDN-2. If validated to be free of transgenes, they are exempt from the current GMO regulations and can be released as a new variety and used for further development and evaluation. In May 2022, the Department of Biotechnology, Government of India, released "Guidelines for the Safety Assessment of Genome Edited Plants, 2022," which provides detailed guidance on the regulatory requirements. Notably, Bangladesh, Nepal, Sri Lanka, and Cambodia have "seeds without borders" agreements in place that will likely lead to harmonization of genome editing guidelines.

One report says that 52 percent of the world's population lives in countries with a positive or partially positive outlook to exempt genome editing from rigorous biosafety assessment. In most of the other countries, details of the procedure for classifying genome edited organisms are still being worked out. The CRISPR/Cas gene

editing tool is undoubtedly very easy to use and fast, the gene editing tool at present having an enormous potential to bring another revolution in science, especially in sustainable agriculture and food security. The most important task for the scientific community is to convey the potential advantages and precision of the tool so that its products are realized in a timely manner to ensure harvesting the benefit of the technology.

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Powdery mildew on wheat leaf. Genome-edited powdery mildew resistance in wheat is an example of gene-edited plants that have been created using SDN1 to generate larger indels © Abdelmoumen Taoutaou | Dreamstime.com

Arctic Fuji Apple: A Special Apple with Prolonged Freshness

Joya Prottasha Das, Brac University

STUDENT SHOWCASE

To encourage written discourse on topics related to biosafety and biotechnology among the younger generation, the SABP Newsletter dedicates space in select issues to spotlight pieces written by students residing in South Asia. Since articles with the "Student Showcase" tag are meant to reflect the actual views and capabilities of the author(s), they are not revised for content and only lightly edited to conform with the newsletter's style guide.

Almost 50% of the apples grown in the United

States is discarded due to this browning or

to unappealing swelling. Addressing this

Apple is considered a very nutritious fruit as it contains high fiber and antioxidants. It has several health advantages, including a reduced risk of many chronic diseases such as diabetes, heart disease, and

cancer. Because of these health benefits and its exquisite flavor, apple is a popular and common snack all over the world and hence, apple trees are grown worldwide. However, due to unappealing swelling by the browning of completely edible apples, this delicious fruit ends up as food waste. Almost 50% of the apples grown in the United States is

discarded due to this browning or to unappealing swelling. Addressing this natural phenomenon of browning of apple, an agrarian biotechnology organization situated in Summerland, British Columbia, Canada named Okanagan Specialty Fruits Inc. (OSF) has genetically modified a

series of apple varieties, which they named Arctic Apples. In 2016, Arctic Fuji Apple, made by modifying the Fuji apple cultivar, was OSF's third non-browning apple, following Arctic Golden Apple and Arctic Granny

> Apple of the Arctic Apple series. Fuji apple was chosen for this trait improvement because of its popularity, due to its sweetness and crispiness. To keep the Fuji apple fresh and nonbrowning for longer, even with bruising and cutting, OSF inserted a chimeric Polyphenol Oxidase (PPO) suppression element derived from apple PPO sequences.

Scientists found that the browning of apple flesh occurs following damage, cutting, or bruising due to an enzymatic reaction catalyzed by polyphenol oxidase (PPO). In the cell, phenolic substrates and PPO

natural phenomenon of browning of apple, [...] Okanagan Specialty Fruits Inc. (OSF) has genetically modified a series of apple varieties, which they named Arctic Apples. are separately compartmentalized, with phenolic substrates located in



Browning apple sliced in half © Dpullman | Dreamstime.com

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the vacuole and PPO in plastids. When cells are damaged, this compartmentalization is lost, and browning happens due to their interaction. However, if there is little to no presence of PPO in the cell, then the cell disruption can be avoided, keeping the apple fresh. Considering this hypothesis, OSF employed the technique of reducing the activities of PPO by inserting a PPO suppression cassette. Transcription of the PPO transgene suppression construct resulted in suppression of endogenous genes, which form dsRNA and trigger an RNA interference response. As a result of the destruction of endogenous mRNAs, reduced levels of the PPO protein accumulate in the plastids, leading to decreased PPO activity in the transformed apples.

Now, to determine the level of enzymatic activity in Arctic Fuji in comparison to unmodified Fuji, the PPO levels and enzymatic browning

in response to mechanical bruising of mature fruit were studied. Compositional and nutritional evaluations were also performed to determine whether Arctic Apples are equivalent to untransformed apples. According to the published data provided by the United States Department of Agriculture (USDA) on nutrient values for

apples, the composition of Arctic Apples and their natural counterpart are approximately close in terms of fat, protein, moisture, ash, carbohydrates, calories, and sugar profile and nutritionally equivalent for dietary fiber. However, Arctic Apples were found to have significantly higher levels of potassium, phenolics, and vitamin C than conventional Fuji apples, though the difference for phenolics was not statistically significant.

OSF did an FDA consultation regarding Arctic Fuji Apples since accumulated evidence showed substantial equivalence, i.e., no difference from their traditional counterparts in terms of safety and nutritional characteristics. FDA evaluated OSF's submission to determine if Arctic Fuji Apples raise any safety or regulatory issues with respect to the intended modifications or with respect to their use in human or animal food. FDA did not identify any safety or regulatory issues under the United States Federal Food, Drug and Cosmetic Act that would require further evaluation based on the information provided by the company and other information available to the agency. The USDA Animal and Plant Health Inspection Service (USDA APHIS) granted approval to Arctic Fuji on September 23, 2016, and the Canadian Food Inspection Agency and Health Canada gave approval on January 30, 2018 for food, feed, and cultivation.

USDA APHIS evaluated the plant pest risk of Artic Fuji Apples by assessing their similarity to the deregulated OSF ancestor apple events. From the similarity assessment, they concluded that from the transformation process, the insertion or expression of new genetic material, or from changes in metabolism, there is no plant pest risk in modified apples. They also concluded that there are no plant pest effects expected on these or other agricultural products and no impacts are expected to APHIS pest control programs. From the similarity studies, Artic Fuji Apples are unlikely to adversely impact non-target organisms and unlikely to become weedier than the antecedents, which are not weedy. Additionally, these were found not to pose any significant changes to agricultural or cultivation practices. Thus, modified Arctic Fuji Apples can be consumed as a great nutritious snack option that can be kept fresh without any further processing.

As Arctic Apple is found to be similar to the natural apple, it has successfully entered supermarkets in the USA and Canada.

> The improvement of apple is not restricted to the use of genetic engineering technology. Recently, researchers have used genome editing to demonstrate its application in apple to introduce beneficial characteristics, such as virus resistance, overall quality improvement, and reduced fire blight susceptibility. Gala Apple is

one such success story.

Globally, food and nutritional security is a huge challenge. Food waste makes this more challenging. Improvement of food through modern biotechnology can contribute to addressing food security.

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overall quality improvement, and

reduced fire blight susceptibility.

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Fire blight on apple tree © Viesturs Kalvans | Dreamstime.com

CALENDAR OF EVENTS			
EVENT	ORGANIZED BY	DATE	WEBSITE
BANGLADESH Conference on Genome Editing in Plants: Harnessing the Benefits for Bangladesh	Bangladesh Academy of Sciences (BAS), Bangladesh Agricultural Research Council (BARC), South Asia Biosafety Program (SABP), Agriculture & Food Systems Institute (AFSI), and Biotech Consortium India Ltd. (BCIL)	October 18-19, 2022 Dhaka	https://foodsystems.org/event/ ge-ag-bangladesh-2022- conference/
INDIA			
SUGARCON 2022 and 7 th IAPSIT International Sugar Conference	ICAR-Indian Institute of Sugarcane Research and Society for Sugar Research & Promotion	October 16-19, 2022 Lucknow	http://sugarcon2022.com/
International Conference on Advances in Biotechnology: Research, Innovations, and Entrepreneurial Venues - An Outlook	PG Department of Biotechnology, Dwaraka Doss Goverdhan Doss Vaishnav College	October 19-20, 2022 Online	https://forms.gle/ QWLJa4UZukWRTP2q9
International Conference on Existing Climate Change Scenario & Its Arising Risks	Mahima Research Foundation and Social Welfare and Sher-e-Kashmir University of Agricultural Sciences & Technology	October 21-22, 2022 Jammu	http://www.skuast.org/events. php
Hands On Training: Basics of DNA Fingerprinting	Tamil Nadu Agricultural University (TNAU)	November 14-18, 2022 Coimbatore	https://tnau.ac.in/news-events/ https://docs.google.com/forms/ d/e/1FAlpQLSdvcL8lpdxYEQ3gD lf2o6J3wfxem8gGdJ41kEqVflyQ V9kWTg/viewform
19 th Biennial International Conference on Nutritional Technologies to Augment Livestock, Poultry, Canine and Fish Production for Global Competitiveness	Animal Nutrition Society of India (ANSI) and Guru Angad Dev Veterinary and Animal Sciences University (GADVASU)	November 16-18, 2022 Ludhiana	https://ansi.org.in/conferences/
1st National Conference on Plant Genetic Resource Management (NCPGRM 2022)	Indian Society of Plant Genetic Resources (ISPGR), ICAR-IARI, ICAR- National Bureau of Plant Genetic Resources (NBPGR), Alliance of Bioversity International, and CIAT-India Office	November 22-24, 2022 New Delhi	http://www.nbpgr.ernet.in/
National Conference on Biotechnology for Sustainable Development and Human Welfare	Department of Biotechnology, School of Chemical and Life Sciences, Jamia Hamdard	November 23-24, 2022 New Delhi	https://jamiahamdard.edu/
International Conference on Biotechnology, Sustainable Bioresources, and Bioeconomy	Indian Institute of Technology Guwahati	December 7-11, 2022, Guwahati	https://www.iitg.ac.in/iitg_ events_all https://www.bsb2iitg2022.in/
International Conference on System of Crop Intensification (ICSCI 2022) for Climate-Smart Livelihood and Nutritional Security	The Society for Advancement of Rice Research	December 12-14, 2022, Hyderabad	https://www.icar-iirr.org/ ICSCI%20Brochure-2022.pdf
International Conference on Food and Nutritional Security (IFANS-2023)	National Agri-Food Biotechnology Institute (NABI), Centre for Innovative and Applied Bioprocessing (CIAB), Indian Council for Agricultural Research-National Institute of Plant Biotechnology (ICAR-NIPB), and International Centre for Genetic Engineering and Biotechnology (ICGEB)	January 6-9, 2023 Mohali	https://neist.res.in/iswcpc/

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CALENDAR OF EVENTS

EVENT	ORGANIZED BY	DATE	WEBSITE	
INDIA				
International Seminar and Workshop on CRISPR/Cas Based Plant Functional Genomics and Computational Modeling (ISWCPC-2023)	CSIR-North East Institute of Science and Technology (NEIST)	January 18-21, 2023 Jorhat (in-person and online)	https://neist.res.in/iswcpc/	
International Conference on Pulses: Smart Crops for Agricultural Sustainability and Nutritional Security	Indian Society of Pulses Research and Development (ISPRD), ICAR- Indian Institute of Pulses Research (IIPR), and Indian Council of Agricultural Research (ICAR)	February 10-12, 2023 New Delhi	https://iipr.icar.gov.in/	
2 nd Indian Rice Congress - Transforming Rice Research: Learning from Recent Scientific Developments and Global Food Crisis	The Association of Rice Research Workers (ARRW) and ICAR-National Rice Research Institute (NRRI)	February 11-14, 2023 Cuttack	https://icar-nrri.in/	
INTERNATIONAL				
8 th Plant Genomics and Gene Editing Congress: Asia	Global Engage Ltd.	November 15-16, 2022 Kuala Lumpur, Malaysia	https://www.global-engage. com/event/plant-genomics- asia/?inf_contact_key=b30e05 13e19017c3427d97d729e061a 1dcd43eaff8ca03dc1d15424b3 c75c21d	
Asian Seed Congress 2022	Asia and Pacific Seed Association (APSA)	November 14-18, 2022 Bangkok, Thailand	https://web.apsaseed.org/ asc2022	
Fifteenth Meeting of the Conference of the Parties to the Convention on Biological Diversity (Part Two) Tenth Meeting of the Conference of the Parties serving as the Meeting of the Parties to the Cartagena Protocol on Biosafety (Part Two) Fourth Meeting of the Conference of the Parties Serving as the Meeting of the Parties to the Nagoya Protocol on Access and Benefit-Sharing (Part Two)	CBD Secretariat	December 7-19, 2022 Montreal, Canada	https://www.cbd.int/meetings/	
16 th ISBR Symposium	International Society for Biosafety Research	April 30-May 4, 2023 St Louis, Missouri	https://isbr.info/symposium	



The South Asia Biosafety Program (SABP) is an international development program implemented in India and Bangladesh with support from the United States Agency for International Development (USAID). SABP aims to work with national governmental agencies and other public sector partners to facilitate the implementation of transparent, efficient, and responsive regulatory frameworks for products of modern biotechnology that meet national goals as regards the safety of novel foods and feeds, and environmental protection.

Agriculture &

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SOUTH ASIA



BIOSAFETY PROGRAM

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