CHAPTER 14

APPLICATION FORMAT FOR IMPORT OF GENETICALLY MODIFIED ORGANISMS AND PRODUCTS THEREOF FOR FOOD, FEED AND PROCESSING (FFP)

When GMOs and/or products thereof have been adequately field tested and enough data accumulated to show that the experimental organisms and its products are free from any risk, an application may be made requesting that the said GMO or its products are safe and be allowed to considered for or crushing for food, feed and processing. The application/project proposal must contain sufficient detail, field performance data, as well as relevant molecular biology data, to ensure that the reader would be able to ascertain that the material poses no risk. The proposal/application may be including data obtained elsewhere particularly by the technology developer himself or his nominated person/Lab/institute/country (not necessarily by the applicant) whether favorable or unfavorable to the position of "no risk status".

The succeeding pages present an updated format for preparing an application.

IBC No.

National Bio-safety Committee (NBC),
Pakistan Environmental Protection Agency
Ministry of Climate Change &
Environmental Coordination,
Government of Pakistan, Islamabad.

Application for Permission to Import Genetically Modified Organisms (GMOs)/Living Modified Organisms (LMOs) Intended forDirect Use as Food or Feed, or for Processing (FFP)

GMO PRODUCTS TO BE IMPORTED FOR USE AS:

☐ Food ☐ Feed

☐ Processing ☐ Food, Feed and Processing

COUNTRY OF ORIGIN (Make separate entry for each Country)	NAME OF GMO PRODUCTS Scientific or English names must be included (*colloquial names are* not acceptable) List of Products, by-product and substances	QUANTITY PER ANNUM (in metric tons)	PAKISTANI PORT OF ARRIVAL/ DISCHARGE

Use additional pages if more space is needed and "X" box ☐

2	
	MEANS OF IMPORTATION/ Air Mail or Parcel Post [ ] Air Freight [ ] Car/ [ ] Surface Mail or Parcel Post [ ] Truck, Rail, or Ship [ ] Baggage [ ]

	Name & Signature of Applicant			
CNIC: NTN/STN				

	ENCLOSURES	ENCLOSED ("X")	IF PREVIOUSLY SUBMITTED LIST DATE & PERMIT/DIARY NO.
a.	The name and contact details of the applicant for a decision to grant the import license/permit.		
b.	Names, addresses, and telephone numbers of developer/supplier/distributor/exporter of the products, by-product and substances etc. of genetically modified organism (GMO) to Pakistan for the purpose of FFP.		
c.	Name and identity of the source GMO from which products, by- product and substances etc. have been obtained.		
d.	Description of the source GMO including: i) gene ii) gene modification/s (GM)and/or GM event/s iii) Event Code iv) Trade Name v) Gene Source		
e.	Description of the technique used for the gene modification of the source GMO (if available with the developer/exporter/supplier)		
f.	Description of resulting characteristics/trait of source GMO.		

g.	Detail of any unique identification of the source GMO.		
h.	Taxonomic status, common name and characteristics of recipient/parental organisms of the source GMO.		
i.	Center (s) of origin, IF KNOWN, of the recipient/parental organisms and a description of the habitats where the recipient/parental organisms may persist or proliferate.		
j.	Name of three (3) countries where approvals have been granted for use of this GMO trait/stack and the nature of approval in each of the countries [*e.g., for cultivation and/or for use as food, feed, or for processing (FFP)*		
k.	A risk assessment report of the source GMO which has been done at its country of origin. The risk assessment should have been evaluated and certified by the regulatory bodies of the country of origin of the source GMO.		
l.	Details of suggested methods for the safe handling, storage, transport and use, including packaging, labelling, documentation, disposal and contingency procedures.		
m.	Emergency response procedures that will be applied in Pakistan in the event of adverse consequences/ misuse of the product.		
n.	Details of the final disposition/use of the regulated article within purview of FFP		
o.	Name and Qualification of the Molecular Biologist/Biotechnologist/Agri-technologist who has prepared the dossier for the grant of the license.		
p.	Name of the Institutional Biosafety Committee (IBC) through which the dossiers of the application have been processed.		

* IBC may require additional details of gene(s), gene modification(s), genetic engineering processes, gene modification events, details of recipient/parental organisms of the source GMO, risk assessment and other data etc.

* In case of an ambiguity TAC/NBC may require samples and randomly perform/outsource (with justification) appropriate genetic identification test(s) to testify applicant's claims at the applicant's cost.

For more information, please refer to National Biosafety Guidelines 2005 (amended 2024), Pakistan Biosafety Rules 2005 (amended 2024) and Annexure II of Cartagena Protocol on Biosafety to the Convention on Biological Diversity.

CERTIFICATION

(To be submitted along with application on Rs.100 Stamp Paper)

I certify that the information given in this application is correct and I understand the consequence of giving false information, or violation of any terms of the import license for FFP shall subject the undersigned to:

(i) confiscation of the consignment;

(ii) penalty/fine under laws of Pakistan;

(iii) cancellation of the import license;

(iv) any other consequences under the laws of Pakistan.

Signed by applicant:

CNIC:_ _____

NTN/STN: _____

Date: _____

IBC No: _____

National Bio-safety Committee
(NBC) Pakistan Environmental
Protection Agency
Ministry of Climate Change &
Environmental Coordination,
Government of Pakistan, Islamabad.

IBC Permission

To Import GMOs for use as Food	To Import GMOs for use as Feed	To Import GMOs for Processing	Import of GMOs for use as Food, Feed, Processing

IBC Review

Specific Comments if any

Recommendation with/without Provisos
Signature of IBC Secretary
Date / /

14.1 THE APPLICATION MUST BE SUPPORTED BY THE FOLLOWING DATA/DOCUMENTS

1. Rationale for Development of the Organism/Product

2. The Organism or its Product
   a) Modified, Unmodified organism (e.g., Soybean, Maize, Mustard and canola etc.)
   b) Taxonomy of the organism
   c) Genetics of the organism
   d) Mode of reproduction
   e) Characteristics of the unmodified organism/product If the organism is a crop plant, please proceed to (f), (g), (h)and (i)
   f) Modes of gene escape in the environment
   g) Weediness (the organism is a crop or plant)
   h) Effect on Narrow or Broad leaf weeds
   i) Mode of action on Target and non-target insect pest

3. Description of the Modification/Transformation

4. Donor Genes and Regulatory Sequences
   a) Gene of interest
   b) Marker gene

5. Genetic Analyses of Field Performance
   a) Southern, Western and Northern Blot Analyses
   b) Expression/ELISA analysis of inserted genes
   c) Characteristics of the modification/improvement
   d) Characteristics of the specialized feature introduced as a modification.
   e) Mendelian inheritance
   f) Mycotoxins (in the case of plants)

6. Environmental Consequences of the Modification
   a) Specific advantages
   b) Current uses of unmodified organism/product, normal practices and disadvantages
   c) Proposed use of the modified organism/product with economic and social advantages
   d) Possible side-effects
   e) Effects on related organisms
   f) Vertical transfer of the new genes
   g) Horizontal transfer of the new genes

7. Adverse Consequences of the Modification

8. References

14.2 INSTRUCTION FOR THE PREPARATION OF THE APPLICATION FOR IMPORT OF GENETICALLY MODIFIED ORGANISMS AND PRODUCTS THEREOF FOR FFP

14.2.1 Description of the biology of the non-modified & modified recipient organism should include taxonomy, genetics, if the organism is plant, pollination, and evidence of reported weediness (e.g., noting whether the crop or sexually compatible species is listed in the relevant publications of the Weed Society of America) and discussion of sexual compatibility with wild and weedy free-living relatives in natural crosses or crosses with human intervention. The applicant should provide the source of recipient (cultivar name or accession number) and the weed status of its sexually compatible relatives.

14.2.2 The applicant should explicitly identify the lines to be considered in the application and the cultivars from which they are derived. If there are multiple lines, each line must be given a unique identifier and listed in the application. For virus-resistant plants, applicants should provide in an additional section on the nature of the virus that provided the sequences encoding the resistance phenotype:

i) The taxonomic name of the virus including family, genus, and strain designation including any synonyms;
ii) The type of nucleic acid contained in the virus;
iii) Whether the infection is systemic or tissue specific;
iv) Whether the virus is associated with any satellite or helper viruses;
v) The natural host range of the virus;
vi) How the virus is transmitted;
vii) If transmitted by a vector, the identity of the vector including mode of transmission (e.g., persistent or non-persistent);
viii) Whether any synergistic or trans-capsidation interactions with other viruses under field situations have been reported in the literature, and
ix) The location and the name of the host from which plant the virus was originally isolated.

The above information can be provided in a table format. This information can be Supplemented by listing references that report the host range, insect vectors, etc., for the virus.

14.2.3 For *Agrobacterium*-based transformation protocol, the applicant must indicate how Ti plasmid-based vector was disarmed (i.e., all tumorigenic DNA was removed). Applicants can provide citations that describe the transformation procedure. However, any significant modifications of transformation, strain designation, etc. should be described.

For other methods of transformation, the applicant can describe the sources of various components of the plasmid (or other DNA including possible carrier DNA) and method of transformation by citation. However, any significant modifications in transformation, tissue regeneration, etc. should be described.

14.2.4 The applicant must provide a detailed restriction map along with gene(s) name, gene host, insertion site of the gene(s) description and sequences of detection primers of the plasmid that is sufficient to be used in the analysis of Southern data. Description

of added restriction sites is helpful in interpretation of Southern data and should be provided.

14.2.5 In general, it is always prudent to analyze data statistically when such analysis is possible. When unpublished information or an opinion has been supplied by a scientific expert, a letter communicating the information should be included in the application. If the unpublished information provided is data resulting from scientific research, then these data can be provided as a personal communication either in a letter from the researcher or in the text of the project/application. In either case the materials and methods, data analysis, and discussion of the data analysis should be provided in detail. Unsupported assertions about the results of the experiment are not acceptable.

Applicants must report any differences noted between transgenic and non-transgenic plants that are not directly attributed to the expected phenotype. Differences observed could include changes in morphology, rates, other changes in overwintering capabilities, insect susceptibilities, diseases resistance, yield [if plants, pollen viability, seed germination and agronomic performance etc]. Applicants must also note the types of characteristics that were compared between unmodified and modified organism.

The applicant should describe whether data submitted are from inbred or hybrid plants; if hybrid plants, state which generation.

If the organism is Plant, please address the following:

14.2.6 State whether data with respect to plant performance were generated in a greenhouse or field environment. If from the field, indicate how many sites, states and number of years the data represents. Seed germination, seed dormancy, seed production, growth rate, and other data relating to the plant's performance will be required when the nature of the gene and the biology of the plant (including sexually-compatible relatives) warrant such data. This type of data will usually not be required for plants that are highly domesticated (e.g., corn), are exclusively self-pollinating (e.g., soybean), are male sterile, and have high seed germination rates (>90%), and whose phenotypes are unlikely to affect performance with respect to weediness or fitness (e.g., delayed ripening or oil seed modification). Phenotypes that might require performance data (depending on the plant) include but are not limited to the following: could tolerance, salt tolerance and tolerance or resistance to other biotic or abiotic stresses.

14.2.7 Southern analysis should include DNA isolated from non-modified & modified recipient, all or selected transformed lines, and the vector. Parental plasmid DNA (e.g., PUC 18) not containing intended donor genes may be labeled and hybridized to Southern blots to demonstrate that only the intended sequences have been incorporated in the genome of the transgenic plant. Restriction enzymes to be used might include enzymes that do not cut within the transforming plasmid but will cut the entire insert into one fragment from the DNA of the transgenic plant.

In the case of an *Agrobacterium*-based transformation system, the applicant should determine if genes that reside outside the LB/RB are inserted in the genome of the regulated cultivar. If a complete copy of any of these genes is present, the applicant should determine

whether it is expressed in the plant. For direct transformation systems, applicants should determine which sequences are inserted in transgenic plants and whether they are expressed. PCR analysis may be used to prove that only the targeted DNA has been incorporated. Sequencing of the transgene in plant and adjacent sequences is not required. Determination of the number of copies of integrated transgenes is not required, but the number of insertions may be used to support analysis of inheritance data.

14.2.8 If the inserted DNA sequence order is complex, as is often the case for plants engineered via direct transformation systems (e.g., electroporation, polyethylene glycol transformation of protoplasts, or particle bombardment techniques), the applicants should summarize the data by providing the following information for the all genes (whether under the direction of plant or bacterial promoters), is there a complete copy of the gene present in regulated article? Is the protein expressed in the plant? If multiple complete copies of a gene are present, applicants do not have to determine if each copy of the gene is expressed. It is very helpful to provide a table, that summarizes the results and indicates where specific data is to be found.

14.2.9 Mendelian inheritance data and Chi square analysis for at least 2 generations are appropriate to demonstrate whether the transgene is stable inserted and inherited in Mendelian fashion. Such data are generally not necessary for infertile vegetatively propagated crops such as male-sterile potatoes.

14.2.10 RNA-Northern analysis is generally not required except for virus-resistant plants. However, such analysis may be necessary for ribozyme, truncated sense, or antisense constructs, when protein levels cannot be provided.

14.2.11 Proteins-Expression levels of gene(s) of interest and marker genes in various tissues, developmental stages of plant, and experimental conditions (induced or non-induced) are required. Assays can be of enzyme activity. Serology, ELISA, and Western blots may also be used. Describing the source of the immunogenic is critical for serological analysis.

For virus resistant plants, the amount of viral transgene RNA produced should be determined and compared to the amount of the RNA produced by the viral gene in an infected non-transgenic plant. Applicants should address whether the transgenic RNA (or protein) is present in the same tissues as are infected during natural infections. In addition, provide the amount of both coat proteins (i.e., from the transgene and the naturally infecting virus) produced in the transgenic plant singly infected with the widely prevalent viruses in the U.S. that normally infect the recipient plant (contact NBC for the list of these viruses). For comparison, provide the amount of both coat proteins produced in the non-engineered plant in mixed infections of the virus from which the coat protein gene was derived and the same widely prevalent viruses used in the single infection study. Provide description of symptoms of infected plants in all cases.

14.2.12 For all diseases and pathogens surveyed, names of the diseases and the scientific names of the pathogens should be provided. Data from field tests in foreign countries are acceptable. If the data on diseases and pests were obtained in the foreign country, the applicant should submit information about the distribution of those pests; disease or pathogens in Pakistan or the sub-continent. Disease and pathogen susceptibility on wild type and transgenic plants should be determined preferably from natural infestations. However, if applicant must use direct

inoculations; i.e., with virus resistant transgenic plant, the source and taxonomic classification of the virus should be provided.

14.2.13 Certain plants have minute quantities of known toxicants which may adverselyimpact nontarget organisms and beneficial insects; e.g., tomatine in tomatoes, cucurbit in in cucurbits, gossypol in cotton etc., if such plants are recipients of transgenes, the applicant should provide information as to whether the level of toxicants is altered. If the plant produces no known toxicant, the applicant should state so and provide data or the reference to support the claim. Plant toxins can be assessed by the tests and criteria that plant breeders traditionally use in the crop. In some instances, this may be done qualitatively e.g., taste testing of cucurbits.

14.2.14 Assuming that the levels of known toxicants in the regulated organism (plants) are in acceptable range; that there were no notable differences reported between transgenic and nontragic plant; and that the gene(s) engineered into the recipient plant have no known reported toxic properties; then, toxicological data on effects of the plant on nontarget organisms and threatened and endangered species will usually not be required.

14.3 *Special Consideration*

Following special consideration shall be followed while considering the import for FFP.

i. To stop the leakage of imported grains the importer will maintain the traceability record of imported grains consumption this will be added in the terms and conditions of license issued in respect of biosafety clearance.
ii. The importer/developer of technology will submit cases for deregulation of genetic events related to imported grains, to NBC accompanied by complete certified risk assessment data report generated in the country of origin.
iii. The certified risk assessment report by relevant agency in the country of origin for imported grains meant for Food, Feed and Processing will be acceptable in Pakistan. However, PARC may conduct retest in exceptional cases, if a need arises.
iv. PARC will screen the candidate lines of soybean submitted in National Uniform Yield Trial for GM Testing.

These guidelines are contingent upon the applicability of the revision in Pakistan Biosafety Rules 2005 (amended 2024) and if the Rules are not revised as per Sunset Clause, as mentioned in sub-rule 2(2A) of Rule 14 of S.R.O 45(1)(2024) dated 18th Jan 2024, these guidelines will become infructuous in consistent with the Pakistan Biosafety Rules, 2005 (amended 2024).