Food and Agriculture Organization
of the United Nations

Technical summary report

Training Workshop on GM Food Safety Assessment in Bhutan: Using a Real Case Study

4-15 February 2019
Paro, Bhutan

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Regional Office for Asia and the Pacific
Food and Agriculture Organization of the United Nations
Bangkok, 2019

Required citation:
FAO. 2019. *Technical summary report - Training Workshop on GM food safety assessment: Using a Real Case Study.* Bangkok, 32 pp. License: CC BY-NC-SA 3.0 IGO.

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Abstract

Upon the official request of the Royal Government of the Kingdom of Bhutan, a training workshop entitled "Training workshop on GM food safety assessment: Using a real case study" was co-organized by the Bhutan Agriculture and Food Regulatory Authority (BAFRA) and the Food and Agriculture Organization of the United Nations (FAO) on 4 – 15 February 2019 in Paro, Bhutan. A total of 12 participants comprising Bhutan Biosafety Technical Working Group (TWG) members or their nominated representatives and BAFRA officials attended the workshop, which was run by an FAO Technical Panel Expert from Food Standards Australia New Zealand assisted by an FAO Food Safety Officer.

The International Rice Research Institute had provided its complete regulatory dossier (already submitted formally to several regulatory agencies) on biofortified rice event GR2E (Golden Rice) as an in-kind contribution to FAO, for the express purpose of providing an example of a 'real' application for developing countries to work through. Using this dossier, workshop participants were able to meet the objectives of the workshop, which were to provide:

1. An understanding of the various techniques that are used to generate the data for a genetically modified (GM) food application;
2. An appreciation of how to go about assessing the safety data
3. A understanding of the administrative, legal and communication activities associated with handling a GM food application
4. Recommendations for finalizing a guideline and information document for potential applicants.

Key words:
GM foods; food safety; capacity building

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Acknowledgements

Food and Agriculture Organization of the United Nations (FAO) would like to express its appreciation to the many people who contributed to the report. This report was prepared for FAO, and the development process was coordinated by, Masami Takeuchi (FAO). The first draft was prepared by Janet Gorst, FAO Technical Panel Expert from Food Standards Australia New Zealand (FSANZ, Australia) with inputs from Jambay Dorji, Bhutan Agriculture and Food Regulatory Authority (BAFRA) and finalized with review comments and feedback received from the participants and relevant government officials of Bhutan. Overall guidance from Somsak Pipoppinyo, FAO Representative to Bhutan/Nepal and contributions from several FAO Bhutan colleagues are also herewith acknowledged.

Abbreviations and acronyms

BAFRA	Bhutan Agriculture and Food Regulatory Authority
BLAST	Basic Local Alignment Search Tool
CCI	Confidential Commercial Information
DNA	deoxyribose nucleic acid
FAO	Food and Agriculture Organization of the United Nations
ELISA	Enzyme Linked Immunosorbent Assay
FASTA	Fast Alignment and Search Tool-All
FSANZ	Food Standards Australia New Zealand
GM	genetically modified
IRRI	International Rice Research Institute
MALDI-TOF MS	Matrix-assisted laser desorption/ionization-Time of Flight Mass Spectrometry
MRL	Maximum Residue Level
NBTs	New Breeding Techniques
OECD	Organisation for Economic Co-operation and Development
SDS-PAGE	Sodium dodecyl-Polyacrylamide Gel Electrophoresis
TWG	Bhutan Biosafety Technical Working Group

Executive summary

In Bhutan, non-viable forms of genetically modified (GM) foods and feeds are permitted if approved by the National Biosafety Board, while the environmental release of viable genetically modified organisms (GMOs) is prohibited. Ensuring the safety of imported GM food in the market is one of the main priorities for the Bhutan Agriculture and Food Regulatory Authority (BAFRA) within the Ministry of Agriculture and Forests, especially given Bhutan's reliance on significant imports to satisfy food needs. While BAFRA now has a basic enabling structure that includes a legal framework, aligned protocols and standard operating procedures it has yet to receive an application for a GM food safety assessment.

The body charged with assessing a GM food application under section 15(2) of the Bhutan *Biosafety Rules and Regulations 2018* is Biosafety Technical Working Group (TWG). This Group comprises members from a number of key agencies within Bhutan and is also responsible for advising on the technical and scientific issues related to GMOs.

Given that no application has yet been received, the International Rice Research Institute (IRRI) had provided its complete regulatory dossier on biofortified rice event GR2E (Golden Rice) as an in-kind contribution to Food and Agriculture Organization of the United Nations (FAO), for the express purpose of providing an example of a 'real' application for the Biosafety TWG to work through and gain experience from.

A training workshop entitled "Training workshop on GM Food Safety Assessment: Using a Real Case Study" was co-organized by BAFRA and the FAO from4 – 15 February 2019 in Paro, Bhutan. A total of 12 participants including Biosafety TWG members or their nominated representatives, and BAFRA officials attended the workshop, which was conducted by FAO Technical Panel Expert from Food Standards Australia New Zealand (FSANZ) assisted by an FAO Food Safety Officer.

Using the IRRI GR2E dossier, workshop participants were able to meet the objectives of the workshop, which were to provide:

1) An understanding of the various techniques that are used to generate the data for a GM food application;
2) An appreciation of how to go about assessing the safety data
3) A understanding of the administrative, legal and communication activities associated with handling a GM food application
4) Recommendations for finalizing a guideline and information document for potential applicants.

1. Introduction

1.1. Overview

Upon the official request of the Royal Government of the Kingdom of Bhutan, a training workshop entitled "Training workshop on GM Food Safety Assessment: Using a Real Case Study" was co-organized by the Bhutan Agriculture and Food Regulatory Authority (BAFRA) and the Food and Agriculture Organization of the United Nations (FAO) from4 – 15 February 2019 in Paro, Bhutan. A total of 12 participants including Bhutan Biosafety Technical Working Group (TWG) members or their nominated representatives and BAFRA officials attended the workshop. The list of participants is attached as Annex 1. The workshop was conducted by Janet Gorst, FAO Technical Panel Expert from Food Standards Australia New Zealand (FSANZ) assisted by Masami Takeuchi, FAO Food Safety Officer.

This workshop was follow-up of the introductory week-long training presentation on genetically modified (GM) food for a much wider audience in July 2018, which was focused specifically on the procedures involved for assessing a GM food application. The final report of the previous workshop is available at http://www.fao.org/3/CA1436EN/ca1436en.pdf.

The objectives of the present workshop were to provide:

1. An understanding of the various techniques that are used to generate the data for a GM food application;
2. An appreciation of how to go about assessing the safety data
3. An understanding of the administrative, legal and communication activities associated with handling a GM food application
4. Recommendations for finalizing a guideline and information document for potential applicants.

Prior to the workshop, a number of preliminary questions from the Technical Panel Expert were submitted to, and answered by, BAFRA headquarters staff in order to frame and scope the topics for the scheduled training workshop. These questions were mainly concerned with data requirements, administrative requirements and timeframes. Answers to some of the questions are articulated in legal documents such as the *Guideline on Risk Assessment of Food and Feed Products Derived from Genetically Modified Plants*, *Biosafety Rules & Regulations*, *Guideline for Handling Applications for Activities Involving Genetically Modified Organisms (GMOs)/Living Modified Organisms (LMOs) and Products Thereof*. Some answers required further clarification and were discussed during the workshop.

Over the ten days of the workshop, participants were introduced to and able to follow various stages involved in receiving and processing an application i.e. pre-application-lodgement interaction with a potential applicant, administrative assessment of a received application prior to acceptance, safety assessment of the data provided, preparation for public consultation, final report preparation and gazettal. In addition, as the level of biotechnology technical expertise of the participants was very basic, time was spent in explaining the principles of the range of techniques used to generate data for an application. The final agenda of the workshop is attached as Annex 2.

1.2. Background

BAFRA, an authority under Ministry of Agriculture and Forests, within the Royal Government of Bhutan, is the designated national competent authority for the implementation and enforcement of biosafety-related activities as provided by Chapter II, section 14 of the *Biosafety Act of Bhutan 2015*. Following the endorsement of *Biosafety Rules and Regulations of Bhutan 2018*, BAFRA has a role in carrying out risk assessment (environment, food and feed), risk management and risk communication associated with biosafety. Specifically, in relation to genetically modified organisms (GMOs) and GM products, BAFRA is tasked with handling applications, inspection and monitoring, and laboratory detection. One of the main mandates of BAFRA is to protect biological diversity, the environment, and the health of humans and animals.

In Bhutan, non-viable forms of genetically modified (GM) foods and feeds are permitted if approved by the National Biosafety Board, while the environmental release of viable GMOs is prohibited. Ensuring the safety of GM food in the market is one of the main priorities for BAFRA, especially given Bhutan's reliance on significant imports to satisfy food needs. While BAFRA now has a basic enabling structure that includes a legal framework, aligned protocols and standard operating procedures it has yet to receive an application for a GM food safety assessment.

The body charged specifically with assessing a GM food application under section 15(2) of the Bhutan *Biosafety Rules and Regulations*, is the Biosafety TWG. This Group comprises members from a number of key agencies within Bhutan and is responsible for advising the National Biosafety Board on any technical and scientific issues related to GMOs.

Given that no application has yet been received, the International Rice Research Institute (IRRI) had provided its complete regulatory dossier on biofortified rice event GR2E (Golden Rice) as an in-kind contribution to the FAO, for the express purpose of providing an example of a 'real' application for the Biosafety TWG to work through. The dossier was exactly the same as that provided to FSANZ in November 2016 for consideration for approval of GR2E. FSANZ subsequently gazetted the GR2E food approval in February 2018.

2. Highlights of the training

2.1 Day 1

The workshop was opened by Jambay Dorji (Senior Planning Officer/ Focal for Biosafety, BAFRA) who outlined the objectives of the workshop – which were to provide, for participants, an understanding of how to prepare a safety assessment using the Golden Rice dossier provided by IRRI, using the existing legislation already developed in Bhutan. Following introductions by the participants, the Technical Panel Expert gave a short presentation on the basics of the genetic modification of plants and the principles involved in undertaking a safety assessment. There was also discussion on food derived using the so-called 'new breeding techniques'(NBTs) including genome editing (oligonucleotide mutagenesis, use of site directed nucleases such as Transcription Activator-Like Effector Nucleases and Clustered Regularly Interspaced Short Palindromic Repeats], cisgenesis/intragenesis, grafting of a non-GM scion on a GM rootstock, and the problems being faced by regulators, globally, in deciding whether and how such food may be regulated. Bhutan too, may need to address this in its own legislation at some point of time in the future.

Discussions continued to walk through the legislation, procedures, and principles that need to be in place before the first 'real' (transgenesis) application is received by Bhutan, and the Technical Panel Expert asked a number of questions throughout the day designed to provoke thought about how BAFRA might deal with a number of issues related to the handling of applications in general and specific issues that have not been addressed (or may need to be addressed).The data requirements were also a topic of the focus as BAFRA will need to use these as the basis for the safety assessment and they will also provide information for prospective applicants. Using information available in Annexure A of the Bhutan *Guideline on Risk Assessment of Food and Feed Products Derived from Genetically Modified Plants*, and examples from Health Canada and FSANZ data requirements, the participants worked their way through a consideration of the molecular characterization, new substances, compositional analyses and nutritional data A number of legal aspects of application handling and communication were also discussed. Using information collected and inputs/feedback received on the Day1, the Technical Panel Expert has compiled a list of General and Data Requirements that could form the basis of information that BAFRA could provide to potential applicants. The list was frequently revised throughout the workshop and finalized as Annex 3.

2.2 Day 2

The first draft of the Data Requirements list was presented and participants highlighted several areas where BAFRA will need to make a decision about the data they require on the following points:

1. For novel proteins, will an oral toxicity study be routinely required (or only required if other evidence such as bioinformatics analysis and protein stability flag a toxicity concern)?
2. Will a whole food animal feeding study be a mandatory requirement?
3. If there are endogenous allergens present in the host, will data be required on changes to levels in the GM plant?
4. For traits involving herbicide tolerance, will a residue study be required, will the potential toxicity of novel metabolites need to be considered, who will deal with any maximum residue level (MRL) requests?
5. If there has been a nutritional change, what information, will be required to undertake a dietary intake assessment or equivalent for the Bhutan diet.
6. How will food from breeding stacks be considered if the GM parents have been approved? Section 34 of the *Biosafety Rules and Regulations*2018 states that food containing a stacked event requires a new application – it is not obvious from this wording whether molecular or breeding stacks (or both) are encompassed. If food from breeding stacks with multiple GM parents does require assessment, will every permutation and combination of stacks need to be assessed or only the 'master' stack? Will the data requirements for food from a breeding stack be the same as those for a single event?
7. Will there be a lighter touch for re-transformation (where the same cassette is inserted into a different host)
8. Will information from other country assessments (that are available on the web) be used in the Bhutan safety assessment? If so, how will this information be incorporated/ acknowledged?
9. May need to think about GM animals

10. May need to think about food produced using NBTs and whether a process or product approach is more appropriate (noting the current regulatory environment is process-based).
11. May need to think about whether a GM comparator or GM reference lines would be acceptable in the compositional analyses.

The usefulness of having a pre-application contact/meeting with an applicant and approaches of regulators in other Countries to the conduct of a pre-application meeting were discussed. While BAFRA can adopt any administrative process it chooses for handling pre-application contacts with applicants, it was stressed that a legal waiver regarding any information provided by BAFRA to an applicant, is a necessary inclusion in that process. An example was given of the sorts of information that could be discussed at such a meeting.

Participants prepared for the training on Day 3by starting on the Administrative Assessment of the GR2E dossier, using the Data Requirements list as a checklist of what to look for in the dossier.

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2.3 Day 3

The components of the Organisation for Economic Co-operation and Development (OECD) Unique Identifier were explained and as part of an exercise, the participants confirmed that the verification number used for GR2E was the correct one. There was also discussion about the Golden Rice Licensing Arrangements (with information being taken from the Golden Rice website at http://www.goldenrice.org/Content1-Who/who4_IP.php. The European Union Joint Research Commission website (http://gmo-crl.jrc.ec.europa.eu/gmomethods/) where detection methodology is referenced was visited to look at the event specific detection method for GR2E - http://gmo-crl.jrc.ec.europa.eu/gmomethods/entry?db=gmometh&id=qt-eve-os-001&q=id%3aQT-eve-OS*

All participants continued working through the Administrative Assessment of the GR2E dossier, guided by the Technical Panel Expert, who also provided detailed examples of the various reports

and documents that could be generated by a regulator once an application had been accepted for pre-market assessment.

A progress summary was provided on the principles, documentation and policies that need to be in place before Bhutan receives its first GM food application, the considerations for pre-application communication with an applicant and the considerations for conducting an Administrative Assessment once an application is submitted.

2.4 Day 4

Participants began going through the GR2E dossier as they would do in preparing a safety assessment, following the order and subject matter in the draft Data Requirements. These requirements were updated as appropriate, to reflect points raised in discussing the data in the dossier. The Technical Panel Expert highlighted the desirability of BAFRA maintaining a consistent appearance (i.e. formatting, language, document headings and layout) in all safety assessments (or part thereof) that will be released publicly, as this presents a professional image. It was also stressed that assessors should always use the associated unpublished studies, supplied as part of the dossier, as the source of information being evaluated and that, where possible, the language used in the safety assessment should be that of the assessor rather than of the applicant. It is acceptable, however, to directly copy figures and tables from the dossier as appropriate for summarizing results and concepts.

The Technical Panel Expert suggested a general structure for presenting information in the safety assessment, while suggesting BAFRA should decide for itself how it would like to implement this, since there is no set protocol. Stress was also made of BAFRA, using Bhutan-specific information where appropriate.

The safety assessment areas covered in the relevant discussions were:

- the information in the general introduction – which is essentially a description of the recombinant DNA plant
- description of the host organism – where there is particular scope for presenting Bhutan-specific information
- description of the donor organism
- description of the inserted DNA

Techniques and experimental approaches used by the applicant were explained and several short videos from YouTube were presented:

- DNA isolation (https://www.youtube.com/watch?v=J5vEP7oUUwY)
- Agarose gel electrophoresis (https://www.youtube.com/watch?v=vq759wKCCUQ)
- Southern blotting (https://www.youtube.com/watch?v=uUFXXxI5qMs)
- Basics of polymerase chain reaction (https://www.youtube.com/watch?v=iQsu3Kz9NYo)

Participants worked individually on the part of the dossier dealing with the stability of the phenotype (in this case, beta-carotene production) and then discussed the points that can be appropriate to include in the safety assessment.

2.5 Day 5

The remainder of the dossier on stability and Mendelian inheritance was discussed and participants further discussed what information should be included in the conclusion about the molecular characterization.

Discussion on communication with the general public was also held, since this forms an important adjunct to the process of assessing an application. Two videos were shown, dealing specifically with Golden Rice, with the aim of presenting some alternative views in the context of the Philippines, a country that shares with Bhutan a similar society and organic approach to food production.

GMO debate grows over golden rice in the Philippines:
https://www.youtube.com/watch?v=Ayv_EYi43E8
All that glitters is not gold [a Greenpeace production]:
https://www.youtube.com/watch?v=GxSGKD50ioE

It was noted that BAFRA (with assistance from the FAO) is in the final stages of developing a Biosafety Communication Strategy that should be released within 2019.

2.6 Day 6

With participants now more familiar with the approach of an assessor in handling the data, the session began with their going through the list of unpublished studies and checking off which part(s) of the Data Requirements the studies pertained to.

Discussions were held on Data Requirement #10 which pertains to the characterization of any new substances. Information on some of the various techniques used in protein characterization was discussed and included YouTube videos on:

- SDS-PAGE: cartoon explanation of the principle- https://www.youtube.com/watch?v=IWZN_G_pC8U; demonstration of equipment in the lab https://www.youtube.com/watch?v=eaETFKXtNRA
- monoclonal antibody production https://www.youtube.com/watch?v=WLHlmY4eWg0
- western blottinghttps://www.youtube.com/watch?v=VgAuZ6dBOfs
- Enzyme-linked immunosorbent assay (ELISA): https://www.youtube.com/watch?v=lUWpWKVcmc4; https://www.youtube.com/watch?v=zR_xlV5v_f4

The use of the ExPASY web tool (https://web.expasy.org/translate/) for translating a DNA sequence into a deduced amino acid sequence was demonstrated using the *Zmpsy1* and *crtI* gene sequences. Having identified the relevant new substance to consider, participants then discussed each of the sub-groups within #10 i.e. function and phenotypic effects; sites and levels of expression in GR2E; history of consumption; confirmation of identity.

Participants also started looking into Data Requirement #11(potential toxicity) which pertains to the characterization of a protein that needs to be produced in a heterologous system (e.g. bacterial) in order to obtain sufficient quantity to undertake some of the studies. In the case of GR2E, this had been done and some explanation was required about the purpose of various ‘additions’ that

may need to be made to the microbially-expressed protein e.g. $(His)_6$-tag; Factor Xa cleavage site; linker sequence.

2.7 Day 7

A number of more technical points carrying over from the previous days were explained further before the resumption of working through the GR2E studies associated with Data Requirement #11 (potential toxicity). Two new protein analysis techniques were explained in some detail – MALDI-TOF MS and N-terminal sequencing by Edman degradation.

Studies dealing with toxicity and allergenicity (Data Requirements #12 and #13) were explained and the principle of bioinformatics searches using the FASTA and BLAST algorithms were introduced to the participants. One of the participants from the National Food Testing Laboratory had experience in using both types of searches and provided a short tutorial on their use within the National Centre for Biotechnology Information website. Work was concluded on Data Requirement #12 and discussion on the bioinformatics searches dealing with allergenicity, and the studies dealing with thermal stability were also completed.

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2.8 Day 8

Data Requirements around allergenicity was discussed, in particular on glycosylation. The use of the NetNGlyc website (http://www.cbs.dtu.dk/services/NetNGlyc/) was introduced for determining potential glycosylation sites in proteins that cannot be obtained from the plant in sufficient quantities for a chemical glycosylation analysis. A final conclusion about the new proteins present in GR2E (covering Data Requirements #10, #11, #12 and #13) was then discussed before moving on to the compositional analysis.

Participants were introduced to the OECD consensus documents on compositional analysis (http://www.oecd.org/chemicalsafety/biotrack/consensus-documents-safety-of-novel-foods-and-feeds.htm) and then went through the rice consensus document in some detail. They then

considered the data in the GR2E dossier pertaining to the compositional analyses. There was particular discussion about whether the inclusion of an oral toxicity study and a whole food study should be mandatory and the question needs to be further addressed at the national level.

The Technical Panel Expert indicated that a basic safety assessment had been prepared as the participants worked their way through the GR2E dossier over the previous four days and that this could be used by BAFRA as a guide to what to include in a safety assessment.

2.9 Day 9

Following on from the discussion about the need, or not, to make the acute oral toxicity study and 90-day whole food feeding study a compulsory data requirement for BAFRA, the Technical Panel Expert provided some opinions from other regulators and scientists and left it to the Biosafety TWG to weigh up the arguments and make a decision.

The administrative/communication considerations that would accompany the release of a draft safety assessment for public consultation should be undertaken. Examples of documents used by FSANZ were provided and included the Call for Submissions Report (a public document which provides information on how to make a submission, along with a summary of the application, the safety assessment findings, risk management (labelling, trade considerations), risk communication strategy and the proposed legal drafting should the application be approved); the Decision Paper (an internal document outlining the various legal considerations that need to be addressed for an application and that requires sign-off by senior management), website information, and a template for acknowledging any submissions received.

2.10 Day 10

During the first session of the morning, the steps and likely documentation involved in finalizing the safety assessment and moving towards approval of the application were discussed. Examples of documents used by FSANZ were provided and included the Approval Report (which is similar to the Call for Submissions Report but also addresses issues raised in the consultation) and Decision Paper (similar to the consultation papers), and a template for notification of the final decision to the applicant.

The workshop was concluded with the workshop summary provided by the Technical Panel Expert together with conclusions and future recommendations for consideration by BAFRA in finalizing its GM food application protocols and documentation. Participants were also awarded with certificate of participation.

3. Conclusions and recommendations

3.1 Overview

BAFRA now has a basic enabling institutional framework that includes a legal framework, aligned protocols, standard operating procedures and a near-finalized communication strategy document. This workshop provided an opportunity, for those who will be tasked with assessing a GM food application, to experience the approach to undertaking a safety assessment using an actual dossier.

In going through the IRRI dossier, participants also gained knowledge about both a variety of techniques used to generate the data for a GM food application and the administrative, legal and communication factors that accompany the receipt and assessment of such an application. A major tangible outcome was the generation of a draft Application Requirements List (Annex 3) that will be invaluable to both any potential applicant (for unambiguously setting out what information is required by BAFRA) and BAFRA (for providing a checklist that can be used at Administrative Assessment for checking that the relevant information has been addressed). By the end of the workshop, a rudimentary safety assessment had been compiled addressing a basic framework for considering the data, using the GR2E data as an example of what information to include.

A number of factors that BAFRA could consider in finalizing the documentation for handling a GM food application were raised during the workshop. Those pertaining to data requirements are discussed in section 2.2 (Day 2) of this report. Points raised in regard to two other areas – General and Legal - are itemized below.

3.2 General points

- Who will undertake the administrative assessment?
- Will a single safety assessment report be produced or, because it is unlikely a single person will complete the safety assessment, will there be a number of individual contributions. If a single safety assessment report, who will compile it?
- What information about the application (eg. the application itself, the BAFRA reports, submissions received during consultation) will be made publicly available?
- Is the timeframe that has been designated for Administrative Assessment, preparing a safety assessment, public consultation and reaching a decision on an application, going to be sufficient? Currently that timeframe is 128 days from receipt of application until issue of decision to applicant.
- Are the provisions for stopping the clock, should further information/data need to be obtained from the applicant, clearly articulated?
- For the final approval decision, will there be a need to consider factors other than safety (e.g. social impact, World Trade Organization, cost/benefit (economic); religious; Regulatory Impact)? If yes, how will they be evaluated and by whom?
- BAFRA could consider expanding the *Guideline on Risk Assessment of Food and Feed Products Derived from Genetically Modified Plants* (or maybe creating a whole new document – or maybe incorporating the information into the new *Communication Strategy*

document)to cover all of the kinds of information it is useful for applicants to know, that includes Data Requirements and encompasses, for example (and in no particular order),

- Relevant reference to legal documents (e.g. *Biosafety Act of Bhutan 2015*; *Biosafety Rules and Regulations of Bhutan 2018)*
- Maybe some information about the Bhutan Biosafety Clearing House (since this is where any GM food approvals will be listed),
- The inter-relationship between an event approval and an import permit,
- The role of BAFRA and the Biosafety Board in approving a GM food,
- Timeframes and stages for the handling of an application (from receipt of application to approval), including what happens when a stop clock is imposed (and under what circumstances it is imposed). Flow chart would be appropriate to include.
- Definition of confidential commercial information (CCI) [refer to relevant legal document]and the process for considering CCI
- It is usual these days for the names/contact details of any individual appearing on a document (e.g. the applicant details, or a study) to be redacted if that document is going to be made public. The redaction could be done by BAFRA or by the applicant – if the latter, as for CCI (see General Requirements), it would be appropriate for the applicant to provide both a redacted and unredacted copy.
- Applicants are encouraged to discuss their proposed application with BAFRA prior to submission in order to clarify the nature of the application and to assist in identifying the information required.
- The pre-application process
- Contact details for any application enquiries,
- BAFRA's obligation to applicants,
- Communication
- The basis for withdrawal or rejection of an application,
- The general administrative requirements that need to be met in an application
- Risk management strategies e.g. labelling
- Information about what information in an application will be made public (e.g. the application itself, the safety assessment [which will contain non-CCI data from the application])

As a guide, similar documents prepared by other jurisdictions could be consulted (e.g. Food Standards Australia New Zealand *Application Handbook* – available at http://www.foodstandards.gov.au/code/changes/pages/applicationshandbook.aspx)

3.3 Legal points

- Are any people outside BAFRA/government going to be involved (especially in the safety assessment) – if so what is the legal basis of their involvement (financial, competing interests etc).
- Need to develop a waiver regarding advice given at any pre-application contact with the applicant
- The conditions around confidential commercial information need to be clearly articulated and standard templates prepared for both internal purposes (outlining any CCI and why it

meets the definition) and for the applicant (formal letter of notification re acceptance/rejection of requested CCI).

- It would be advisable for BAFRA to develop standard wording to be included in a Statutory Declaration (that each applicant must sign and include in a GM food application) regarding the truthfulness of information contained in the application.
- How does current legislation deal with food from new breeding techniques – will changes need to be made?

BAFRA will face a number of challenges in implementing its GM food application process, and the most significant is the lack of experience in handling a dossier. This will impact on the efficiency with which the first few applications will be handled. It is likely that the current Biosafety TWG may require recruitment of alternate representatives and additional experts such as Nutritionist, Toxicologist, Molecular Biologist, to supplement the knowledge base required for fully assessing a dossier and that, even with this addition, it may be necessary, depending on the trait(s) involved, to outsource the assessment of some areas of a dossier. Implicit in this is the likelihood that the outsourcing will require a significant extra financial commitment since personnel outside BAFRA will justifiably expect remunerations. Finally, it needs to be understood at the management level, that assessing a dossier involves a significant time commitment which will need to be factored in, along with the existing work commitments of those Biosafety TWG members undertaking the safety assessment.

Annex 1. List of Participants

No	Name	Agency
1.	Ms. Dechen Choden	Assistant Lecturer, College of Natural Resources, Royal University of Bhutan, Lobeysa, Punakha
2.	Ms. Ugyen Wangmo	Horticulture Officer, Research and Development Center, Department of Agriculture, Ministry of Agriculture and Forests, Yusipang, Thimphu
3.	Mr. Tek Bahadur Rai	Sr. Forest Officer, Department of Forests and Park Services, Ministry of Agriculture and Forests, Thimphu
4.	Mr. Mani Prasad Nirola	Sr. Biodiversity Officer, National Biodiversity Centre, Ministry of Agriculture and Forests, Serbithang, Thimphu
5.	Ms. Jamyang Lhamo	Sr. Dietician, Jigme Dorji Wangchuck National Referral Hospital, Thimphu
6.	Ms. Dechen Wangmo	Officer In-charge, National Food Testing Laboratory, Bhutan Agriculture and Food Regulatory Authority, Ministry of Agriculture and Forests, Yusipang, Thimphu
7.	Ms. Tashi Yangzom	Sr. Regulatory and Quarantine Officer, Bhutan Agriculture and Food Regulatory Authority, Ministry of Agriculture and Forests, BAFRA Head Office, Thimphu
8.	Mr. Norbu Jamtsho	Assistant Laboratory Officer, National Food Testing Laboratory, Bhutan Agriculture and Food Regulatory Authority, Ministry of Agriculture and Forests, Yusipang, Thimphu
9.	Dr. Sherub Phuntsho	Sr. Regulatory and Quarantine Officer, Bhutan Agriculture and Food Regulatory Authority, Ministry of Agriculture and Forests, BAFRA Samdrup Jongkhar District
10.	Mr. Pasang Wangdi	Sr. Regulatory and Quarantine Inspector, Bhutan Agriculture and Food Regulatory Authority, Ministry of Agriculture and Forests, Thimphu District
11.	Ms. Barsha Gurung	Deputy Chief Regulatory and Quarantine Officer, Bhutan Agriculture and Food Regulatory Authority, Ministry of Agriculture and Forests, Paro District
12.	Mr. Jambay Dorji	Sr. Planning Officer/Focal for Biosafety, Bhutan Agriculture and Food Regulatory Authority, Ministry of Agriculture and Forests, BAFRA Head Office, Thimphu.

Annex 2. Final workshop agenda

Training workshop on GM food safety assessment: using a real case study
4-15 February 2019, Metta Resort, Paro, Bhutan

4 February 2019

Time	Item
09.00 – 09.30	Registration
09.30 – 10.15	Objectives of the workshop, introduction of the resource person; introduction to basics of genetic modification and assessment of safety
10.15 – 10.45	Tea break
10:45 – 11:30	Pre-Application Considerations: Data Requirements 1 – Interactive discussion on Molecular characterization
11:30 – 12:30	Pre-Application Considerations: Data Requirements 2 – Interactive discussion on characterization of new substances (including proteins, RNA, new herbicide metabolites)
12:30 – 13:00	General discussion of topics raised in the morning – MRLs; herbicide metabolites; food derived using new breeding techniques
13.00 – 14.00	Lunch
14:00 – 14:30	Pre-Application Considerations: Data requirements 3 – Interactive discussion on Compositional Analysis and Nutritional Impact
14:30 – 15:00	Pre-Application Considerations: legal aspects, timeframes, communication, general requirements
15.00 – 15:30	Tea break
15:30 – 17:00	Pre-Application Considerations: legal aspects, timeframes, communication, general requirements (continued)

5 February 2019

Time	Item
9:00 – 9:30	Further discussion about herbicide metabolites
9:30 – 10:00	Discussion about the draft Application Requirements lists (including General and Data requirements) drawn up as a result of considerations of the previous day
10.00 – 10.30	Tea break
10:30 – 13:00	Pre-Application advice and meetings; background to the GR2E dossier
13.00 – 14.00	Lunch
14:00 – 15:30	General discussion about the Administrative Assessment; Participants to make their own start on Administrative Assessment of GR2E dossier using the Application Requirements checklists
15.30 – 16:00	Tea break
	Conclude for the day

6 February 2019

Time	Item
9:00 – 9:30	Discussion about the OECD Unique Identifier (and calculation of the verification code); discussion about the Golden Rice Licensing agreement; updating of Data Requirements list
9:30 – 10:00	Discussion about the Administrative Assessment – General Requirements of the GR2E dossier; discussion about detection methodology and use of the EU JRC website
10.00 – 10.45	Tea break
10:45 – 13:00	The Administrative Assessment of GR2E: Molecular data
13.00 – 14.00	Lunch
14:00 – 15:40	The Administrative Assessment of GR2E: New substance data
15.40 – 16:15	Tea break
16:15 – 16:45	The Administrative Assessment of GR2E: Compositional and nutritional data; Preparing the Administrative Assessment Report – Interactive discussion on what to put into the Report, documentation, notification to applicant
16.45 – 17.00	Summary of Days 2 and 3

7 February 2019

Time	Item
9:00 – 10:00	The Safety Assessment: Interactive discussion on the structure of the information in the Safety Assessment document GR2E Safety Assessment: consideration of the description of the rDNA plant, host and donor organisms
10.00 – 10:30	Tea break
10:30 – 13:10	Explanation of various DNA techniques – DNA isolation, agarose gel electrophoresis, Southern blotting GR2E Safety Assessment: description of the inserted DNA
13.10 – 14.15	Lunch
14:00 – 15:30	Explanation of polymerase chain reaction (PCR) GR2E Safety Assessment: description of inserted DNA (continued)
15.30 – 16:10	Tea break
16:10 – 16:30	GR2E Safety Assessment: explanation of the breeding diagram
16.30 – 17.00	Participants to read through the section of the GR2E dossier on the stability of the beta-carotene phenotype and discuss what to include in the safety assessment

8 February 2019

Time	Item
9:00 – 10:00	GR2E Safety Assessment: stability of the inserted genetic material; discussion about phenotype stability (from previous day) Questions FSANZ asked IRRI re molecular characterisation
10.00 – 10:30	Tea break
10:30 – 13:00	Viewing of two YouTube videos on Golden Rice and discussion of any issues raised in the videos that would be pertinent to Bhutan
13.00 – 14.00	Lunch
	Conclude for the day

9-10 February 2019
Self-reading of GR2E dossiers by the participants

11 February 2019

Time	Item
9:00 – 9:30	Participants to go through the GR2E Study Catalogue and decide which studies belong to which part of the Data Requirements
9:30 – 10:30	Explanation of SDS-PAGE
10.30 – 11:00	Tea break
10:30 – 12:00	Explanation of various protein techniques – nature of antibodies; production of monoclonal and polyclonal antibodies; western blotting; ELISA
12:00 – 13:00	GR2E Safety Assessment: function ad phenotypic effects of new substances
13.00 – 14.00	Lunch
14:00 – 15:15	GR2E Safety Assessment: sites and levels of expression of new substances; history of consumption; amino acid sequence
15.15 – 15.45	Tea break
15:45 – 16:30	Explanation of the use of a heterologous system for protein expression; His-tag. Questions FSANZ asked IRRI renew substance results

12 February 2019

Time	Item
9:00 – 10:30	Demonstration of ExPASY for translating DNA sequences GR2E Safety Assessment – SDS PAGE and western blot Explanation of protein techniques – HPLC, MALDI-TOF – results for GR2E
10:30 – 11:30	Tea break
11:30 – 12:15	Explanation of techniques – Edman degradation and N-terminal sequencing – results for GR2E GR2E – enzymatic activity of new proteins
12:15 – 13:00	Explanation of bioinformatics – FASTA, BLAST GR2E Safety Assessment – toxicity of new proteins
13:00 – 14:00	Lunch

14:00 – 14:30	Demonstration of BLAST searches by Norbu Jamtsho, a participant at the workshop
14:30 – 15.30	GR2E Safety Assessment – allergenicity of new proteins
15:30 – 16:00	Tea break
	Conclude for the day

13 February 2019

Time	**Item**
9:00 – 9:30	Explanation of glycosylation Demonstration of NetNGlyc for determining potential glycosylation sites in an intractable protein
9:30 – 10:30	OECD Rice compositional document GR2E Safety Assessment: Compositional considerations
10.30 – 11:00	Tea break
11:00 – 13:00	Participants to work through the compositional analyses in one of the GR2E studies Discussion of results and then comparison with the combined results from the 2 compositional studies
13.00 – 14.00	Lunch
14:00 – 14:30	Introduction to ILSI crop composition database – using rice as an example
14:30 – 15.00	GR2E nutritional assessment – go through what was considered by FSANZ
15:00 – 15:30	Discussion of the GR2E safety assessment Revisiting the proposed Data requirements and update as appropriate
15:30 – 16.00	Presentation of Certificates of Attendance to participants (to coincide with the visit of the FAO Safety Officer)

14 February 2019

Time	**Item**
11:30 – 12:30	Considerations of acute oral toxicity studies and whole food animal feeding studies and discussion about whether Bhutan should mandate these in the Data Requirements
12:30 – 13:00	GR2E Safety Assessment 4: Group activity – Compositional analyses and discussion of any issues (continued)
12:30 – 13:00	Documents and processes to be considered once the safety assessment is completed and if a consultation will be undertaken
13.00 – 14.00	Lunch
14:00 – 15:30	Documents and processes to be considered once the safety assessment is completed and if a consultation will be undertaken (continued) Examples of submission received by FSANZ during the consultation period for GR2E
15.30 – 16:00	Tea break
	Conclude for the day

15 February 2019

Time	Item
9:30 – 10:30	Steps to approval – Interactive discussion including: considerations for finalizing the safety assessment, accompanying reports, legal aspects, communication, consultation, administration, decision-making, notification, gazettal
10.30 – 11:00	Tea break
11:00 – 13:00	Summary of the workshop, Conclusions and a way forward
13.00 – 14.00	Lunch
	Conclude for the day

Annex 3. Suggested Considerations for Applications involving Food Products Derived from Genetically Modified Plants

General Requirements

1. Language: can be in English or Dzongkha
2. Format: Table of Contents is necessary, other things like Table of Figures, Abbreviations may not be considered necessary by BAFRA but are useful; pages sequentially numbered
3. Form of application: electronic, hard copy or both? If hard copy, how many copies? If electronic, the documents must be searchable by word and phrase. If electronic, is there a size limit above which there will be problems with the BAFRA computer system in receiving the application?
4. Applicant details – e.g. as per Annex A of *Guideline for Risk Assessment of Food and Feed Products Derived from Genetically Modified Plants*
5. If applicable, list of similar applications made to other countries and the status of the applications
6. Information to support the application – as per Data Requirements. All requirements must be addressed and justification given if they are not addressed
7. Unpublished studies containing laboratory analyses should be designed and conducted in accordance with Good Laboratory Practice (GLP) and be accompanied by evidence (e.g. a statement signed by a suitably qualified person) of a quality control programme.
8. Confidential Commercial Information must be identified and the applicant must provide a written explanation of why and how that identified information is CCI. If there is CCI, the applicant must provide both a full (unexpurgated) copy of the relevant document(s) as well as an expurgated copy (e.g. CCI blacked out) for both hard and electronic applications [depending on which type was submitted].
9. A signed statutory declaration (signed by a senior officer) MUST be provided and include the following sorts of statements
    - *The information provided in this application fully sets out the matters required.*
    - *The information provided in this application is true to the best of my knowledge and belief.*
    - *No information has been withheld that might prejudice this application, to the best of my knowledge and belief.*
10. A checklist must be completed by the applicant and include page numbers where the relevant information can be found in the application [BAFRA can develop a high level checklist summarizing the General Requirements and Data Requirements – see example in Appendix 1]

Data Requirements

The following information is required to support an application for a new genetically modified food. This information is in addition to that specified for General Requirements.

1. Description of the GM plant
   (a) Nature and purpose of the genetic modification
   (b) OECD Unique Identifier plus any trade name or line name of the plant and trade name (if known) of the food

2. Description of the host plant
   (a) Common name
   (b) Scientific name
   (c) Taxonomic classification
   (d) History of cultivation
   (e) History of food use
   (f) Any toxicity or allergenicity concerns
   (g) Whole foods derived from the plant (e.g. seeds, fruit)
   (h) Products derived directly from the whole food (e.g. oil, protein isolate, sugar, meal)
   (i) Processed products containing (g) or (h) – e.g. spreads, infant formula, bakery foods, breakfast cereals, tinned foods

3. Description of donor organisms from which genetic elements are derived
   (a) Common name
   (b) Scientific name
   (c) Taxonomic classification
   (d) Any known pathogenicity, allergenicity or toxicity of relevance to the food
   (e) History of use in the food supply or history of human exposure to the organism through other than intended food use e.g. normal contaminant

4. Description of the DNA to be introduced and transformation process
   (a) Method used for transformation process
   (b) Description of the construct and transformation vectors used
      (1) The size, source and function of all genetic components including marker genes and regulatory elements
      (2) A detailed map of the location and orientation of all the genetic components contained within the construct and vector, including the location of relevant restriction sites

5. Description of how the line from which the food was obtained was bred from the original transformant and identification (where relevant) of which generations were used for each study provided

6. Description of the DNA inserted into the plant genome
   (a) Number of insertion sites and the number of copies at each insertion site
   (b) Identification of all transferred genetic material and whether it has undergone any rearrangements

(c) Full annotated DNA sequence of each insertion site, including junction regions with the host DNA
(d) A map depicting the organisation of the inserted genetic material at each insertion site
(e) Demonstration of the absence of plasmid/vector backbone (if transformation was Agrobacterium-mediated)
(f) An analysis of the insert and junction regions for the occurrence of any open reading frames (ORFs)
(g) If any ORFs are identified, bioinformatic analyses must be done to indicate whether there is any similarity of the potentially expressed protein to known toxins or allergens.

7. Analysis of expressed RNA transcripts, where RNA interference has been used.

8. Provision of an event-specific detection methodology for the GM derived agricultural commodities.

9. Demonstration that new traits are expressed and inherited in a manner that is stable through several generations consistent with laws of inheritance.
   (a) stability of the transferred gene(s)
   (b) stability of the phenotype

10. Characterisation of new substances (e.g. protein, untranslated RNA, new metabolite)
   (a) Description of the biochemical function and phenotypic effects of all new substances expressed
   (b) Sites and levels of expression in the GM plant
   (c) History of safe use - prior history of consumption of the new substances, or their similarity to substances previously consumed in food
   (d) Confirmation of the identity of the new substance in the plant
      i) For a protein that can be obtained in sufficient quantity from the host, this must include at least molecular weight, immunochemical property, amino acid sequence, enzyme activity
      ii) Where RNA interference(RNAi) has been used, information must be provided on
      - The role of any endogenous target gene, and any changes to the food as a result of silencing of that gene
      - The specificity of the RNA interference

11. If a new protein has been produced in an alternative organism (e.g a microbial expression system) in order to obtain sufficient quantities for e.g. digestibility/heat lability studies, then information (such as in 10(d)(i)) must be provided to demonstrate that the microbially-produced protein is biochemically, structurally and functionally equivalent to that expressed in the food derived from the GMO.

12. Potential toxicity of any new protein(s)
   (a) Sequence homology with known toxicants/anti-nutrients (bioinformatic analysis)
   (b) Stability of the protein to degradation in appropriate gastrointestinal model systems

(c) Acute oral toxicity testing if 10(a) and 10(b) indicate either a relationship with known protein toxins/anti-nutrients or resistance to proteolysis [BAFRA TO DECIDE WHETHER TO MAKE THIS COMPULSORY]

13. Potential allergenicity of any new protein(s)
    (a) Sequence homology with known allergens (bioinformatic analysis)
    (b) Stability of the protein to heat
    (c) Specific serum screening where a new protein is derived from a source known to be allergenic or if the new protein has sequence homology with a known allergen
    (d) Elicitation of gluten sensitive enteropathy if protein from wheat, rye, barley, oats
    (e) Post-translational modification – glycosylation

14. If a herbicide tolerance trait has been introduced, (BAFRA to decide)
    (a) residue data must be provided if the trait has not previously been assessed
    (b) any novel metabolite [1] must be identified and, if present, appropriate toxicity information must be provided.

15. Compositional analyses must provide analyses for both food and feed and include:
    (a) levels of relevant key nutrients, toxicants and anti-nutrients in the food/feed produced form the GMO compared with levels in an appropriate comparator. A statistical analysis must be provided. For herbicide-tolerant plants, the levels of each constituent must be determined for both sprayed and unsprayed plants.
    (b) Information on the range of natural variation for each constituent measured to allow for assessment of biological significance should statistical significance be identified.
    (c) The levels of any other constituents that may potentially be influenced by the genetic modification compared with levels in the comparator.

16. If there has been a deliberate nutritional change or the compositional analyses indicate biologically significant changes to the levels of certain nutrients in the food produced from a GMO, the nutritional impact of the changes must be determined by including
    (a) Information that will allow a dietary intake assessment to be undertaken (BAFRA TO DECIDE WHETHER AN INTAKE ASSESSMENT WILL BE DONE) (e.g. food groups or foods proposed to contain the nutrient; the maximum proposed level (or range) of the nutrient in the food groups/foods; the likely level of consumption of the food groups/foods[2]; the market share of the foods likely to contain the nutrient).
    (b) Information on any change to the bioavailability of the nutrient(s) in the GM food
    (c) Where an intended nutritional change has been made, information on the likely nutritional impact on health

    There is no requirement to conduct animal feeding or whole food toxicity studies. [THIS IS FSANZ's POSITION - BAFRA TO DECIDE WHETHER THIS IS ACCEPTABLE]

[1] A novel metabolite is one that is not normally found in non-GM crops sprayed with the same herbicide.
[2] This could be based on foods from a similar market in another country

Annex 4. Example of a Checklist for Applicants

This Checklist will assist you in determining if you have met the mandatory format and data requirements. All applications must include this Checklist.

Check	Page No.	Requirements
		A Form of application
		□ Application in English or Dzongkha
□		□ Pages sequentially numbered
		□ Electronic copy (searchable)
		□ Format as specified
□		B Applicant details
		C Information to support the application
□		□ Data requirements addressed
□		D. List of applications to other countries (if applicable)
		E Confidential commercial information
		□ CCI material separated from other application material
□		□ Documents containing full CCI provided
		□ Documents with CCI removed/blacked out provided
		□ Formal request for CCI, including reasons
□		F Checklist provided with application
□		G Statutory declaration
		H. Data Requirements
		□ Description of the GM plant
		□ Description and use of host and donor organisms
		□ Description of the DNA to be introduced
		□ Breeding tree
		□ Description of the DNA inserted into the plant
		□ Analysis of RNA transcripts where applicable
		□ Detection methodology
□		□ Stability of the genetic modification
		□ Characterisation of new substance
		□ Equivalence of plant- and microbially-produced proteins where applicable
		□ Potential toxicity and allergenicity of new proteins
		□ Information on herbicide tolerance trait where applicable
		□ Compositional analyses for food and feed
		□ Nutritional impact where applicable

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CA442EN/1/05.19