Implementing National Biosafety Frameworks in the Caribbean Sub-Region

SAFETY ASSESSMENT OF GENETICALLY MODIFIED FOODS

UWI

ICGEB

UNEP

gef

Executive Summary

This Guideline applies the approach outlined in "Foods derived from modern biotechnology" (Codex, 2009), which itself is a compilation of internationally-agreed principles and guidelines for GM food safety assessment:

- Principles for the risk analysis of foods derived from modern biotechnology (CAC/GL 44-2003).
- Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants (CAC/GL 45-2003).
- Guideline for the conduct of food safety assessment of foods produced using recombinant-DNA micro-organisms (CAC/GL 46-2003).
- Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA animals (CAC/GL 68-2008).

This approach differs from the traditional risk assessment approach which was elaborated specifically to investigate chemical hazards and was not intended to apply to whole foods, such as GM foods. The traditional approach relies extensively on animal toxicity testing and is mainly used for the assessment of single substances of known purity such as food additives, pesticides and contaminants. This approach cannot easily be applied to whole foods. In fact, few conventional foods safely consumed today have been assessed scientifically in a manner that would fully characterise all potential risks associated with the food. It is also the case that many foods contain substances (e.g. natural toxicants) that would likely be found harmful if subjected to conventional approaches to safety testing.

This activity was carried out with support from the United Nations Environment Programme (UNEP) and the Global Environment Facility (GEF), under project GFL/2328-2716-4C20, "Regional Project For Implementing National Biosafety Frameworks in the Caribbean Sub-region".

1. INTRODUCTION

The Codex Alimentarius multidisciplinary approach to GM food safety assessment (Codex, 2009) uses the concept of a scientific comparison of the GM food to a conventional counterpart having a history of safe use. The basic principles behind this comparative approach1 were first discussed internationally at a Joint FAO/WHO Consultation in 1991 (FAO/WHO, 1991) and were further elaborated by the Organisation for Economic Cooperation and Development (OECD, 1993).

A Joint FAO/WHO Expert Consultation on Safety Aspects of Genetically Modified Foods of Plant Origin re-evaluated the usefulness of the comparative approach and concluded that **"there were presently no alternative strategies that would provide a better assurance of safety of GM foods"** (FAO/WHO 2000). This approach was subsequently endorsed for use with foods derived from GM microorganisms (FAO/WHO 2001a) and GM animals (FAO/WHO 2004).

The main purpose of the GM food safety assessment is to identify new or altered hazards associated with the food as a result of the genetic modification. If a new or altered hazard, nutritional or other food safety concern is identified, further investigation is undertaken to determine its relevance to human health.

The assessment itself is characterised by:

1. **Case-by-case consideration.** The key issues requiring consideration in a safety assessment will depend on the nature of the food being evaluated and the particular genetic modification. Application of the safety assessment guidelines therefore needs to remain flexible so the specific and unique issues that can arise as a result of different genetic modifications can be addressed. This means that the data requirements can be adjusted (e.g. either expanded or contracted) to suit the case being assessed.
2. **Consideration of the intended and unintended effects of the genetic modification.** Intended effects are the changes introduced as a direct consequence of the genetic modification e.g. herbicide tolerance. There may also be other changes associated with the genetic modification that were unintended (see subsection 1.2). The human health impact of both types of changes is considered in the safety assessment.
3. **Comparisons with other foods having an acceptable standard of safety.** These comparisons aid in the identification of similarities and differences between the GM food and an appropriate comparator (see subsection 1.1). Any identified differences become the focus of further scrutiny to determine if they raise potential safety and nutritional issues. The extent of this further scrutiny will depend on the nature of the identified differences, and could include relevant comparisons with other foods or additional testing of the nutritional or toxicological properties of the GM food. This will need to be decided on a case-by-case basis.

Use of the comparative approach relies upon:

(i) consideration of the molecular characterisation of the genetic modification;

(ii) phenotypic characterisation of the new organism, compared with an appropriate comparator;

(iii) consideration of the safety of new substances produced in the food by the introduction of new genetic material, and;

(iv) compositional analysis of the food.

1 Also referred to as "substantial equivalence".

This activity was carried out with support from the United Nations Environment Programme (UNEP) and the Global Environment Facility (GEF), under project GFL/2328-2716-4C20, "Regional Project For Implementing National Biosafety Frameworks in the Caribbean Sub-region".

It is important to note that the key focus of the assessment is ultimately on whether the GM food is safe rather than how different/similar it is to the chosen comparator(s). If the identified differences do not raise any safety or nutritional concerns then it can be concluded the GM food is comparable to other foods already in the food supply in terms of its safety for human consumption.

1.1. Selection and use of comparators

The comparative approach requires identifying a suitable comparator(s) against which the GM product will be compared throughout the safety assessment. It is acceptable for more than one comparator to be used across different studies, or within a single study. Comparators are particularly important for informing the molecular characterisation (see subsection 1.3), compositional analyses (see subsection 1.5), and any corresponding nutritional assessment (see subsection 1.6).

In the first instance, the relevant comparison should be between the GM organism and its nearest non-GM genetic relative, otherwise known as the near-isogenic line. Use of a close genetic relative as an experimental control optimises the sensitivity of the comparison because it minimises differences due to germplasm alone. Ideally, the near-isogenic line will be the original transformed (parental) line. However in practice, it may be more relevant to use a line with a similar genetic background to the GM line undergoing assessment. This is particularly true for GM crops in which complex conventional breeding steps have occurred to arrive at the GM line of greatest commercial value.

For the molecular characterisation, usually only one comparator, the parental line, is relevant for assessment. For compositional analyses it is necessary to establish the extent of natural variation already present in the food supply; in addition to the near-isogenic line, a number of commercial varieties (often referred to as reference lines) are almost always included in field trials and analysed in the same way as the GM and near-isogenic lines. It is also acceptable

This activity was carried out with support from the United Nations Environment Programme (UNEP) and the Global Environment Facility (GEF), under project GFL/2328-2716-4C20, "Regional Project For Implementing National Biosafety Frameworks in the Caribbean Sub-region".

to use published data to establish a literature range representative of variability in commercial crop composition. Comparing data from a GM plant against the range of natural variability already present in the food supply assists in interpreting whether any identified differences between the GM line and its near-isogenic comparator are biologically significant.

While it has generally been accepted the comparators should all be non-GM, in certain circumstances this may be impractical or unsound from a scientific perspective. For example where:

- **The parental line itself is already a GM line.** *This situation would most likely require a three-way comparison between the original non-GM line, the GM parental line and the new re-transformed line;*
- **GM lines make up the bulk of commercial plantings.** *In these situations it may be appropriate to include approved commercial GM lines to determine a reference range that more accurately reflects the existing food supply;*
- **The breeding tree is complex.** *It can sometimes be difficult to identify a non-GM comparator that has both a history of safe use as food, and which is also closely-related to the line from which the GM food is derived. This would occur if the breeding programme had been so complex that the final food-producing line may no longer be closely-related to the original transformed line;*
- **The food-producing line may be a hybrid that has been crossed then backcrossed to elite lines.** *In this case it would be acceptable for the comparator used for the compositional analyses to be a null (or negative) segregant*[2] *with an equivalent breeding history.*

A number of other issues may also be relevant when selecting an appropriate comparator or comparators:

- *If it is not possible to maintain a genetically "pure" line because of a high frequency of inbreeding depression, the comparator used for the compositional analyses could consist of a population of genetically-similar but not identical (isogenic) individuals;*
- *For logistical reasons, the comparator(s) for GM animals may be more appropriately sourced from traditionally-bred animals of the same species not necessarily closely-related. However, the comparator should ideally be matched in housing and husbandry conditions, breed, age, sex, parity, lactation, or laying cycle (where appropriate), although this will be species-specific and may not be feasible in all cases;*
- *In the case of a food produced from a GM microorganism, it is likely the food will be highly purified (e.g. a protein) and, except in the case of proteins that are protein-engineered, will be identical to that produced in its natural source (nature identical). Any comparator would either have to be the same purified substance (from any source) as used already in the food industry (recognising that the amino acid sequence of the protein may exhibit natural variation), or the same substance produced using a near-isogenic strain of microorganism.*

Because of the complexity of the technology, it is not possible to envisage all potential scenarios for the selection and use of comparators. Ultimately, the comparators that are used should be those best suited to the particular GM food in question. The Regulatory Authority should assess the appropriateness and acceptability of the selected comparators on a case-by-case basis.

2 A null or negative segregant is an individual selected from the progeny that has not inherited the introduced gene.

This activity was carried out with support from the United Nations Environment Programme (UNEP) and the Global Environment Facility (GEF), under project GFL/2328-2716-4C20, "Regional Project For Implementing National Biosafety Frameworks in the Caribbean Sub-region".

1.2. Unintended effects

One of the objectives of the safety assessment is to consider the unintended effects of the genetic modification (Section 1), and in particular whether these raise any food safety concerns.

Gene technology is frequently used to introduce new DNA expressing one or more genes into genomes to produce a novel trait or phenotype. The insertion of such sequences can often be accompanied by other genetic changes, such as the insertion of additional DNA, deletions and/or rearrangements. These changes are collectively referred to as insertional effects and are an unavoidable consequence of genetic engineering (Schnell et al., 2015). While such changes have the potential to give rise to unintended effects, experience to date with GM plants indicates very few in fact give rise to discernible changes to plant phenotype. In cases where unintended changes to plant phenotype have occurred, these have not led to any safety concerns. For any single GM line that is commercialised, it is likely that during the course of development more than 1,000 individual lines would have been screened (Phillips McDougall, 2011) and any exhibiting unintended effects discarded from further review. This is consistent with the common practice of discarding lines of conventionally-bred plants exhibiting undesirable properties during the course of a commercial selection programme.

The phenotypic and other comparisons that are routinely required for the GM food safety assessment therefore primarily serve as confirmation that the selection process has been effective and that any potentially hazardous unintended changes are absent from the food being assessed.

A variety of data can be used to derive information about the occurrence of unintended effects in the new GM food. A thorough characterisation of the genetic modification, and any newly-expressed substances, along with a comprehensive analysis of the composition of the food is essential to ensure that any important differences between the GM food and the non-GM counterpart are identified. Where unintended differences are observed that

This activity was carried out with support from the United Nations Environment Programme (UNEP) and the Global Environment Facility (GEF), under project GFL/2328-2716-4C20, "Regional Project For Implementing National Biosafety Frameworks in the Caribbean Sub-region".

are not consistent with normal biological variation, further assessment should be done to determine if they raise any food safety concerns. The type of further assessment required will depend on the differences identified and is therefore decided on a case-by-case basis.

Notwithstanding the above, it is important to note that the occurrence of genetic changes as a result of the insertion of new DNA, and any consequent unintended effects, is not restricted to the insertion of new DNA using gene technology but may also occur spontaneously or when conventional breeding techniques are used (Schnell et al., 2015). Gene technology therefore presents a similar level of risk, in terms of the occurrence of unintended effects, to other genetic changes that can occur in plants as a result of natural processes or conventional breeding practices.

1.3. Molecular characterisation

Molecular characterisation provides an understanding of the DNA introduced into the host genome and helps to inform the safety assessment in relation to both the intended and possible unintended effects resulting from the transformation (OECD, 2010).

Molecular characterisation typically addresses the following:

- **The transformation method together with a detailed description of any DNA sequences that could potentially transfer to the host genome.** *A breeding pedigree for the various generations produced during selection of the lead event is also important. This information is essential for analysing and interpreting the data from the characterisation of the inserted DNA and insertion site (below).*
- **A characterisation of the inserted DNA and the insertion site.** *This information is used to describe the configuration of genetic elements introduced into the host organism. The characterisation includes information about the nature and number of expression cassettes and the number of insertion sites, including a description of any rearrangements or deletions that may have occurred as a result of the transformation. It also includes the identification and analysis of any unintended open reading frames of significant length created as a result of the insertion event.*
- **Inheritance and genetic stability of the inserted DNA.** *Analysis of inheritance includes consideration of whether the inserted DNA has been stably integrated into the host genome and inherited from one generation to the next and provides assurance that the safety assessment is applicable to future generations. The stability of the genetic modification may be analysed at the genotypic and/or phenotypic level.*

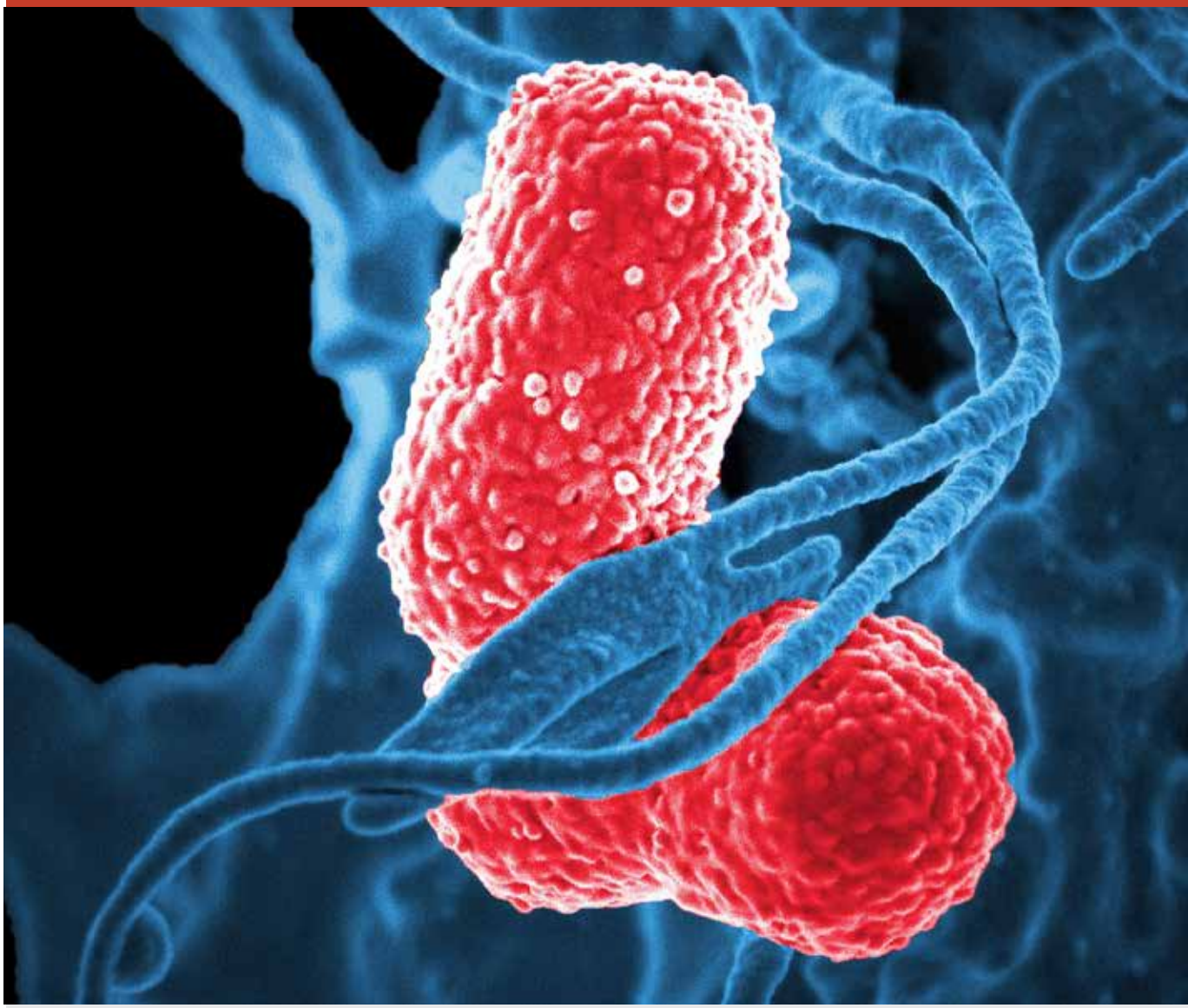
In cases where RNA interference (RNAi) has been used, the molecular characterisation can also include consideration of any transcripts that are produced, and/or evidence of silencing (where an endogenous gene has been targeted).

1.4. Characterisation of new substances

This part of the assessment provides an understanding of any newly-expressed substances that are produced in the food as a consequence of the genetic modification and assists in the identification of any potential hazards. Typically, the main focus of the characterisation will be on newly-expressed proteins, however, other (non-protein) substances may also be included on a case-by-case basis.

The main purpose of the characterisation is to describe the nature of any new substances and their phenotypic and biochemical effects on the organism in which they are expressed,

This activity was carried out with support from the United Nations Environment Programme (UNEP) and the Global Environment Facility (GEF), under project GFL/2328-2716-4C20, "Regional Project For Implementing National Biosafety Frameworks in the Caribbean Sub-region".



particularly in the parts of the organism consumed as food. The level and site of expression of any new substances is useful information for determining potential exposure, should a particular hazard be identified. Where appropriate, the level of expression of new substances in processed food fractions can also provide useful information on potential exposure. It is also important to determine if any new substances are expressed as expected, including, in the case of proteins, whether any post-translational modifications have occurred.

1.4.a. Safety assessment of newly-expressed proteins

One of the key considerations in relation to proteins that are expressed as a result of the genetic modification will be to determine if the expressed protein is new to the organism. Proteins expressed from genes derived from unrelated organisms may require greater assessment than those derived from related organisms, especially those that already have a history of safe use as food.

This issue was discussed at a workshop to discuss new plant breeding techniques (FSANZ, 2012). It was concluded that the source of the gene (i.e. whether it is from the same or a

This activity was carried out with support from the United Nations Environment Programme (UNEP) and the Global Environment Facility (GEF), under project GFL/2328-2716-4C20, "Regional Project For Implementing National Biosafety Frameworks in the Caribbean Sub-region".

different species) could potentially influence the type of safety assessment that would be required. Where the transferred gene is derived from either the same or a cross-compatible species (i.e. a species that is able to be crossed with the host organism by traditional plant breeding to obtain fertile offspring), as would be the case where either cisgenesis3 or intragenesis[4] had been used, and the gene donor belongs to a species that is commonly used as food and has a history of safe use, it can be reasonably assumed that the expressed protein is safe. Subjecting the expressed protein to the full battery of safety studies would therefore serve no legitimate safety assessment purpose. In this situation, evidence would need to be provided to demonstrate the equivalence of the newly-expressed protein to one that has already previously been safely consumed.

The issue of exposure to the expressed protein was also discussed at the workshop on new plant breeding techniques. If the expressed protein is absent from the parts of the organism consumed as food, consideration could be given to waiving the assessment of potential toxicity and allergenicity. This could occur for example in the case of a grafted plant where a transgenic rootstock has been used in combination with a non-transgenic scion. If food is only derived from the scion, and it can be shown that the newly-expressed protein does not translocate to the scion, then there will be no need to consider the safety of the expressed protein.

When assessing the safety of any new proteins, it is important to acknowledge that a large and diverse range of proteins are ingested as part of the normal human diet without any adverse effects. Only a relatively small number of proteins have the potential to impair health. As proteins perform a wide variety of functions in organisms, different possible effects have to be considered during the safety assessment including potential toxic, anti-nutritional and allergenic effects.

Where specific safety studies are undertaken using isolated protein, this protein should ideally be the same as the protein expressed in the new GM organism. If it is not possible to obtain sufficient quantities of protein from the new GM organism for testing, then an equivalent protein produced in a microbial expression system may be used as a substitute. In these circumstances, a range of studies should be undertaken to demonstrate that the microbially expressed protein is structurally, functionally and biochemically equivalent to that expressed in the new GM organism. Collectively, these are referred to as equivalence studies, and they serve to fully characterise the newly expressed protein in the GM organism.

1.4.b. Assessment of potential toxicity

All ingested proteins are subject to the same digestive processes, irrespective of their source or function, including any new proteins expressed in GM foods. Most proteins that are ingested have a predictable metabolic fate. They are typically broken down by proteolytic enzymes in the stomach and small intestine to amino acids and small peptides (di-peptides and tri-peptides), which are readily absorbed.

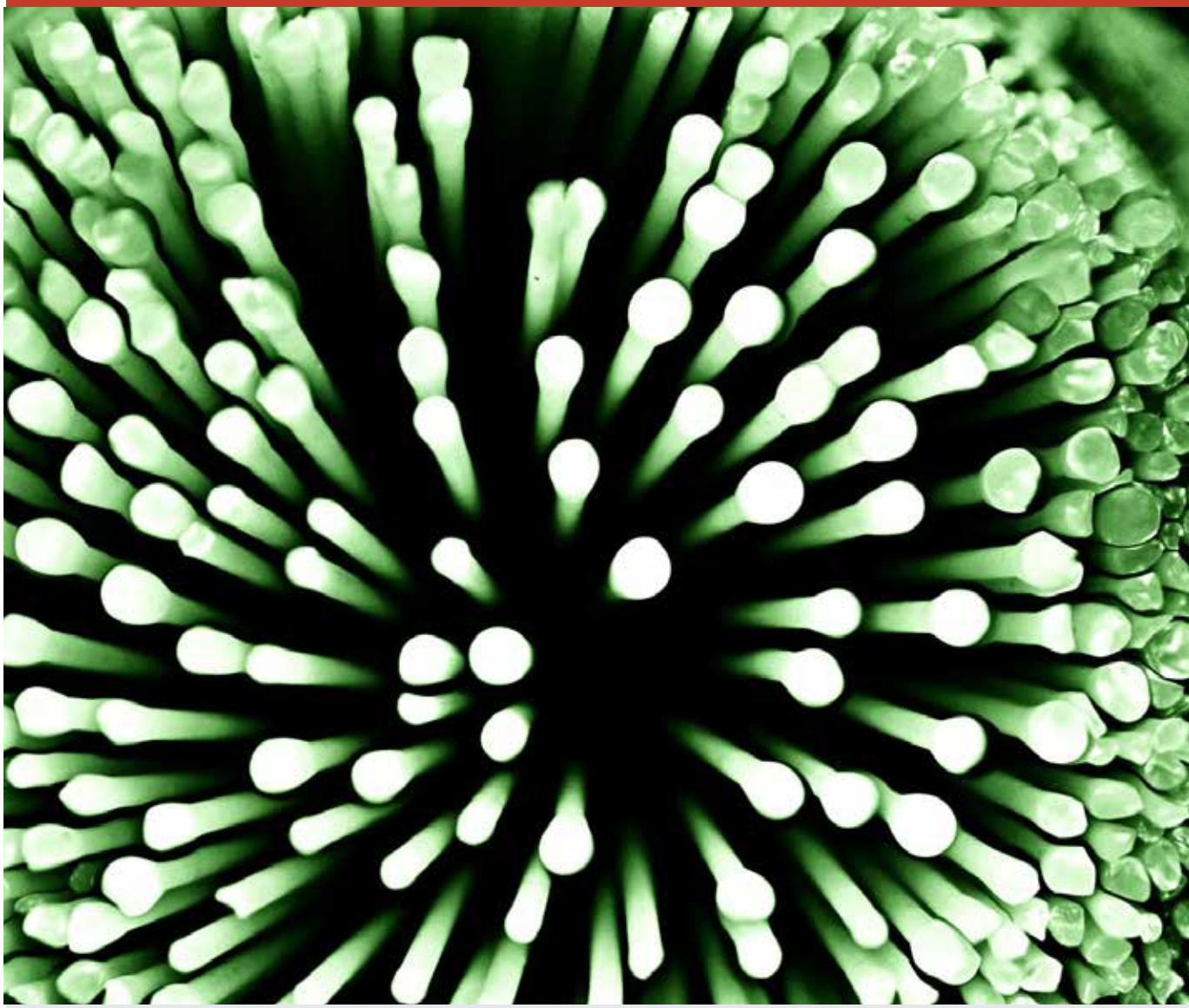
While the vast majority of dietary proteins are innocuous, a small number may be harmful. Of these, the bacterial toxins (e.g. botulinum toxin) are the best described. A number of toxic or anti-nutritional proteins are also produced by plants, an example being ricin, a highly toxic plant lectin found in the seeds of ***Ricinus communis***, commonly known as the castor oil plant.

If the GM food differs from the conventional counterpart food by the presence of one or

3 Cisgenesis involves transferring DNA to a plant where that DNA has been derived from the same species or a cross-compatible species. To qualify as cisgenic, the introduced DNA must comprise a natural genomic fragment containing the gene of interest with its own introns as well as regulatory sequences (promoter, terminator).

4 Intragenesis involves the use of donor DNA from the same or a cross-compatible species, however, new combinations of DNA fragments are acceptable, for example using non-native promoters.

This activity was carried out with support from the United Nations Environment Programme (UNEP) and the Global Environment Facility (GEF), under project GFL/2328-2716-4C20, "Regional Project For Implementing National Biosafety Frameworks in the Caribbean Sub-region".



more new proteins, then these proteins should be fully characterised and assessed for their potential toxicity. The main purpose of an assessment of potential toxicity is to establish, using a weight of evidence approach, that the new protein will behave like any other innocuous dietary protein once ingested. If questions remain following this assessment, additional investigation should be undertaken.

An assessment of potential toxicity of a new protein should consider the following:

1. **History of safe use (HOSU).** This part of the assessment considers whether the new protein has a prior history of safe human consumption. This can be assumed if the protein is identical to proteins present in foods that have a long HOSU. Where amino acid changes have been introduced into the protein but it retains the same biological function as related proteins with a HOSU in food, and the exposure level is similar to functionally-related proteins, then the modified protein could also be considered to be sufficiently similar to proteins with a HOSU.
2. **Amino acid sequence similarity between the new protein and known protein toxins and antinutrients.** This assessment is typically undertaken using bioinformatic analysis where the amino acid sequence of the new protein is compared to the amino acid sequence of known protein toxins and anti-nutrients in public domain databases. This analysis can demonstrate whether the new protein shares any sequence or structural similarity with proteins already identified as toxins and known to pose toxicological hazards.

This activity was carried out with support from the United Nations Environment Programme (UNEP) and the Global Environment Facility (GEF), under project GFL/2328-2716-4C20, "Regional Project For Implementing National Biosafety Frameworks in the Caribbean Sub-region".

3. **Resistance to digestion of the new protein.** This is typically assessed through the use of ***in vitro*** digestibility studies, particularly pepsin digestion. Evidence of slow or limited protein digestibility does not necessarily indicate a safety concern, however proteins that are resistant to proteolysis may be more likely to be absorbed in a biologically intact form or exert effects directly on the GI tract.

If a new protein is found to have no significant sequence similarity to known protein toxins, is readily digested in validated ***in vitro*** digestibility tests, is sufficiently similar to proteins that have been safely consumed in food, and its biological function does not raise any safety concerns, it can be reasonably concluded that the protein is non-toxic to humans and no further investigations would be required.

Where results from the protein characterisation and assessment of potential toxicity indicate the need for further investigation, appropriate acute oral toxicity studies in animals might also be considered. The need for, and nature of, such studies should be discussed with the Regulatory Authority prior to submitting an application.

1.4.c. Assessment of potential allergenicity

Food allergies are abnormal immunological responses mostly to particular proteins in foods, and are a significant public health concern. Virtually all food allergies are caused by a small number of common allergenic foods including peanuts, soybeans, milk, eggs, fish, crustacea, cereals and tree nuts. These eight foods account for over 90 % of all moderate to severe allergic reactions to foods in susceptible individuals.

Although food allergens are generally proteins, the human diet contains many thousands of proteins that are not allergenic. Dietary proteins come from a diverse array of plant, animal and microbial food sources; some of these are staple foods consumed widely around the world, while others are more exotic (e.g. insects) with consumption limited to certain geographical regions. It should be noted that additional protein diversity is sometimes introduced into the food supply through conventional plant breeding techniques. For example, since commercialisation, the conventionally-bred kiwi fruit has proven to be an additional source of food allergens.

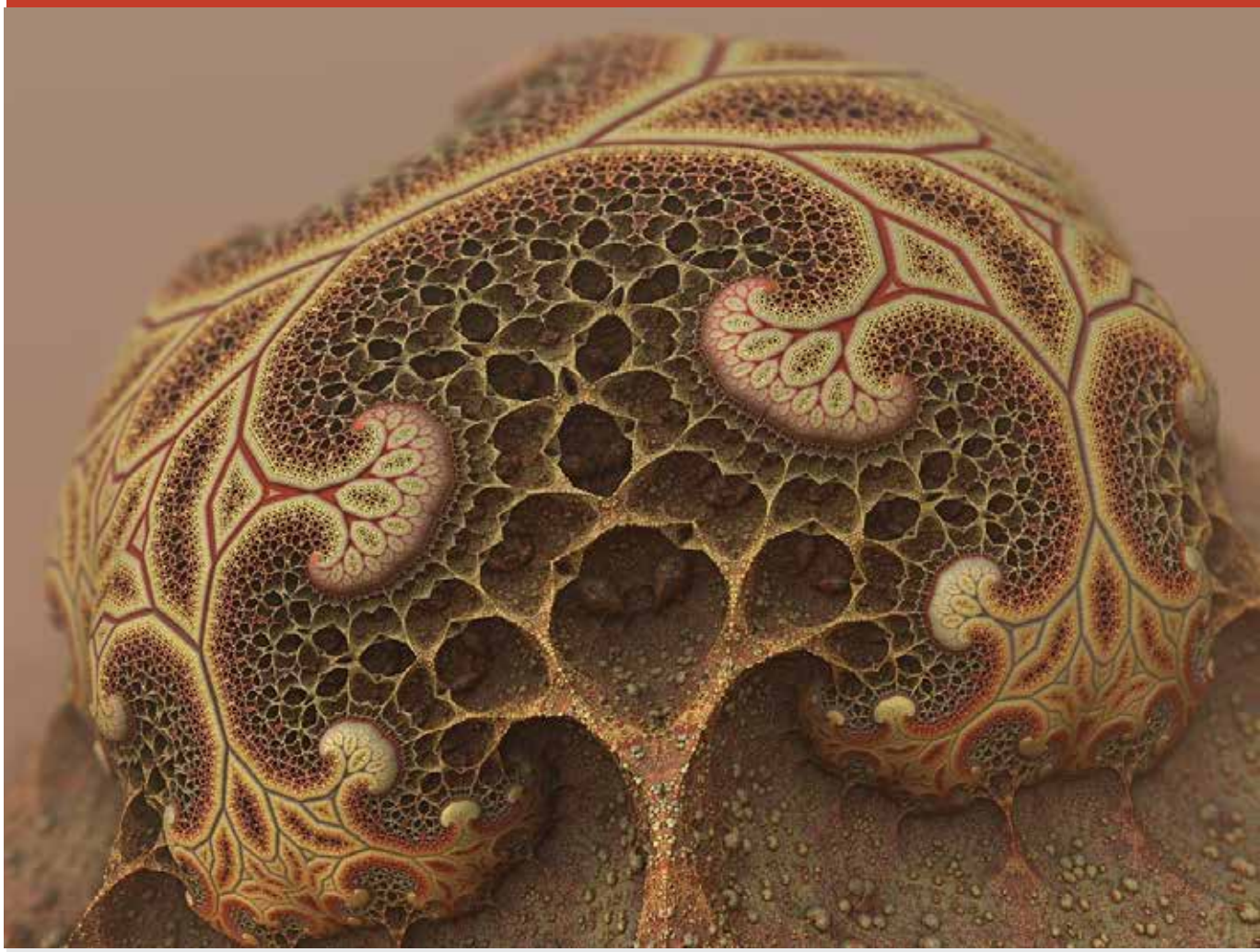
The consumption of food produced using gene technology can contribute in a defined way to the diversity of proteins in the diet. Consequently, the possible allergenicity of any new protein should be part of the safety evaluation. This should include whether the new protein:

- ***is one to which certain individuals are already known to be sensitive;***
- ***is considered likely to cause an allergic reaction in some individuals.***

There are presently no reliable animal models for the assessment of allergenicity, and no single test that can be applied to a protein to predict whether it is likely to be allergenic in humans. The evaluation of new proteins for potential allergenicity was the subject of a Joint FAO/WHO Expert Consultation in 2001 (FAO/WHO, 2001b). The outcome of this consultation was subsequently used by the Codex Ad Hoc Intergovernmental Task Force on Foods Derived from Biotechnology to develop guidance on the best possible scientific approach to assess protein allergenicity (see e.g. Annex 1 in Codex [2003a]). As a result, there is general agreement that a step-wise, weight-of-evidence approach can be used to indicate whether a newly-expressed protein is a possible allergen.

The weight of evidence approach takes into account data and information derived from several types of analysis and includes consideration of the following[5]:

5 Previously, the level of expression of a novel protein was thought to be an important factor to consider in assessing potential allergenicity (Metcalfe et al., 1996), however very little information currently exists on threshold levels of pro-



1. **Source of the newly expressed protein.** It is important to determine if the source of the protein (i.e. the donor organism) is associated with allergic reactions in humans. Genes derived from sources known to be allergenic would in particular be a focus of investigation.
2. **Amino acid sequence similarity between the newly expressed protein and known allergens.** The amino acid sequences of many allergenic proteins are readily-available through public domain databases, which are updated and expanded on a regular basis. **In silico** bioinformatic analyses are done to determine the overall level of similarity. The possibility of IgE cross-reactivity between the new protein and a known allergen should be considered when there is greater than 35 % identity in a segment of 80 or more amino acids (FAO/WHO, 2001b; Codex, 2009).
3. **Physicochemical properties of the newly-expressed protein.** This includes, but is not limited to, its susceptibility to digestion, heat stability and/or acid and enzymatic treatment. Resistance to hydrolysis by digestive proteases has been observed in several food allergens (Astwood et al., 1996). The standard test that has been advocated to determine susceptibility to digestion is pepsin hydrolysis (Codex, 2003a; Codex, 2003b; Thomas et al., 2004). Recent evidence however indicates there may not be a strong association between stability to digestion and allergenicity (Herman et al., 2007) therefore the results of the digestibility assay by itself should not be relied upon as an indicator or allergenicity.

teins required for sensitisation and subsequent elicitation of an allergic response. As a consequence, it is currently not possible to consider the level of expression of a novel protein as a relevant factor in the assessment of potential allergenicity. This may change in the futureas knowledge improves.

This activity was carried out with support from the United Nations Environment Programme (UNEP) and the Global Environment Facility (GEF), under project GFL/2328-2716-4C20, "Regional Project For Implementing National Biosafety Frameworks in the Caribbean Sub-region".

The presence of any post-translational modifications, e.g. glycosylation, and their impact on the allergenic potential of the novel protein would also be a relevant consideration. Glycosylation may affect the susceptibility of a protein to processing and proteolysis and may introduce glycan peptides, which are known to be highly cross-reactive epitopes (FAO/WHO, 2001b)

4. **Specific serum screening.** This should be undertaken when a newly-expressed protein is derived from a source known to be allergenic or has amino acid sequence similarity with a known allergen. Specific serum screening involves testing the immunoreactivity of the newly-expressed protein against IgE antibodies in sera obtained from individuals with an allergy to the source of the protein, or a known allergen identified in the bioinformatics analysis. Such tests are contingent on the availability of sera from well-characterised patients. Additional testing, for example using skin prick tests, may be necessary to confirm a negative result from the serum screening.

The potential exposure to the new protein and the effects of relevant food processing will contribute to an overall conclusion about any human health risk. In this regard, the nature of the food product intended for consumption should be taken into consideration to determine whether normal processing would have an effect on the integrity of the protein or remove it altogether from the final food product.

This assessment strategy is not applicable to assessing whether a new protein is capable of inducing gluten-sensitive or other enteropathies. In cases where the introduced gene is obtained from wheat, rye, barley, oats or related cereal grains, newly-expressed proteins should be evaluated for any possible role in the elicitation of gluten-sensitive enteropathy.

If the assessment of allergenicity risk results in a conclusion that the newly-expressed protein is a potential allergen, the Regulatory Authority should take appropriate regulatory action to manage the risk to susceptible population groups. On a case-by-case basis, management could involve mandatory labelling to inform consumers of an identified risk, other measures to limit exposure in vulnerable groups, or could mean the GM food would not be approved.

1.4.d. Other (non-protein) substances

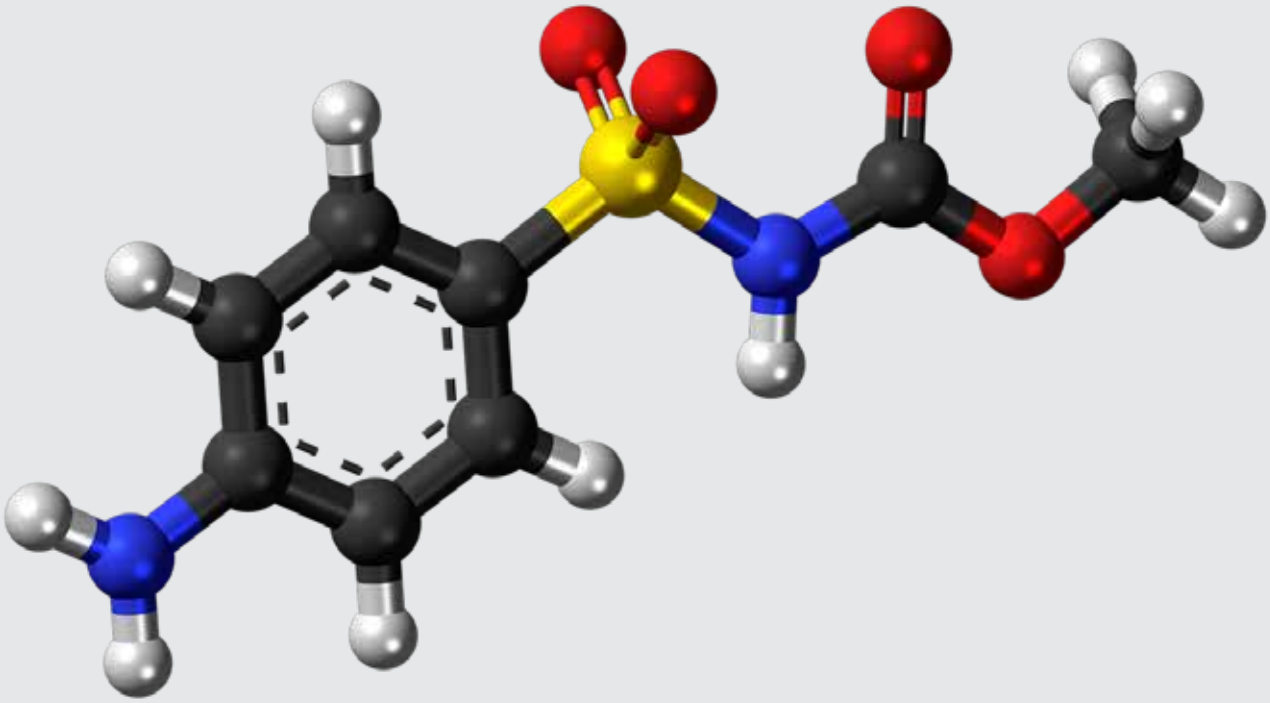
The safety of other (non-protein) substances should be assessed on a case-by-case basis, taking into account the identity and biological function of the substance, whether it has previously been safely consumed in food or has a HOSU, and potential dietary exposure. The types of studies or information that might be relevant to the assessment will depend on the substance in question and should be discussed with the Regulatory Authority prior to submitting an application.

In cases where RNAi has been used, considerations may include a discussion of the role of any target gene, the expression level of the transcript in various plant parts, and the specificity of the RNAi effect.

1.4.e. Novel herbicide metabolites

In the case of herbicide-tolerant plants, where tolerance is achieved by metabolism of the herbicide, it is possible that one or more novel metabolites may accumulate in the GM plant following the application of herbicide. In these situations, the assessment should consider whether these are present in food products and whether their presence raises any toxicological concerns.

This activity was carried out with support from the United Nations Environment Programme (UNEP) and the Global Environment Facility (GEF), under project GFL/2328-2716-4C20, "Regional Project For Implementing National Biosafety Frameworks in the Caribbean Sub-region".



In particular, the assessment should consider if appropriate health-based guidance values (i.e. an Acceptable Daily Intake[6] or Acute Reference Dose[7]) need to be established.

The assessment will also need to consider residue data in order to confirm the concentration of the novel metabolite relative to the parent herbicide in the final food. The amount of herbicide residue that is allowed to be present on the food however is addressed under a process that is separate from the GM food safety assessment and will involve a separate standard (usually set by the Regulatory Authority with responsibility for pesticides). Residues can only legally be present in food if they comply with specific maximum legally-tolerated residue levels (MRLs). The MRLs apply equally to foods regardless of their source i.e. whether they were produced from non-GM or GM crops. Where necessary, an MRL pertaining to a particular herbicide on a crop may have to be set (this is this responsibility of the Regulatory Authority for pesticides).

Mechanisms of herbicide tolerance that do not rely upon the conversion of the herbicide into herbicidally-inactive forms (e.g. where a gene encoding an herbicide insensitive form of an endogenous enzyme has been introduced into the plant) would not be expected to result in the accumulation of novel metabolites.

6 An estimate of an amount of a chemical substance in food that can be ingested daily over a lifetime without appreciable health risk to the consumer.
7 The amount of a chemical substance that can be ingested in one day without appreciable health risk to the consumer.

This activity was carried out with support from the United Nations Environment Programme (UNEP) and the Global Environment Facility (GEF), under project GFL/2328-2716-4C20, "Regional Project For Implementing National Biosafety Frameworks in the Caribbean Sub-region".

1.5. Compositional analysis

The main purpose of the compositional analysis is to determine if any unintended changes in composition have occurred in the food. Compositional analysis is also used for evaluating deliberate changes to food composition, since it can confirm whether the introduced trait is being expressed appropriately, and quantify the magnitude of the change.

Compositional analysis of food is often limited by available analytical methodologies. It is therefore important that appropriate validated analytical methods are used and referenced and that the sensitivity (e.g. limit of detection and limit of quantitation) is documented (Rogers, 2013).

The classic approach to the compositional analysis of GM food is targeted. Rather than analysing every single constituent, which would be impractical, the aim is to analyse only those constituents (analytes) most relevant to the nutritional profile of the food in question. The focus is therefore on key nutrients, natural toxicants and anti-nutrients – components in a particular food that have been identified as important in the context of the diet. They may be major constituents (fats, proteins, carbohydrates or enzyme inhibitors such as anti-nutrients) or quantitatively more minor constituents (minerals, vitamins). Key toxicants are those toxicologically-significant compounds known to be inherently present in an organism, such as compounds whose toxic potency and level may be significant to health (e.g. solanine in potatoes). It is recognised that animals, unlike plants, do not generally contain pathways involved in producing toxins or anti-nutrients and animal metabolites are therefore not considered to raise human health or food safety concerns. There are, however exceptions, particularly with aquatic organisms.

The OECD has developed a series of Consensus Documents[8] to aid in the compositional analysis of foods derived from GM plants. These documents provide information on the key constituents for particular crops and also provide baseline data on the concentration range for each constituent. The International Life Sciences Institute (ILSI) has developed a Crop Composition Database[9] that is regularly updated and provides high quality data that can be used to inform the range of levels for important crop analytes in corn, cotton and soya bean varieties already in commercial production. To date this database has not included information from commercialised GM lines.

Depending on the nature of the genetic modification, or the characteristics of a new protein, additional constituents may need to be analysed. This will need to be determined on a case-by-case basis. For example, if a gene is transferred which results in increased production of a particular nutrient (e.g. the amino acid lysine), the levels of other constituents resulting from metabolism of that nutrient should also be determined for comparison with an appropriate comparator.

Analyses of concentrations of key components of the GM food should be compared with an equivalent analysis of food derived from an appropriate comparator (typically this would be the conventional counterpart) produced under the same conditions (see subsection 1.1). This may not be feasible in all cases, however a line as close as possible should be chosen. The relevance of any observed differences should be assessed in the context of the range of natural variation for that parameter to determine its biological significance (see subsection 1.1).

When the genetic modification results in a food with significant compositional changes, it may be appropriate to select relevant comparator products that are more closely matched in terms of the key nutrient composition in order to assess the nutritional impact of the food (e.g. in the

8 http://www.oecd.org/science/biotrackconsensusdocumentsfortheworkonthesafetyofnovelfoodsandfeeds.htm

9 https://www.cropcomposition.org/query/index.html

This activity was carried out with support from the United Nations Environment Programme (UNEP) and the Global Environment Facility (GEF), under project GFL/2328-2716-4C20, "Regional Project For Implementing National Biosafety Frameworks in the Caribbean Sub-region".

case of high oleic acid soya bean oil, olive oil may provide a more suitable comparison than standard soya bean oil). Such comparator products should have a history of safe use as food, but they do not need to come from close genetic relatives.

It is important to recognise that food composition is not due entirely to the genes of an organism (germplasm) but is also known to be influenced by numerous environmental factors (Privalle et al., 2013). For example, the mineral composition of many plant-derived foods is heavily influenced by soil type and fertiliser practices. In the case of animals, diet is known to influence the composition of food products. For example, supplementation of the diet of chickens with omega-3 fatty acids has been used to increase the omega-3 content of eggs. Studies for the collection of compositional data should be designed and conducted in such a way as to minimise differences that could be attributed to these external factors.

For GM plants, field trial sites should be generally representative of the range of environmental conditions under which the crop would be grown commercially. Standard agronomic practices should be employed but in addition, a comparison with the GM plant grown under its expected agronomic conditions may need to be considered. The number of trial sites should be sufficient to allow accurate assessment of phenotypic/agronomic characteristics over this range and an adequate number of plants should be sampled. Where the number of trial sites is limited, consideration should be given to repeating the trials over more than one season. Each trial site should include replicates and the treatments should be randomised (e.g. randomised complete block design).

For GM animals, it is recognised that a compositional analysis is likely to require sacrifice

This activity was carried out with support from the United Nations Environment Programme (UNEP) and the Global Environment Facility (GEF), under project GFL/2328-2716-4C20, "Regional Project For Implementing National Biosafety Frameworks in the Caribbean Sub-region".

of the whole animal in cases where the meat/organ is the food product. In cases where the animal is not sacrificed, the available number of samples for compositional analysis may be limited. A number of different foods may also need to be sampled (e.g. meat, milk, eggs) which adds complexity to the overall compositional analysis. There is likely to be larger variation between individual samples derived from animals, even those bred and raised under the same husbandry conditions and, to date, there are no compositional databases available, as there are for plants, which may provide information on the normal ranges of analytes.

Statistical analysis of the GM line and the comparator should be appropriate to the experimental design and be documented. In addition to supplying raw data, summary descriptive statistics (e.g. mean, standard error) for each analyte in each treatment should be provided. It is also appropriate, but not essential, to provide 95 % tolerance intervals. Comparisons to reference and/or literature ranges are made to determine the range of natural variation and to establish the biological significance of any identified statistical differences. Through this process it is then possible to determine whether any statistically-significant differences require further investigation.

1.6. Nutritional considerations

GM foods that have altered nutritional characteristics should be subjected to additional nutritional assessment in order to investigate the consequences of the introduced changes and determine whether nutrient intakes are likely to be altered by the introduction of such foods into the food supply (Codex, 2009).

If necessary, the Regulatory Authority should undertake a dietary exposure assessment of the nutrients in the GM food by combining food consumption data from the latest pertinent National Nutrition Surveys (if available) together with food nutrient composition data.

When the modification results in a food product, such as vegetable oil, with a composition that is significantly different from its conventional counterpart, it will be appropriate to use additional foods or food components (i.e. foods or food components whose nutritional composition is closer to that of the GM food) as comparators to assess the nutritional impact of the food (see also subsection 1.5).

Further assessment of nutritional impact, using information gained from volunteer human studies, will be necessary if changes in the bioavailability of nutrients are expected or if the composition is not comparable to other foods in the food supply. If the assessment indicates the available data are insufficient for a thorough safety assessment, the Regulatory Authority should consider the need for additional studies, including whether whole food animal feeding studies are likely to be informative.

1.7. Whole food animal feeding studies

Animal toxicity studies with whole GM foods are not routinely required to complete a safety assessment. There is clear evidence that, in a majority of circumstances, a scientifically-informed comparative assessment using a relevant comparator can generally identify any potential adverse health effects or differences in the GM food requiring specific evaluation (Bartholomaeus et al., 2013; Herman and Ekmay, 2014).

Notwithstanding this evidence, there may be some GM foods, particularly those involving intentional modifications to nutrient composition, where the results of well-designed feeding studies in appropriate animal species may be informative. This would be likely to apply for example to a GM crop developed specifically to improve the nutritional quality of feed for the production of livestock for human consumption, or for use in aquaculture.

This activity was carried out with support from the United Nations Environment Programme (UNEP) and the Global Environment Facility (GEF), under project GFL/2328-2716-4C20, "Regional Project For Implementing National Biosafety Frameworks in the Caribbean Sub-region".

The need for whole food studies will therefore continue to be determined on a case-by-case basis, taking into account the nature and purpose of the genetic modification and the results of the compositional analyses and overall comparative assessment. Discussion with the Regulatory Authority prior to the submission of a data package in support of an application is therefore indicated. It should be noted that studies of any duration in rodents using whole foods would rarely be warranted.

1.8. GM MICROORGANISMS

Microorganisms can be consumed as foods (e.g. edible cultures) or used for the production of substances added to foods to achieve a technological function. Predominantly, such substances derived from GM microorganisms are usually subject to regulation as food additives or processing aids by a different Regulatory Authority with the relevant mandate. Food additives and processing aids undergo a pre-market safety assessment and their use in foods is usually restricted according to their technological function. Unless specifically modified (e.g. by protein engineering), the substance may be indistinguishable from the equivalent, naturally-occurring (nature identical) product. In these instances, the safety assessment of the substance produced from a GM microorganism may therefore be essentially the same as that from a non-GM source, or one that is chemically synthesised.

This activity was carried out with support from the United Nations Environment Programme (UNEP) and the Global Environment Facility (GEF), under project GFL/2328-2716-4C20, "Regional Project For Implementing National Biosafety Frameworks in the Caribbean Sub-region".

The GM microorganisms that are typically used to produce food additives and processing aids (e.g. enzymes used in the manufacture of cheese) are generally strains that have a history of safe use in food production. Where the recipient strains do not have a history of safe use, particular attention to the integrity of the consumed substance needs to be applied. This entails a detailed characterisation of the substance and its degree of compliance with established specifications for purity, including consideration of any possible contaminants carried over from the GM production organism.

The requirement for a safety assessment of a GM microorganism is applicable also to those that are present in foods or constitute the final food e.g. probiotic bacteria, fermentation cultures, etc. The food may contain either viable or non-viable GM microorganisms (e.g. heat-inactivated), or both forms may be present in the food.

Where GM microorganisms remain in or constitute the final food (e.g. lactic acid bacteria in yoghurt), the safety assessment considers:

- **The possibility of gene transfer, including bacterial antibiotic resistance genes, between organisms.** ***Molecular characterisation of the inserted genetic elements in the GM microorganism allows an assessment of the possible consequences of a transfer of functional DNA to commensal organisms in the human gastrointestinal tract. It is important to establish that strains carrying antibiotic resistance genes are not used, particularly where viable microorganisms are present in the final food. Any indication of the presence of transmissible elements such as plasmids, transposons and integrons containing such resistance genes should be specifically investigated.***
- **The possibility of a change in pathogenicity as a result of the genetic modification.** ***In most cases, the recipient microorganism will have a history of safe use as food. The safety assessment considers whether the genetic changes could cause a change in the viability of the organism or produce a toxin. A detailed characterisation of the introduced gene/s and gene product/s, together with studies demonstrating the biological effects in the organism address these safety issues.***

Permanent, life-long colonisation of the digestive tract by ingested microorganisms is rare, however the possibility remains that an ingested GM microorganism could influence the gastrointestinal microflora of the human host (FAO/WHO, 2001a). For this reason, the viability and residence of the GM microorganism may need to be examined. If processing (such as baking) of the final food eliminates viable microorganisms, or if accumulation of end-products toxic to the microorganism (such as alcohol or acids) extinguishes viability, then this scenario need not be examined in any detail.

1.9. GM ANIMALS

The safety assessment of foods derived from GM animals can largely be performed along the lines that have already been established for food from GM plants, using a comparative safety assessment approach (FAO/WHO, 2004).

Given the diverse range of animals used as food (e.g. mammals, birds, finfish and shellfish) and the combined impacts of their genetic diversity, husbandry and conditions under which they are raised or harvested, the safety assessment framework that has been developed is intended to address the general safety issues that are common to all types of GM animals (Codex, 2009). Additional issues that may relate only to one type of animal or species would need to be considered on a case-by-case basis.

In assessing the safety of food from GM animals, the approach takes into account the nature of the DNA construct and its expression products (if any), the health status of the GM animal, and the composition of the food.

This activity was carried out with support from the United Nations Environment Programme (UNEP) and the Global Environment Facility (GEF), under project GFL/2328-2716-4C20, "Regional Project For Implementing National Biosafety Frameworks in the Caribbean Sub-region".

In contrast to plants, an evaluation of the health of the animal is one of the essential steps in ensuring the safety of food derived from GM animals. This is because, unlike plants, animals that have a history of safe use as sources of food generally do not contain genes encoding toxic substances. The health of an animal is therefore a useful indicator of food safety and the practice of only allowing animals with an acceptable health status to enter the human food supply is an essential step in ensuring safe food.

In undertaking the health assessment, it is important to compare the health status of the GM animal with the health status of an appropriate counterpart (Section 1.1), taking into account the developmental stage. The assessment includes consideration of general health and performance indicators, including behaviour, growth and development, general anatomy, and reproductive function (if appropriate), physiological measures, including clinical and analytical parameters and species-specific considerations, where appropriate.

1.10. Review of safety assessments

The Regulatory Authority should routinely monitor and review the scientific literature and other information about GM foods to determine if there is any new scientific information that might alter the conclusions of previous safety assessments. This analysis also extends to any

This activity was carried out with support from the United Nations Environment Programme (UNEP) and the Global Environment Facility (GEF), under project GFL/2328-2716-4C20, "Regional Project For Implementing National Biosafety Frameworks in the Caribbean Sub-region".

new scientific developments in relation to the technology in general which may be relevant to the safety assessment approach. These reviews should be made publicly-available, usually from the website of the Regulatory Authority.

The Regulatory Authority should also liaise with other national or regional food agencies to maintain a watching brief on any potential safety issues with internationally-traded foods, whether GM or not. This ensures that appropriate action can be initiated by relevant agencies if necessary to prevent unsafe or non-compliant food from entering the food supply or, if already present, to have the food removed.

REFERENCES

Astwood JD, Leach JN, Fuchs RL (1996). Stability of food allergens to digestion ***in vitro***. ***Nature Biotechnology*** 14(10): 1269–1273.

Bartholomaeus A, Parrott W, Bondy G, Walker K (2013). The use of whole food animal studies in the safety assessment of genetically modified crops: Limitations and recommendations. ***Critical Reviews in Toxicology*** 43(S2): 1–24.

Batista R, Oliveira M (2010). Plant natural variability may affect safety assessment data. ***Regulatory Toxicologyand Pharmacology*** 58: S8–S12.

Chassy BM (2010). Can -omics inform a food safety assessment? ***Regulatory Toxicology and Pharmacology*** 58(3S): S62-S70.

Codex (2003a). ***Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants.*** CAC/GL 45-2003. Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization of the United Nations, Rome, Italy.

Codex (2003b). ***Guideline for the conduct of food safety assessment of foods produced using recombinant-DNA microorganisms.*** CAC/GL 46-2003. Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization of the United Nations, Rome, Italy.

Codex (2009). ***Foods derived from modern biotechnology,*** 2nd edition. Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization of the United Nations, Rome, Italy. http://www.fao.org/docrep/011/a1554e/a1554e00.htm.

FAO/WHO (1991). ***Report of a joint FAO/WHO consultation: Strategies for assessing the safety of food processed by biotechnology.*** World Health Organisation, Geneva, Switzerland. www.who.int/foodsafety/publications/biotech/en/1990.pdf.

FAO/WHO (2000). ***Safety aspects of genetically modified foods of plant origin.*** Report of a Joint FAO/WHO Expert Consultation. World Health Organization, Geneva, Switzerland. www.who.int/foodsafety/publications/biotech/.../ec_june2000_en.pdf.

FAO/WHO (2001a). ***Safety assessment of foods derived from genetically modified microorganisms.*** WHO/SDE/PHE/FOS/01.3. Report of a Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology. Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization of the United Nations, Rome, Italy. http://www.fao.org/3/a-ae585e.pdf.

FAO/WHO (2001b). ***Evaluation of allergenicity of genetically modified foods***. Report of a Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology, January 22 - 25, 2001, Rome, Italy.

This activity was carried out with support from the United Nations Environment Programme (UNEP) and the Global Environment Facility (GEF), under project GFL/2328-2716-4C20, "Regional Project For Implementing National Biosafety Frameworks in the Caribbean Sub-region".

FAO/WHO (2004). Safety assessment of foods derived from genetically modified animals: Report of the FAO/WHO Expert Consultation, November 2003. Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization of the United Nations, Rome, Italy.

Hepburn P, Howlett J, Boeing H, Cockburn A, Constable A, Davi A, de Jong N, Moseley B, Oberdörfer R, Robertson C, Wal JM, Samuels F (2008). The application of post-market monitoring to novel foods. ***Food and Chemical Toxicology*** 46: 9–33.

Herman RA, Ekmay R (2014). Do whole-food animal feeding studies have any value in the safety assessment of GM crops? ***Regulatory Toxicology and Pharmacology*** 68: 171–174.

Herman RA, Woolhiser MM, Ladics GS, Korjagin VA, Schafer BW, Storer NP, Green SB, Kan L (2007). Stability of a set of allergens and non-allergens in simulated gastric fluid. ***International Journal of Food Sciences and Nutrition*** 58: 125–141.

Metcalfe DD, Astwood JD, Townsend R, Sampson HA, Taylor SL, Fuchs RL (1996). Assessment of the allergenic potential of foods derived from genetically engineered crop plants. ***Critical Reviews in Food Science and Nutrition*** 36(S): S165–S186.

OECD (1993). ***Safety evaluation of foods derived by modern biotechnology: Concepts and principles***. Organisation for Economic Co-operation and Development, Paris, France. https://www.oecd.org/science/biotrack/41036698.pdf.

OECD (2010). ***Consensus document on molecular characterisation of plants derived from modern biotechnology***. ENV/JM/MONO(2010)41. Organisation for Economic Co-operation and Development, Paris, France. http://www.oecd.org/env/ehs/biotrack/46815346.pdf.

Phillips McDougall (2011). ***The cost and time involved in the discovery, development and authorisation of a new plant biotechnology derived trait.*** A consultancy study for Croplife International Phillips McDougall, Midlothian, UK. https://croplife.org/wp-content/uploads/2014/04/Getting-a-Biotech-Crop-to-Market-Phillips-McDougall-Study.pdf.

Privalle LS, Gillikin N, Wandelt C (2013). Bringing a transgenic crop to market: Where compositional analysis fits. ***Journal of Agricultural and Food Chemistry*** 61: 8260–8266.

Ricroch AE (2013). Assessment of GE food safety using '-omics' techniques and long-term animal feeding studies. ***New Biotechnology*** 30(4): 349–354.

Rogers HA (2013). How composition methods are developed and validated. ***Journal of Agricultural and Food Chemistry*** 61(35): 8312–8316.

Schnell J, Steele M, Bean J, Neuspiel M, Girard C, Dormann N, Pearson C, Savoie A, Bourbonniére L, MacDonald P (2015). A comparative analysis of insertional effects in genetically engineered plants: Considerations for pre-market assessments. ***Transgenic Research*** 24: 1–17.

Thomas K, Aalbers M, Bannon GA, Bartels M, Dearman RJ, Esdaile DJ, Fu T-J, Glatt CM, Hadfield N, Hatzos C, Hefle SL, Heylings JR, Goodman RE, Henry B, Herouet C, Holsapple M, Ladics GS, Landry TD, MacIntosh SC, Rice EA, Privalle LS, Steiner HY, Teshima R, Van Ree R, Woolhiser M, Zawodny J (2004). A multilaboratory evaluation of a common ***in vitro*** pepsin digestion assay protocol used in assessing the safety of novel proteins. ***Regulatory Toxicology and Pharmacology*** 39: 87–98.

This activity was carried out with support from the United Nations Environment Programme (UNEP) and the Global Environment Facility (GEF), under project GFL/2328-2716-4C20, "Regional Project For Implementing National Biosafety Frameworks in the Caribbean Sub-region".

This activity was carried out with support from the United Nations Environment Programme (UNEP) and the Global Environment Facility (GEF), under project GFL/2328-2716-4C20, "Regional Project For Implementing National Biosafety Frameworks in the Caribbean Sub-region".

For further information please contact:
Regional Biosafety Project
info@caribbeanbiosafety.org