Safety Assessment of Food and Feed Derived from GM Crops: Using Problem Formulation to Ensure "Fit for Purpose" Risk Assessments

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Abstract

All genetically modified (GM) crops intended for use in food and feed must be assessed for their safety to humans and animals. The data and methodology used to conduct these assessments has been developed over many years. International organisations like the Food and Agriculture Organization (FAO) of the United Nations, the World Health Organization (WHO) and the Organisation for Economic Cooperation and Development (OECD) have been facilitating the harmonisation of food and feed risk assessment methodologies. The Codex Alimentarius Commission, established by FAO and WHO in 1963, has developed harmonised international food standards, guidelines and codes of practice and promoted coordination of all food standards work undertaken by international governmental and non-governmental organisations. The risk assessments undertaken to assess the safety of GM crops used in food and feed follow these standards. These risk assessments are conducted to support national regulatory authorities in making decisions on whether or not to approve the use of a GM crop in their country. Thus, the risk assessments must comply with every requirement outlined in the country's regulatory framework and provide clearly laid out robust scientific information to facilitate this decision-making process. In other words, the risk assessment must be "fit for purpose", clearly providing the necessary information.

Using the problem formulation methodology in risk assessments provides a tool to ensure that the assessments are fit for purpose. Problem formulation takes into account national protection goals and key regulatory requirements and drives the compilation of information relevant for the assessment.

Given the wealth of information on food and feed risk assessment methods and the existence of internationally-agreed consensus documents, the aim of this review is not to provide a comprehensive guide on how to conduct

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food and feed risk assessments. The aim is rather to demonstrate how problem formulation can be used in these assessments to ensure that they are fit for purpose and can indeed facilitate decision-making.

Keywords: biosafety, food and feed safety assessment, genetically modified crops, problem formulation, regulation.

Riassunto

Tutte le colture geneticamente modificate (GM) destinate ad essere utilizzate in alimenti e mangimi devono essere valutate per la loro sicurezza per l'uomo e gli animali. I dati e la metodologia applicata per condurre queste valutazioni sono stati sviluppati nel corso di molti anni. Le organizzazioni internazionali come l'Organizzazione delle Nazioni Unite per l'alimentazione e l'agricoltura (FAO), l'Organizzazione mondiale della sanità (OMS) e l'Organizzazione per la cooperazione e lo sviluppo economico (OCSE) hanno contribuito a facilitare l'armonizzazione delle metodologie di valutazione del rischio per alimenti e mangimi. La Commissione del Codex Alimentarius, istituita dalla FAO e dall'OMS nel 1963, ha sviluppato standard alimentari internazionali, linee guida e regole di condotta e promosso il coordinamento di tutte le norme alimentari adottate da organizzazioni governative e non governative internazionali. Le valutazioni del rischio utilizzate per stimare la sicurezza delle colture geneticamente modificate destinate all'alimentazione umana e animale seguono questi standard. Esse sono condotte per aiutare le autorità nazionali di regolamentazione nel prendere decisioni sull'opportunità o meno di approvare l'uso di una coltura GM nel Paese di appartenenza. Pertanto, le valutazioni dei rischi devono rispettare ogni richiesta delineata nel quadro normativo del Paese e fornire chiaramente informazioni scientifiche rilevanti per facilitare questo processo decisionale. In altre parole, la valutazione del rischio deve essere "adatta allo scopo", fornendo chiaramente tutte le informazioni necessarie.

Nella valutazione del rischio, il "problem formulation" fornisce uno strumento per assicurare che le valutazioni siano adatte allo scopo. La formulazione del problema tiene conto degli obiettivi nazionali di protezione e dei requisiti normativi fondamentali, e guida alla compilazione di informazioni rilevanti ai fini della valutazione.

Data la ricchezza di informazioni sui metodi di valutazione del rischio per prodotti alimentari e mangimi e l'esistenza di documenti di consenso concordati a livello internazionale, l'obiettivo di questo articolo non è quello di fornire una guida completa su come condurre la valutazione del rischio di alimenti e mangimi. L'obiettivo è piuttosto quello di dimostrare come la formulazione del problema è un metodo che può essere utilizzato in queste valutazioni al fine di garantire che siano adatte allo scopo facilitandone il processo decisionale.

1. INTRODUCTION

When a conventional crop is modified using modern biotechnology techniques there is a concern that the resulting crop may lead to harm to humans or animals if the crop products are consumed in food or feed. Thus, the use of genetically modified (GM) crops for food and feed is strictly regulated and most regulatory authorities around the world require a comprehensive safety assessment. The main aim of the safety assessment is to provide relevant information to allow regulatory authorities to make informed decisions concerning their eventual use. Therefore these safety assessments need to take into account the data requirements and protection goals established within the relevant regulatory framework to ensure that they are "fit for purpose" and indeed facilitate decision-making.

The process and methodology to follow in risk assessments of GM crops has been developed over many years. Generic methods for these risk assessment have been developed after much research, and build upon the experience of previous evaluations, approvals and the commercialisation of GM crops over more than twenty years. International organisations like the Food and Agriculture Organization (FAO) of the United Nations, the World Health Organization (WHO) and the Organisation for Economic Cooperation and Development (OECD), have been facilitating the harmonisation of food and feed risk assessment methodologies. The Codex Alimentarius Commission, established by FAO and WHO in 1963, has developed harmonised international food standards, guidelines and codes of practice and promoted coordination of all food standards work undertaken by international governmental and non-governmental organisations. Using this guidance and applying the problem formulation methodology, a methodology often used in environmental risk assessments, provides a useful way to collect and organise relevant information for the assessment. Problem formulation takes into account relevant protection goals and national regulatory requirements to guide the compilation of relevant information for the specific crop/trait combination under evaluation. The problem formulation step is also where an initial risk characterisation, using available information, is conducted to establish whether enough data is available to characterise the risk or to require that additional data be gathered. If the latter is concluded, problem formulation provides a good methodology to develop an analysis plan based on specific assessment endpoints from which scientific hypotheses and measurement endpoints can be defined. This increases the probability

that the risk assessment is fit for purpose and will be useful for decision-making. This process is widely used in risk assessments and is now part of the guidance provided by some regulatory authorities. This review provides an overview of how problem formulation can be used in GM food and feed risk assessments to facilitate regulatory decision-making.

2. KEY CONCEPTS

It is accepted that the relevant risks of food and feed that has been consumed by humans and animals for years are known and that overall they are generally regarded as safe; this is the basis for the concepts of "familiarity" (OECD, 1993a) and "history of safe use" (OECD, 1993b). This includes foods that are known to contain toxins, like cassava, potatoes and dried beans that can cause health problems when raw, but which are considered safe for consumption once correct processing procedures have been followed.

As for conventional foods, the safety of GM food or feed cannot be determined by a single study (OECD, 1993b). Whole foods are complex mixtures of compounds, often characterised by wide variations in composition and nutritional values. Often environmental and agronomic factors influence the composition of components in similar varieties of the same conventional crop. Therefore, the safety assessment of food is usually based on the evaluation of specific components (Codex Alimentarius, 2003a).

It is widely accepted that the best approach to begin the safety assessment of GM food and feed is a comparative approach (OECD, 1993b; Schauzu, 2000; Codex Alimentarius, 2003a; Codex Alimentarius, 2003b; Kok & Kuiper, 2003; Konig et al., 2004; Hammond, 2008; Kleter & Kok, 2010), where the GM crop is compared with its conventional counterpart to determine potential changes in composition of key components. This process is known as the evaluation of "substantial equivalence" (Schauzu, 2000) and has been adopted by leading food and regulatory bodies worldwide. According to this principle, if a new food or feed derived from a GM crop is shown to be substantially equivalent to its conventional counterpart, then it is considered to be as safe as the food or feed from the conventional crop, and the assessment then focusses on the safety of the introduced traits (Codex Alimentarius, 2003b).

3. THE COMPARATIVE ASSESSMENT

As discussed in the previous section, the results of a single study alone cannot conclusively establish whether a GM crop food or feed poses a low risk to human or animal health. Therefore the internationally-agreed approach proposed by experts in the field is to conduct a comparative assessment (OECD, 1993b; Codex Alimentarius, 2003a). The comparative assessment allows the identification of any differences between the GM crop and its conventional counterparts that may have arisen due to the genetic modification. The process follows a "weight of evidence" approach, where numerous sources of information are used to identify these potential differences and their implications for food or feed safety (see Figure 1.) (Cockburn, 2002; Konig et al., 2004; Herman et al., 2009; Parrott et al., 2010; Kuntz & Ricroch, 2012; Herman & Price, 2013; Ricroch, 2013).

It is important to note that differences between the GM plant and its conventional counterpart are sometimes detected. However, a difference does not necessarily indicate that an adverse effect will occur. Once differences are detected their biological relevance and the probability that they could lead to an adverse human or animal health effect is evaluated. If the differences are biologically relevant and could lead to adverse effects, then the risk assessment is focused on the risk associated with these differences (Garcia-Alonso, 2010).

The regulatory data package for a new GM crop usually comprises data on molecular characterisation, compositional analysis and agronomic characterisation as these are data that are required in most regulatory frameworks. Therefore these data can be used during problem formulation.

For GM products that have already been commercialised in one country and are being evaluated for approval in another, problem formulation can help establish if the data already available for the comparative assessment is sufficient or if more data has to be generated. If the previous food and feed safety assessment did not reveal any concerns and established that the GM crop was as safe for food and feed consumption as the conventional counterparts, these risk assessment can be used in other geographies and no further data needs to be generated. Exceptions to this occur when specific data requirements established in the national regulatory framework are not

fully met, or where there are specific aspects of food or feed consumption habits in the importing country that need to be taken into account.

For most GM crops the only differences identified are those expected due to the intended effect of the modification; referred to as "intended" differences. However, there is a concern that the transformation process could have led to some "unintended" differences, maybe due to the disruption of an important endogenous gene or metabolic pathway. Such unintended differences are not unique to GM crops and can also occur during conventional breeding.

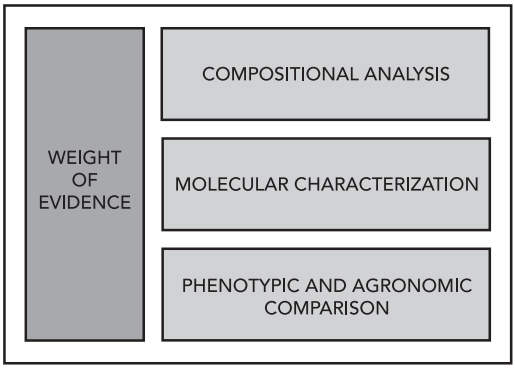

Figure 1. The weight of evidence approach used in comparative assessments takes into account data from molecular characterisation, compositional analysis and phenotypic and agronomic comparisons.

3.1. Molecular characterisation

The use of molecular characterisation for environmental or food and feed risk assessments has been well described in a consensus document published by OECD (OECD, 2010). Molecular characterisation data provides information that can be informative for the risk assessment of GM plants. Although it provides knowledge, at the molecular level, of the inserted DNA, the insertion site and the expressed material and may provide information on intended and possible unintended effects, it is not the primary means to detect such unintended effects (Macdonald, 2012).

The molecular characterisation conducted for GM crops includes an analysis of the transgene sequence to ensure that no sequence changes have occurred during the transformation process and the transgene sequence is as intended. An analysis of the transgene flanking regions is also conducted to establish whether the gene insertion has resulted in any disruption of key endogenous genes. It also includes an open reading frame analysis (ORF) to determine whether proteins other than the intended proteins could be produced by the GM plant. If new proteins (other than the intended protein[s]) were produced, the safety of these unintended proteins must be evaluated (OECD, 2010).

In summary, molecular characterisation provides an indication of whether the genetic modification was as intended, and whether any disruptions at the molecular level occurred after transformation. This information is useful as an indicator of potential issues; however the safety assessment relies more heavily on other sources of information based on the actual phenotypical characteristics of the plant (Macdonald, 2012).

3.2. Compositional analyses

Compositional analyses constitute the core of the risk assessment for GM food and feed (Codex Alimentarius, 2003b). In these studies the composition of a transgenic crop is compared with that of its conventional counterpart that has a history of safe consumption. The aim is to establish if the GM crop is "substantially equivalent" to the conventional crop and if the only differences are those intended by the genetic modification (Schauzu, 2000). If this is the case, the safety assessment focuses on properties of the gene products expressed by the transgenes (OECD, 1993a; OECD, 1993b; Codex Alimentarius, 2003a; Codex Alimentarius, 2003b). If differences are found to be consistent and biologically relevant, and they have the potential to lead to human or animal harm, they are then included in the risk assessment for further risk evaluation (See Section 3.4 below).

In order to generate data for the compositional analysis studies, field trials are conducted in a range of locations representative of different environments where the GM crop may be grown. Tissue samples of tissues that may be consumed in food or feed are collected for analysis of different components. The number of trials can vary from region to region, but the purpose of carrying them out in different locations is to investigate whether

there are environmental factors that could reveal differences between the composition of the GM crop and the conventional counterpart (George et al., 2004; Herman et al., 2009; Ricroch, 2013). There is a large amount of scientific literature and many regulatory submissions that demonstrate the compositional equivalence of numerous GM crops tested in different geographical areas (Harrigan et al.., 2010; Herman and Price, 2010; Ricroch, 2013). The studies often report that statistically significant differences in composition are observed between different varieties of the same crop, but not between the GM crop and its comparator (Reynolds et al., 2005; Herman et al., 2009; Harrigan et al., 2010; Herman & Price, 2010). Once trials have been conducted in a range of different environments, the uncertainty regarding the differences due to environmental factors is better defined and generally reduced. Any regulatory instruction to conduct additional field trials to generate more data must be carefully considered, as this may constitute a duplication of work and may not help in further reducing uncertainty.

The components that are analysed in the plant tissues are chosen using expert knowledge, much of which has been collated and published in consensus documents on compositional characteristics for the crop by the OECD (see Table 1).

Table 1. List of OECD consensus documents available on the compositional considerations and crop biology per crop. Available at www.oecd.org/env/ehs/biotrack/consensusdocumentsfortheworkonthesafetyofnovelfoodsandfeedsplants.htm.

Crop	Compositional Considerations	Biology
Alfalfa	√	
Bananas & plantain		√
Barley	√	
Cassava	√	
Chilli, hot and sweet peppers		√
Cotton	√	√

Cucurbits		√
Grain sorghum	√	
Maize	√	√
Oilseed rape/Canola	√	√
Papaya	√	√
Potato	√	√
Rice	√	√
Soya bean	√	√
Sugar beet	√	√
Sugarcane	√	
Sunflower	√	√
Sweet potato	√	
Tomato	√	
Wheat	√	√

These consensus documents listing key components for different conventional crops are a very valuable source of information, as they provide a harmonised approach concerning the components to test. This contributes to the knowledge on the levels of components that have been used safely as food and feed (history of safe use), thus enabling the construction of databases that provide information on the safe ranges of each component for these crops (ILSI, 2010). For maize, for example (Box 1), specific grain and forage samples are recommended for collection (OECD, 2002), and represent components of nutritional value or indicators of metabolic pathways that are important for the safety assessment. In maize grain, 60-70 components are analysed and statistically compared (OECD, 2002; Reynolds et al., 2005; Herman & Price, 2010). This represents a large number of analyses and comparisons.

BOX 1. MAIZE EXAMPLE

Consider a compositional analysis study for a GM maize variety where four field trials were conducted. In each trial, two entries were included: the GM plant and the comparator planted in four replicate plots per entry. If one type of tissue was collected, for example grain, at least 60 components were analysed per sample. This means that there are a total of 32 samples (4 trials, 2 entries, 4 replicates, 1 tissue), for which 60 components are analysed making a total of 3,840 data analyses and data points. These data are then analysed statistically to establish if differences in component levels are detected between the different entries and between locations.

In some studies, conventional commercial varieties of the crop are also included, increasing the number of entries and thus the number of analyses and comparisons.

Given the large number of comparisons made, it is not uncommon to detect some random statistically-significant differences. Differences in one component that are consistent amongst locations and have a common trend (always higher or lower in one of the entries) are considered relevant, so their potential to cause harm to human or animal health is further evaluated. The levels of the component found in the GM plant are compared with the range of the values normally found in commercial varieties of the crop (Codex Alimentarius, 2003b; Herman & Price, 2010). The range of values found for key components in many crops can be found in online databases like the ILSI database (ILSI, 2010). If the differences observed for the component in the GM plant fall within the ranges considered safe in commercial varieties, it can be concluded that the GM crop will be as safe as the conventional crop varieties apart from the intended trait(s), which is then assessed (Codex Alimentarius, 2003b). If differences are identified that fall outside the ranges, the implications for human and animal health of these different levels are then evaluated for their biological relevance during the risk assessment (Garcia-Alonso, 2010).

3.3. Agronomic and phenotypic comparisons

Most regulatory data packages contain studies aimed at comparing the phenotypic and agronomic characteristics of the GM plant with conventional counterparts. These studies provide useful information for environmental risk assessments, as they allow establishing if the genetic modification has

resulted in changes in any phenotypic or agronomic characteristics that could lead to environmental harm. For example, changes in reproduction characteristics or traits that are linked to weediness are evaluated to assess whether the GM plant could be more persistent or invasive than the conventional counterparts. However, these studies are also used in the comparative safety assessment as an additional component of the weight of evidence approach to identify unintended differences between the GM and conventional counterparts.

In these studies the GM plant is grown alongside the conventional counterpart in several field trials conducted at a range of locations representative of different environments where the GM crop may be grown. The endpoints recommended for measurement are derived from breeder´s experience and cover morphological as well as physiological parameters. They are specifically selected to be sensitive and representative of the key biological features that determine the agronomic behaviour of the crop (Garcia-Alonso, 2010; Gray, 2012; Macdonald, 2012).

Any consistent differences that are identified are assessed for their biological relevance and potential safety implications and are taken as indicators of manifestations of potential unintended effects of the genetic modification. As with compositional analyses, differences between the GM plant and conventional counterparts do sometimes occur and when identified they are assessed for their relevance and potential to cause harm. However, for most GM crops entering the regulatory process, major phenotypic unintended changes in the plant that could lead to adverse effects are very unlikely as developers conduct numerous plant characterisation studies during event selection and development; and identified rogue events are quickly discarded (Gray, 2012).

3.4. Unintended effects of the genetic modification

One of the concerns regarding GM crops is that the genetic modification may have resulted in unintended changes in the crop that may lead to environmental or human or animal harm. The comparative assessment provides a weight of evidence approach that allows the identification of differences between the GM plant and its comparators (Figure 2). For most GM crops, the only differences identified are those expected due to the intended effect of the genetic modification (intended differences). In other cases, other differences may be identified, for example differences

in composition or in agronomic characteristics (unintended differences). A difference does not necessarily constitute an indication of adverse effects, therefore any differences observed, whether intended or unintended, are assessed first for their biological relevance and potential to cause harm. If such differences are identified, they are assessed, regardless of whether they were intended or unintended (Garcia-Alonso, 2010).

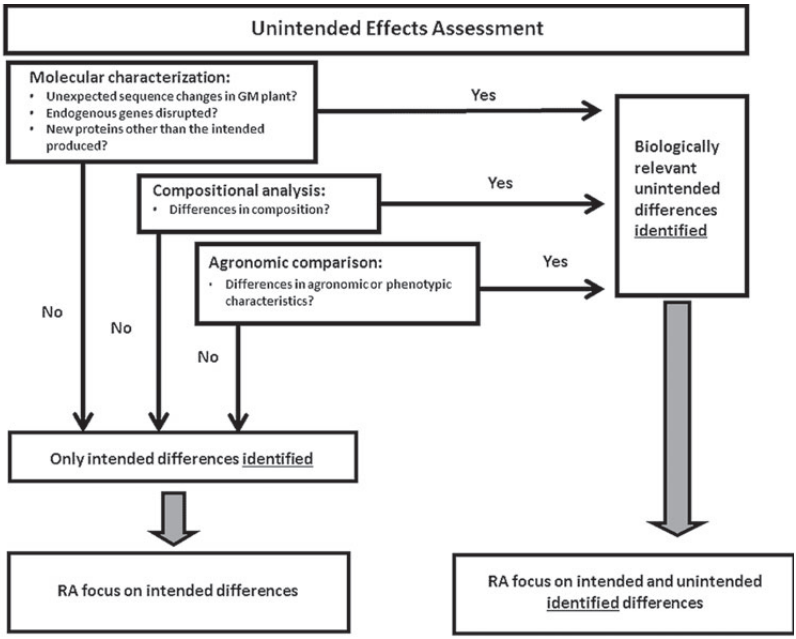

Figure 2. The weight of evidence approach used in comparative assessments facilitates the identification of unintended changes in the GM plant that may have to be assessed in the risk assessment.

4. ALLERGENICITY ASSESSMENT

A key component of the GM food and feed safety assessment is to assess whether the genetic modification has resulted in the introduced gene encoding a major allergenic protein into a food crop that the crop did not previously contain. Some food crops that are widely consumed are known to contain allergens that result in food allergies (for example nuts). Therefore, the assessment also considers whether the genetic modification could have increased the levels of endogenous allergens in the GM crop.

In allergenicity assessments, problem formulation can be applied to focus the assessment on the key questions and allow the gathering and organisation of the information in a logical and structured way. Here the comparative assessment is used to establish the focus of the assessment. Virtually all food allergens known are proteins, therefore the primary focus of the allergenicity assessment for GM crops is to assess the allergenic potential of the new proteins produced by the crop. As for the toxicity, if substantial equivalence has been established and the only novel proteins produced are intended, the allergenicity assessment focuses on those proteins. If unintended new proteins are identified, the potential allergenicity of those proteins is also assessed. In addition, if the conventional crop is known to contain allergens, an assessment is conducted to establish if the genetic modification has led to changes in the levels of those endogenous allergens.

Recognising that there are no single definitive tests for allergenicity, a weight of evidence approach using several sources of information is used. Codex Alimentarius provides guidance (Codex Alimentarius, 2003b) on the types of information and process to follow to assess allergenicity of GM crops. The first step in this approach consists of the collection of available information concerning the proteins under assessment:

- *Donor source* - knowing the source of the genes used in the genetic modification provides information on whether these came from an allergenic source and therefore the potential for allergenicity.

- *Protein sequence* - comparing the sequence of the transgene-derived protein with the sequence of proteins that are known allergens provides an indication of whether cross-sensitisation can occur. Specific

triggers based on the percentage of homology between the sequences are available (Codex Alimentarius, 2003b; Goodman et al., 2008), and can aid deciding whether the degree of homology is significant and whether further evaluations are necessary.

- *Susceptibility to proteolytic digestion* - some known allergens are resistant to pepsin degradation, therefore the degree of such resistance is used in the assessment as an indicator of potential allergenicity. However, it is important to note that not all allergens are resistant to pepsin degradation and not all proteins resistant to such degradation are allergens.

- *Susceptibility to heat degradation* - some allergens remain stable upon heating and can survive cooking procedures.

- *Prevalence in food* - many proteins that are allergens are seed storage proteins expressed as a major constituent of the protein in food crops. Novel protein expression levels in the item of commerce are considered in this context to determine their contribution to the overall food protein profile.

- *History of Safe Exposure* - a review of the source organism, the novel protein, and proteins homologous to the novel protein are all considered for their potential to exist in the human and food chain as safe foods. A novel protein known to already be a safe food and be derived from a safe organism is unlikely to pose an unanticipated risk for allergy.

The main purpose of this approach is to assess the amino acid sequence and other biophysical features of the protein to identify significant similarities with known allergens. If the protein does not come from an allergenic donor, does not have homology to known allergens, is not resistant to pepsin and not resistant to heat degradation, it can be concluded that is not likely to be allergenic (Goodman et al., 2008).

If the protein originates from a source known to be allergenic, or has sequence homology with a known allergen, then the assessment continues. A case-by-case approach is followed to design a suitable analysis plan (see Box 2).

BOX 2. EXAMPLE

Consider a GM maize that produces a new protein. The allergenicity of the protein has been investigated. A significant level of sequence homology between the protein and a known allergen has been detected (e.g. >70% over the length of the protein). In this case, there is a risk of cross-reactivity between the proteins, and humans allergic to the known allergen could have allergic reactions to the protein produced by the GM maize. Following the problem formulation methodology, an analysis plan would have to be put into place where carefully designed studies testing cross-reactivity would have to be conducted.

In summary, using problem formulation provides a logical way to gather the relevant information to conduct an allergenicity assessment and conduct an initial risk characterisation to establish if further data is necessary. If this is the case, problem formulation can be used to design the most suitable analysis plan to complete the assessment so conclusions regarding food and feed safety can be taken.

5. USING THE PROBLEM FORMULATION METHODOLOGY IN FOOD AND FEED RISK ASSESSMENTS

5.1. Problem formulation

Problem formulation is a process which consists of the collection and consideration of all the data available on the GM crop to allow the formulation of testable hypotheses and the design of a plan to test them (Raybould, 2006; Wolt et al., 2010). In other words, problem formulation is a tool for identifying questions relevant to the risk assessment and gathering the relevant information to answer them (Gray, 2012).

Problem formulation takes into consideration the relevant protection goals and regulatory data requirements set in the regulatory framework, thereby focusing the risk assessment on key questions and ensuring that it will be fit for purpose (Figure 3). Problem formulation also includes an initial risk characterisation, conducted using existing data, to establish whether the data gathered is sufficient to characterise the risk or if additional information is necessary. Where a need to generate more data is identified, an analysis plan can then be developed to fulfil the specified needs. If no additional information is required, the risk assessment can stop at this step (Box 3).

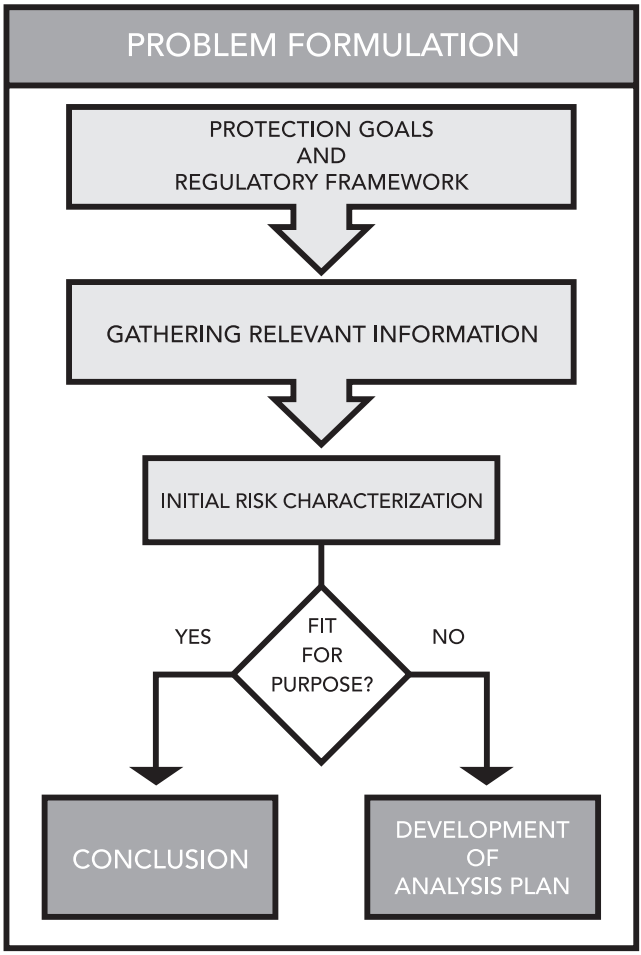

Figure 3. **Problem formulation components.**

BOX 3. EXAMPLE

Consider a GM maize that produces a new protein for pest control. The aim of the safety assessment is to establish that consuming the GM maize will be as safe for humans and animals as conventional maize. The comparative assessment concluded that the GM and non-GM counterpart are substantially equivalent and only differ on the presence of that protein. Two scenarios can arise:

1. The protein is well known and has been introduced previously in other GM crops that are commercialised or used in registered pesticide sprays. Information on the hazard of the protein is therefore available and can be used to conduct an initial risk characterisation using expression data in the GM crop under assessment to do an exposure assessment. If the risk is acceptable, the risk characterisation can be completed at this step (conclusion).

2. The protein is new and there is no hazard data and no data on previous exposure. In this case, it is unlikely that the risk can be fully characterised at this step. An analysis plan can be designed to determine which studies are necessary for the hazard assessment.

5.1.1. Protection goals and regulatory frameworks

When a risk assessment is conducted, it is essential to have a good understanding of the policy protection goals that have been established in the country. In general, for food and feed risk assessment, the protection of human and animal health is a universal policy protection goal. Policy protection goals tend to be formulated in broad terms and cover many different aspects (Evans et al., 2006), for example "Protection of human health". These policy protection goals need to be translated into operational protection goals that can be used in risk assessment, so that assessment endpoints and risk hypotheses can be formulated (Figure 4). For example, an operational protection goal could be "The consumption of GM food or feed should not lead to adverse health effects in humans". This type of operational translation focuses the risk assessment on what is to be protected (humans and animals), from what (GM food or feed), and in what situation (when GM food or feed is consumed). Assessment endpoints are then further elaborated, explicitly expressing the environmental value that is to be protected, specifying the ecological entity that represents the

area of protection (humans or animals), the unit (individuals or populations), the attribute to protect (health), the magnitude (no toxic or allergenic effect), and the temporal and spatial scale (in a particular country, after consumption of GM food or feed) and the degree of certainty (high/low/medium). Assessment endpoints allow the formulation of specific risk hypotheses from which measurement endpoints can be defined (Raybould, 2006; Gray, 2012).

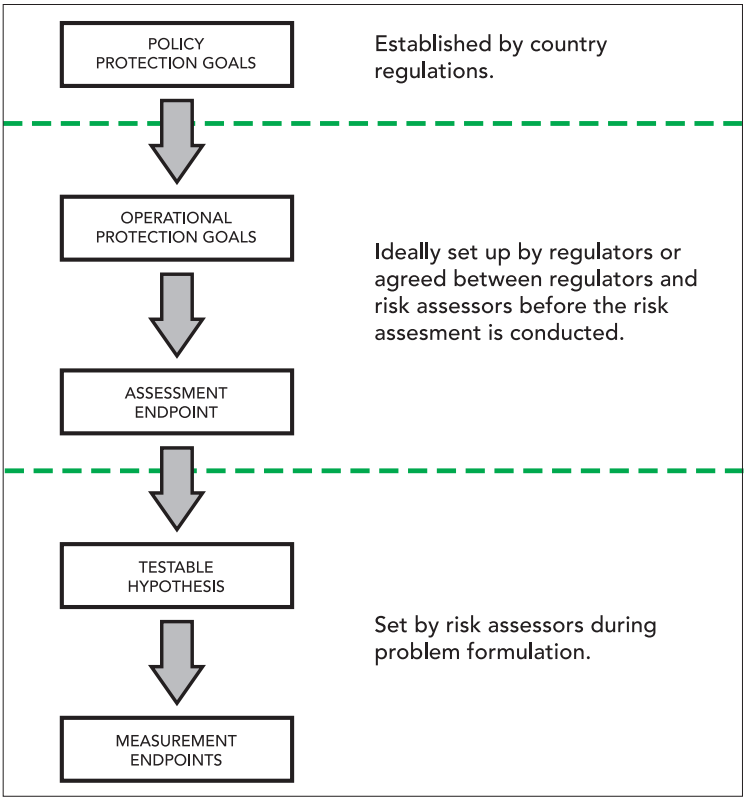

Figure 4. Translating policy protection goals into operational protection goals to use in risk assessment.

In addition to the protection goals, the data requirements set out in the regulatory framework are considered to ensure that the risk assessment will be fit for purpose and allow decision-making.

Regulatory data requirements are not always clearly defined in all regulatory systems, but in the absence of specific regional guidelines, there are some international organisations like OECD and Codex (OECD, 1993b; Codex Alimentarius, 2003b), that have been facilitating the harmonisation of risk assessments procedures. Some regulatory authorities have comprehensive guidance documents that outline the information and studies required for their safety evaluations. Other regulatory agencies are less specific, but still require a comprehensive safety assessment (Macdonald, 2012).

For the safety assessment of food and feed derived from GM crops, the principles and methods used generally follow the recommendations from the Codex Alimentarius (Codex Alimentarius, 2003a; Codex Alimentarius, 2003b); documents developed by international experts in the field of food safety. The documents, providing an assessment framework and guidance on the data necessary to conduct the safety assessment, were developed as a result of several scientific conferences and intergovernmental consultations on the subject of food safety and biotechnology (OECD, 1993a; OECD, 1993b; Codex Alimentarius, 2003a; Codex Alimentarius, 2003b).

In summary, during this stage in the problem formulation it is important to have a clear understanding of the protection goals and regulatory requirements to ensure that the risk assessment is fit for purpose and helps the local regulators making decisions on whether to approve or not the GM food or feed for consumption in that country.

5.1.2. Gathering relevant information

Information used in risk assessments needs to be of high quality, reliable, well referenced so the sources of information can be traced and reviewed by other parties, but above all, the information used must be relevant ("need to know" versus "nice to know") (Raybould, 2012). For example, some information can be of high quality and reliable, but simply not relevant, in which case regulators could spend a large amount of time reviewing information that will not facilitate their conclusion and may delay decision-making (Raybould, 2012).

The sources of relevant information can be very varied and can include regulatory studies generated by the developer, scientific literature, international consensus documents and reports of assessments conducted by regulatory authorities. Regulatory studies aim at providing information conducted using validated protocols that follow international guidelines. These studies provide confidence in the methodology used and are reported in sufficient detail to establish what materials were used, how they were produced and what analytical techniques were used. Studies reported in the literature in peer-reviewed papers may also follow some of these principles, but often reflect innovations in methodology that may not yet be validated and thus require further work before they can be used in regulatory evaluations. When gathering information for the risk assessment, it is therefore key that risk assessors establish the relevance and weight of each piece of information collected.

The Codex Alimentarius and OECD documents previously cited provide useful guidance on the types of information to collect for GM food and feed risk assessments. This usually covers information on the conventional crop, on the transgenes and on the GM crop. A comparison between the GM crop and conventional counterparts is then conducted to establish if there is substantial equivalence and to identify differences that could lead to adverse health effects. This part of the comparative assessment is used to determine if relevant unintended differences were detected and need to be included in the assessment.

In summary, problem formulation guides the compilation of relevant information for the risk assessment.

5.1.3. Initial risk characterisation

During the first step of problem formulation, all gathered information is used to make an initial risk characterisation. For the food and feed risk assessment, the comparative assessment is an essential part of this as it allows identifying the potential hazards that must be included in the assessment (intended and/or unintended differences identified between the GM plant and the conventional counterpart) and can also provide information on previous exposure. Since risk is a function of hazard and exposure, the risk characterisation conducted within problem formulation aims at gathering available information for the evaluation of the adverse

health effects associated with the hazards identified and an estimation of the levels of exposure of those hazards in food or feed. For example, if the only difference observed between the GM plant and the conventional counterpart is the introduced protein, the risk assessment focuses on the protein. The information gathered can be used to establish if the protein can be toxic to humans or animals and if the protein is actually present in the parts of the plant to be consumed in food or feed.

5.1.3.1. Hazard assessment

The first step of hazard assessment is to identify the hazards posed by the GM plant. As explained above, this process is facilitated by the comparative assessment (Figure 2). Hazard assessment aims to establish the intrinsic potential of an agent to cause adverse health effects. Therefore the potential toxicity to humans or animals of any relevant identified differences is evaluated.

As previously discussed in Section 3, in most GM crops evaluated to date, the only differences observed between the GM plant and conventional counterparts are the intended traits. The potential toxicity of these traits has been evaluated. These evaluations have been conducted on a case-by-case basis taking into account the source, function, activity and history of human or animal safe consumption. In general, given the structure and properties of proteins introduced in GM crops so far, the oral bioavailability of these proteins is very low and most proteins are considered non-toxic. However, since there are some proteins that are known toxins, evaluations to determine sequence similarities with known toxins and allergens are usually conducted. Proteins that are known toxins generally exert their effect through acute modes of action. For this reason, some regulatory authorities require confirmatory safety studies. Usually this involves the provision of acute oral toxicity data on novel proteins administered at high doses where "no observed adverse effects levels" (NOAELs) are reported. For most of the proteins used in GM crops, these NOAELs reflect the maximum dose tested in the studies, as no adverse toxic effects have ever been detected even at these high doses (Hammond, 2008). Reviews providing data on toxicity studies conducted on many of the proteins used in GM crops are available, for example, Hammond (2008) provides an excellent review of the methods and process used for characterising the potential toxicity of proteins.

For hazards identified during the comparative assessment for which there is no data available concerning previous exposure or toxicity, problem formulation can help to determine what information has to be generated for the hazard assessment. As described in the Codex Alimentarius (Codex Alimentarius, 2003b), one of the first steps is to gather all information available on the structure and function of the traits or components, and to determine whether there are similarities with known toxins or allergens. For such hazards that are proteins, their sequence is compared to sequences of known toxins, anti-nutrients or allergens. The need to conduct additional toxicity studies is evaluated on a case-by-case basis and will depend on the identity and biological function of the hazard in the GM plant and the likely dietary exposure. Other types of studies can be also considered, such as in vitro studies.

For example if the evaluation is to focus on a transgenic protein produced by a GM plant, information can be gathered on any previous safe exposure in food and feed, and a determination made as to whether it was at similar levels to those found in the GM plant. Such proteins that have a history of safe consumption in food are regarded as safe for consumption (Hammond & Cockburn, 2008).

5.1.3.2. Exposure assessment

In food and feed risk assessments, the exposure assessment is based on the estimation of the dietary intake of the hazards identified (Box 4) and anticipating the effect of food or feed processing. The assessment is conducted in a step-wise process where initially a conservative "worst-case" scenario is considered. The worst-case scenario is built to reflect conditions of much higher levels of exposure than would normally occur to account for most scenarios of exposure. If the risk estimated under this scenario is acceptable, the risk associated with lower exposure will be lower and thus acceptable too. If the risk is not acceptable, exposure estimates can be refined to reflect more realistic levels of exposure.

Expression studies conducted to determine the concentrations of the novel proteins in the GM plant provide data on the expected levels of the proteins in edible tissues. Food consumption databases provide estimates of the expected intake of certain foods in the diet. Knowledge of the uses of the conventional crop in food and feed also provides useful information for the exposure assessment as it helps to determine if the food or feed

is consumed raw or cooked or if only certain processed products from the plant are consumed.

A conservative worst-case exposure scenario can be built considering the following assumptions:

- The food or feed will contain the maximum expression levels detected in the GM plant (maximum exposure as processing or cooking processes that could lower the exposure are not considered).
- All the dietary intake estimated for that food and feed is composed of the GM product under evaluation (maximum exposure as agriculture products are usually co-mingled and 100% purity is rare).

There are a number of databases that provide data collected on human food consumption in different countries, for example the "GEMS/Food Cluster Diets" (WHO, 2010). Other examples of databases can be found in a recent review (Petersen, 2008).

BOX 4. NOVEL PROTEIN EXPOSURE ASSESSMENT

The maximum expression levels of one of the novel proteins introduced in a GM maize is 1.8µg/g fresh weight. Assuming that the consumption of maize in the population for which the assessment is being conducted is estimated at 150g maize/person/day, and considering that an average person weighs 60kg, the consumption of maize is 2.5g maize/kg body weight/day. The amount of novel protein in the diet would be:

150.4g maize/60kg person/day x 1.8µg protein/g maize = 0.0045mg novel protein/day

5.1.3.3. Risk assessment

During the problem formulation step an initial risk characterisation can be conducted using all relevant data available on hazard and exposure. For food and feed risk assessments the potential toxicity of the hazards identified (i.e. relevant differences between the GM plant and its conventional comparators that could lead to harm) is evaluated, as described in Section 4.1.3.1. and exposure to them is estimated as described in Section 4.1.3.2. When possible, the risk is quantified to have an estimation of toxicity exposure

ratios and margins of safety. For example, if it is considered that a protein can be toxic and a toxicity study has been conducted, the endpoint measured, usually a NOAEL, can be compared with the estimated exposure in the diet. This approach is routinely followed to assess the risk of chemicals or toxic substances to obtain a measure of the margins of exposure and estimate how much of the food in which the protein is present would need to be consumed by a person to receive a toxic dose. For proteins this approach is not so useful as usually there are no adverse effects detected in the toxicity studies, therefore the estimation would indicate how much food containing the novel protein a person would have to consume to receive a dose that has been shown not to elicit any adverse effects. Thus the only advantage of this approach is to provide some quantification, but this needs careful interpretation.

Often quantitative measurements of hazard and exposure are not possible and ordered categorical descriptions of risk are used. A good example of risk categorisation using a qualitative approach is described by the Office of the Gene Technology Regulator (OTGR, 2009). This approach estimates the probability that an adverse effect occurs (likelihood assessment) and compares it with an estimate of the consequences if it occurs. A descriptive scale for the likelihood and seriousness of harm in relation to human health is provided (Figure 5). If harm is not expected to occur (for example, because the object of analysis is not toxic or there is no plausible exposure), then risk is considered insubstantial and the impact needs no further analysis.

		RISK ESTIMATE			
LIKELIHOOD ASSESSMENT	Highly likely	Low	Moderate	High	High
	Likely	Low	Low	Moderate	High
	Unlikely	Negligible	Low	Moderate	Moderate
	Highly unlikely	Negligible	Negligible	Low	Moderate
		Marginal	Minor	Intermediate	Major
		CONSEQUENCE ASSESSMENT			

Figure 5: Risk matrix to estimate the level of risk from a combination of outcomes of likelihood and consequence assessments (OGTR, 2009).

In some cases, the initial risk characterisation conducted during problem formulation is not sufficient to characterise the risk and the assessment needs to continue by designing an analysis plan that can provide the information needed to complete the assessment. This usually involves further hazard and exposure characterisation that may require conducting studies aimed at addressing specific test hypotheses. If after completing the assessment the risk or the uncertainty associated with that risk are considered too high, a risk management plan can be implemented. For example, if the risk of causing a toxic effect due to an introduced toxin that is heat labile is detected, the risk could be managed by recommending that the food is not consumed raw, but must be subjected to cooking first (such is the case of many common foods like cassava).

6. CONCLUSIONS

GM crops have been commercialised for over 20 years. Every GM crop commercialised has undergone a strict safety assessment to establish their safety for use in food and feed. The experience gained over this time has led to a better understanding of the data required to conduct safety assessments and there are internationally-agreed guidelines that describe the process to follow. However, the decision of whether or not to approve a GM crop for commercialisation usually takes place at the national level, where policy protection goals and national regulatory frameworks are in place. Therefore, all risk assessments must comply with these national requirements to facilitate the decision-making process. Using problem formulation in these assessments increases the probability that the assessments are fit for purpose and indeed facilitate decision-making, since risk managers receive information that is directly relevant and essential to making informed decisions. Problem formulation takes into account national policy protection goals and data requirements to drive the compilation of relevant data. The initial risk characterisation helps establishing if more data is necessary and if so, problem formulation facilitates the design of analysis plans to address the questions remaining.

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