EuropaBio
The European Association for Bioindustries

Safety Assessment of GM Crops

Document 1.1 Substantial Equivalence - Maize

Issue 2 (July 2003)

Introduction

This document is part of a series on the safety assessment of genetically modified (GM) crops, prepared by the Technical Advisory Group (TAG) of EuropaBio's Plant Biotechnology Unit (PBU). The objective of these documents is to summarise the current consensus of the member companies of PBU on the data necessary for notifications submitted under Council Directive 2001/18/EC, on the deliberate release into the environment of genetically modified organisms, and Regulation (EC) No 258/97, concerning novel foods and novel food ingredients.

The series of documents will focus on technical issues related to the safety of GM crops, such as the assessment of substantial equivalence, provisions for detection and identification, requirements for molecular characterisation and protein safety evaluation, the appropriate use of animal feeding studies and the monitoring of GM crops. All documents will be regularly revised and re-issued with increasing experience on the safety assessment of GM crops.

This document specifically addresses the assessment of substantial equivalence of GM maize (*Zea mays*), particularly the data requirements for compositional analysis and agronomic assessment of GM maize and non-GM maize of comparable genetic background. The selection of data is based on the requirements specified in the above legislation, relevant WHO/FAO (2000) and OECD (1998, 2001, 2002) guidance documents, and published guidance from European regulatory authorities (2003). It also incorporates requests for certain additional data by Member State Competent Authorities expressed in correspondence to companies in respect of different notifications for the approval of GM maize. Finally, the document offers the considered rationale of member companies in compiling the consensus based on these various sources.

Similar documents[1] on the data requirements for the assessment of substantial equivalence of other GM crops have been completed for soybean, oilseed rape, and sugar beet.

1. Compositional analysis

Commission recommendation 97/618/EC, concerning scientific aspects and the presentation of information necessary to support notifications under Regulation (EC) No 258/97, states that compositional analysis "should focus especially on the

[1] Available at http://www.europabio.org/pages/eu_workgroups_detail.asp?wo_id=14

determination of the content of critical nutrients and any critical toxicants and anti-nutritional factors which might be either inherently present or process derived". The International Food Biotechnology Council (IFBC) report (IFBC, 1990) states that "in evaluating a genetically modified food, a comparison with its traditional counterpart will be necessary in order to determine whether the significant nutrients in the new food as consumed will fall within the range typical of the product. If the new product is found to have essential nutrients in the same range as its traditional counterpart, no further nutritional evaluation of the product would be required". This concept, known as substantial equivalence, has been embodied in the regulatory policies such as the US Food and Drug Administration (FDA) policy on GM plant varieties (FDA, 1992) and reaffirmed recently in the Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology (FAO/WHO, 2000).

However, before identifying the data to determine the nutrients and any anti-nutritional factors (see section 1.4) for the assessment of substantial equivalence, it is important to establish a framework under which such data is collected.

1.1 Trial numbers and locations

Based on previous experience, compositional data from a minimum of four locations, consisting of three replicates per treatment, from each of the two growing seasons (total eight trials) would normally be sufficient for the statistically valid assessment of substantial equivalence.

With regard to locations and for notifications under Directive 2001/18/EC for production (cultivation) approval, these data should be collected from trials carried out in the EU and elsewhere, representing a range of agricultural environments which are typical of where the crop is grown. For notifications under Directive 2001/18/EC for import approval only, and for notifications under the "Novel Foods" Regulation (EC) No 258/97, data should be collected from a similar number of trials representing a range of agricultural environments which are typical of where the crop is grown, either in the EU or elsewhere.

These requirements could be streamlined if bridging studies show that different locations do not alter the variables selected for the compositional analysis.

1.2 Experimental comparisons

Trials designed to obtain samples for compositional data should contain the following experimental treatments for comparison:

A. GM maize, conventionally managed;
B. Non-GM maize (comparable genetic background), conventionally managed.

Notwithstanding that compositional data is intended to assess substantial equivalence in the presence of the genetic modification, in the case of herbicide tolerant plants, trials would be designed to obtain samples from herbicide treated plants for analysis, either by inclusion of the following, additional treatment, or substitution of treatment A (above) by the following treatment:

C. GM maize, treated with the herbicide to which tolerance has been introduced.*

** only proximates should be analysed, except where additional analysis can be justified, e.g. where treatment A is omitted.*

In all cases, the comparison of data should be made between the GM and non-GM maize and compared with the range of values given in published literature (e.g. Haytowitz, 1995; Souci *et al*., 1994; USDA, 1993; Notisplus, www; USDA, www). If a range is not available for any particular constituent, an explanation should be given if there is variation of more than 20% from the mean of the non-GM maize (TemaNord, 1998). For those GM crops where the genetic modification results in the substantial change of one of the plant's constituents (e.g. increase or decrease of a specific fatty acid, increase or decrease of a specific carbohydrate) via modification of a branch of a metabolic pathway, substantial equivalence can still be assessed as outlined in this document for all constituents except that of the modified trait. Detailed analysis might be required relating to the specific trait and the metabolic pathway.

1.3 Materials

Composition analysis should be undertaken on maize grain and/or forage, as applicable, and presented on a dry matter basis.

1.4 Data

The specific data recommended for compositional analysis to assess substantial equivalence are given in Annex 1. The selection of data is based on the following considerations and has been developed after detailed consideration of the scientific literature concerning nutrients and toxicants/anti-nutritional factors present in maize.

If the modification of the GM plant under evaluation is designed to change a specific biochemical pathway, additional compositional analysis variables may be included to characterise the effect of the modification.

Proximates

Traditionally, the analysis of the major constituents of maize, or proximates, has been an effective method to determine the nutritional properties of maize grain from different hybrids. Maize is mainly used to produce animal feeds that are characterised by their digestibility, palatability and energy content. The protein content and quality of the feed prepared from maize is usually not sufficient and often needs to be supplemented with protein-rich fractions derived from additional processing of maize or other crops, such as soy bean. Feed formulation takes into account the different nutritional characteristics of the maize grain obtained from different maize hybrids in order to prepare nutritionally balanced rations.

The other major constituents of maize grain are carbohydrates, fibre, fat and ash. Moisture and dry matter are usually measured in order to standardise the values obtained with reference to a known grain moisture content (e.g., at 15 %). These constituents are also applicable to determine the substantial equivalence for forage if the notification includes its use in animal feed.

Carbohydrates

The greatest proportion of carbohydrate in maize grain consists of starch, comprising most of the soluble carbohydrate present, the remainder being fibre and free sugars. The whole fibre content is measured by the neutral detergent fibre (NDF) method, which gives the approximate sum of cellulose and pentosans (hemicellulose). The amount of cellulose and lignin can be estimated by the acid detergent fibre (ADF) method.

Fatty acids

Five fatty acids, which account for 90 % of total lipid in maize (Watson, 1982; 1987), are considered to be important for the compositional analysis of maize grain (Annex 1). They comprise the two most common fatty acids, linoleic and oleic acids, and three other fatty acids which are also found at measurable levels: palmitic, stearic and linolenic acids.

There are other fatty acids detected at very low levels (arachidic, behenic, eicosenoic and palmitoleic) and they cumulatively comprise less than 1 % of total lipids. The fatty acids that are not reliably detectable in maize are arachidonic, capric, caprylic, eicosadienoic, eicosatrienoic, heptadecanoic, lauric, myristic, myristoleic and pentadecanoic acids.

Amino acids

The quality of protein produced by different maize hybrids can be determined by measuring the content of different amino acids. Eighteen amino acids commonly found in maize (Watson, 1982) are considered to be important for the compositional analysis (Annex 1).

With regard to formulation of animal feeds, the most important amino acids are the nutritionally essential methionine and cysteine. Lysine and tryptophan are also important in feed formulation but are present at low concentrations in maize. These two amino acids cannot be produced by non-ruminant animals (such as swine and poultry) or man. Ruminants, however, have microorganisms in the rumen that can synthesise both lysine and tryptophan.

Minerals

A number of mineral ions are recognised as essential plant nutrients and are directly incorporated into organic compounds synthesised by the plant. Of these, calcium, magnesium, phosphorus, potassium and sodium are required by the plant in significant quantities and, as such, these macro-nutrients are recommended for compositional analysis (Annex 1). Other mineral ions, such as iron, copper, zinc and chlorine, are micro-nutrients which are required by plants only in small quantities, and are incorporated in plant tissues only at trace levels.

Vitamins

Maize is not considered an important dietary source of vitamins for either humans or animals. The contribution of maize-based food ingredients to the Recommended Daily Intake (RDI) for humans is calculated to be in the range of only 0.2-1.7 % for a typical daily intake of 14g of maize flour and/or meal. For this and other compounding factors, maize meal and flour are usually enriched with wheat flour and other nutrients to provide a more balanced food for human consumption. Similarly, in modern feed formulation, nutritional balance is achieved by admixture of vitamin supplements (Watson, 1987).

In considering the major carotenoids and tocopherols, only β-carotene (provitamin A) and α-tocopherol (vitamin E) are identified as of potential nutritional importance (Watson, 1987). However, the inherent instability of carotenoids necessitates the admixture of vitamin A to feedstuffs. Moreover, levels of carotenoids and tocopherols in maize can vary substantially according to the maize hybrid.

Nonetheless, four vitamins (B1, B2, E and folic acid) have been identified for which maize makes a minor contribution to the diet and which are considered appropriate for compositional analysis.

Antinutrients

Unlike other crops such as potatoes, oilseed rape, soya bean or cotton, there are no generally recognised anti-nutrients in maize at levels that are considered harmful (toxic or allergenic) and worthy of quantification or risk management (Watson, 1982, 1987; White and Pollak, 1995). However, for the purposes of assessment of substantial equivalence, certain Competent Authorities have asked for the analysis of two anti-nutritional compounds present at higher levels in other plants, trypsin inhibitor and phytic acid (Annex 1). Both compounds are present in extremely low concentrations in maize grain (trypsin inhibitor: 1.9 units/mg dry weight (Del Valle *et al*. 1983); phytate: 0.89 % by dry weight (Cheryan, 1980)).

1.5 Secondary metabolites

Secondary plant metabolites are defined in the literature as those natural products which do not function directly in the primary biochemical activities which support the growth, development, and reproduction of the organism in which they occur (Conn, 1981). Only where they are nutritionally significant toxicants (e.g., solanine in potatoes, glucosinolates in canola, gossypol in cotton) have components from many of these classes of secondary plant metabolites been previously examined, on a routine basis, as part of the compositional analysis.

However, for the purposes of assessment of substantial equivalence under Regulation (EC) No 258/97, the Competent Authority of The Netherlands has asked for analysis of certain secondary metabolites in maize; those specified were coumaric acid, ferulic acid, inositol and raffinose, for which the range of concentrations in maize is known, and furfural. It is recognised that, as *rapporteur* for submissions made under Regulation (EC) No 258/97, the Competent Authority of the Netherlands requires analytical data on these secondary metabolites

2. Agronomic variables

Subject to the specific purpose of the genetic modification, certain agronomic variables based on the plant phenotype are recognised as primary indicators of orderly crop growth and development and have, therefore, been selected for the overall assessment of substantial equivalence of GM maize (Annex 2). The experimental control would normally be a non-GM maize of comparable genetic background.

3. Specific requirements for GM traits combined by traditional breeding

Member States have interpreted the scope of Directive 2001/18/EC and Regulation (EC) No 258/97 to require additional notifications for plants in which two or more genes, originally introduced by separate transformation events, have been combined (stacked) in a single plant by traditional plant breeding methods.

Compositional and phenotypic analysis for these stacked plants would be undertaken over a single growing season (4 sites - see 1.1), and comparison made either with the single-event GM plants or with the non-GM control of comparable genetic background.

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Annex 1 The compositional data recommended for analysis to assess substantial equivalence of GM maize plants

Component	Maize grain	Maize forage
Proximate analysis:		
Protein	yes	yes
Fat	yes	yes
Ash	yes	yes
Moisture	optional	optional
Dry matter	optional	optional
Carbohydrates:		
ADF	optional	yes
NDF	optional	yes
Soluble carbohydrate (by difference)	yes	optional
Fatty acids:		
Linoleic	yes	n/a
Oleic	yes	n/a
Palmitic	yes	n/a
Stearic	yes	n/a
Linolenic	yes	n/a
Amino acids:		
Alanine	yes	n/a
Arginine	yes	n/a
Aspartic acid	yes	n/a
Cystine	yes	n/a
Glutamic acid	yes	n/a
Glycine	yes	n/a
Histidine	yes	n/a
Isoleucine	yes	n/a
Leucine	yes	n/a
Lysine	yes	n/a
Methionine	yes	n/a
Phenylalanine	yes	n/a
Proline	yes	n/a
Serine	yes	n/a
Threonine	yes	n/a
Tryptophan	yes	n/a
Tyrosine	yes	n/a
Valine	yes	n/a

Annex 1 The compositional data recommended for analysis to assess substantial equivalence of GM maize plants

Component	Maize grain	Maize forage
Minerals:		
Calcium	yes	n/a
Magnesium	yes	n/a
Phosphorus	yes	n/a
Potassium	yes	n/a
Sodium	yes	n/a
Vitamins:		
B_1	yes	n/a
B_2	yes	n/a
E	yes	n/a
Folic Acid	yes	n/a
Anti-nutrients/Toxicants*:		
Phytic acid	yes	n/a
Trypsin inhibitor	yes	n/a
*based on requests from certain Competent Authorities		

n/a = not applicable

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Annex 2 The agronomic variables* recommended for measurement to assess substantial equivalence of GM maize plants

1. Plant count at full emergence (e.g. growth stage V3) and/or at harvest

2. Time to flowering (silk emergence and/or pollen shed)

3. Appearance (e.g. vigour/colour/leaf rolling)

4. Susceptibility to pests and diseases

5. Yield at known moisture content

*Variables which have changed as a consequence of a particular genetic modification (e.g. susceptibility to specific insects; yield) would be excluded from this assessment.

Note: that all of the above agronomic variables are highly influenced by the environment.