FOOD AND DRUGS AUTHORITY

GUIDELINES FOR RISK ASSESSMENT OF GENETICALLY MODIFIED FOOD AND FEED

Document No.:	FDA/APBD/GL-RAGM/2013/01
Date of First Adoption:	1st February 2013
Date of Issue:	1st March 2013
Version No.:	01

TABLE OF CONTENT

Contents

INTRODUCTION

In pursuance of sections 13 and 14 of the Biosafety Bill, 2005, this guidance document shall be used for the safety and nutritional assessment of foods and feeds derived from or consisting of Genetically Modified Organisms.

It covers foods and feeds consisting of, or derived from GM plants which have conventional counterparts with a history of safe use.

It outlines information required in the dossier and the sequential steps involved in the risk assessment and characterizations of foods and feeds derived from genetically modified plants.

This document does not address:

- The ethical, environmental or socio-economic aspects of marketing of Food/Feeds derived from GM plants;
- Issues relating to the safety of food production workers handling recombinant-DNA plants;
- Contained use of GM plants;
- Field trials of GM plants.

This document is not conclusive; it will be reviewed as often as new scientific knowledge concerning genetic modification of plants and their derived foods and feeds becomes available.

1. GLOSSARY

For the purpose of this guideline, the following definitions shall apply.

"Biotechnology" means the technological applications that use biological systems, living organisms, or their derivatives to make or modify products or process.

"Modern biotechnology" means the application of:

i) *In vitro* nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles, or,

ii) Fusion of cells beyond the taxonomic family, that overcome natural physiological reproductive or recombinant barriers and that are not techniques used in traditional breeding and selection.

"***Recombinant-DNA Plant***" - means a plant in which the genetic material has been changed through *in vitro* nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles.

"***Conventional Counterpart***" - means a related plant variety, its components and/or products for which there is experience of establishing safety based on common use as food/feed.

"Biosafety" means efforts used to minimize or avoid the potential risks resulting from modern biotechnology and its products.

"GMOs" means an organism whose genetic material has been altered using genetic engineering technique generally known as recombinant (DNA) technology.

"LMOs" means living modified organisms and they are organisms that have been genetically modified through the application of biotechnology including organisms that have been modified by novel recombinant DNA techniques.

"Concept of familiarity"

The concept of familiarity is based on the fact that most genetically engineered organisms are developed from organisms such as crop plants the biology of which is well understood.

"Concept of substantial equivalence"

The concept of substantial equivalence is based on the idea that an existing organism used as food/feed with a history of safe use, can serve as a comparator when assessing the safety of the genetically modified food/feed.

3. REQUIREMENTS

3.1. DOSSIER EVALUATION

The dossier accompanying the GM food or feed shall be a complete document with all information required for the risk assessment of the GM food or feed as outlined in this guidance document.

Data shall be generated in a scientifically sound manner that can withstand any scientific peer review. The pages and appendixes shall be well numbered and a detailed index prepared and attached. Information in dossier shall be clearly legible and all tables, pictures, figures, etc. be clearly labelled.

3.2. RISK ASSESSMENT

Risk assessment can be defined as "a process of evaluation including the identification of the attendant uncertainties, of the likelihood and severity of an adverse effect(s) /event(s) occurring to man or the environment following exposure under defined conditions to a risk source(s)" (European Food Safety Authority (EFSA), 2006).

The sequential steps in risk assessment of GMOs identify characteristics which may cause adverse effects, evaluate their potential consequence, assess the likelihood of occurrence and estimate the risk posed by each identified characteristic of the GMOs

The safety assessment of Foods and feeds derived from GM plants and their derived foods and feeds shall be based on the concept of *substantial equivalence* where the foods and feeds would be compared with their conventional counterpart having a history of safe use *(concept of familiarity)* for any intended or unintended differences by way of new or altered hazards. These are then subject to further safety assessment.

3.2.1. UN-INTENDED EFFECTS

In achieving the objective of conferring a specific target trait (intended effect) to a GM plants by the addition, substitution, removal, or rearrangement of defined

DNA sequences, including those used for the purpose of DNA transfer or maintenance in the recipient organism, additional traits could, in some cases, be acquired or existing traits could be lost or modified.

Unintended effects may be deleterious, beneficial, or neutral with respect to the GM plant's effects on humans after ingestion, or the safety of foods produced using the plant.

A starting point in the identification of potential unintended effects is the sequence analysis of regions flanking the insertion site to establish whether the insertion has occurred within, or in the proximity of, an endogenous gene. Sequence analysis shall extend to identifying whether the introduced DNA interrupts a transcriptional unit as well as whether it causes the synthesis of a fusion protein.

3.2.2. ISSUES TO BE CONSIDERED

The risk assessment of foods and feeds derived from GM plants shall take account of the following:

- the characteristics of the donor and recipient organisms;
- the genetic modification and its functional consequences;
- the potential toxicity and allergenicity of gene products, plant metabolites and the whole GM plant;
- the compositional, nutritional characteristics;
- the influence of processing on the properties of the food or feed;
- the potential for changes in dietary intake;
- the potential for long-term nutritional impact;
- the intended and unintended effects due to the genetic transformation event;
    - potential for horizontal DNA transfer in the appropriate environment.

3.3. INFORMATION REQUIRED FOR RISK ASSESSMENT

3.3.1. DESCRIPTION OF THE RECOMBINANT-DNA PLANT

A description of the recombinant-DNA plant from which the food/feed or ingredient was derived shall be provided. This description shall identify the crop, the transformation event(s) to be reviewed and the type and purpose of the modification. This description shall be sufficient to aid in understanding the nature of the food being submitted for safety assessment.

3.3.2. DESCRIPTION OF THE HOST AND DONOR PLANT AND THEIR USE AS FOOD

Information on both the organisms used as the DNA donor(s) for genetic modification and the recipient organism is required. This information shall include:

i. The most recent taxonomic classification including the family, genus, species, subspecies, cultivar/breeding line or strain. Taxonomic information is important to determine if the donor organism(s) or other closely related members of the family naturally exhibit characteristics of pathogenicity or toxin production, or have other traits that affect human health.

ii. Information on all issues of potential concern, such as the presence of natural toxins, allergens or virulence factors.

iii. Data on the previous use of the donor and the recipient organism including information on

   a. how it is transported and stored,
   b. whether special processing is required to make the plant safe to eat, and
   c. the plant's normal role in the diet (e.g. which part of the plant is used as a food source, whether its consumption is important in particular subgroups of the population, what important macro- or micro-nutrients it contributes to the diet).

3.3.3. DESCRIPTION OF THE GENETIC MODIFICATION(S)

3.3.3.1 Transformation Method

The genetic modification protocol shall be described in detail and relevant references for the genetic modification method shall be provided.

- For *Agrobacterium*-mediated transformation, the ***strain* of *Agrobacterium*** used during the genetic modification process must be indicated, including information or references on how the *Ti/Ri* plasmid based vector was disarmed.
- For genetic modification methods that involve the use of ***helper plasmids,*** a detailed description of these plasmids shall be given.
- In the case of direct transformation, ***pure DNA*** has to be used, implying the absence of carrier DNA. If carrier DNA is used in a transformation event, its source must be stated and a risk assessment provided.

3.3.3.2 Description Of Genetic Material Used For Modification

A physical and genetic map shall detail the position of all functional elements (including coding and non-coding sequences) and other vector components together with the applicant's selected restriction sites for the generation of *probes*, and the position and nucleotide sequence of *primers* used in PCR analysis.

A table identifying each component, its size, its origin and its role shall accompany the map. The region intended for insertion shall be clearly indicated. For each element, the following information is required:

- A description of the genetic element or a citation where the genetic element was isolated and characterized (completed with an accession number in a publicly available database);
- The portion and size of the genetic element that was inserted in the *vector* and its location in the vector;
- Information on whether the genetic element itself codes for proteins responsible for diseases (e.g. Proin, toxicants, allergens, pathogenicity factor or irritant).

- Information about the molecular, biochemical and physiological properties of its products, as known in the donor organism and targeted in the transgenic specie.
- (For direct transformation methods) data on how the part(s) of the vector(s) used was purified and how purity was determined.

3.3.4. CHARACTERIZATION OF THE GENETIC MODIFICATION(S)

In order to provide clear understanding of the impact on the composition and safety of foods derived from recombinant-DNA plants, a comprehensive molecular and biochemical characterization of the genetic modification shall be carried out

3.3.3.3 Information On The Sequences Actually Inserted or Deleted

Applicant shall provide information on;

- the size and copy number of all detectable inserts, both complete and partial. This is typically determined by Southern analysis. Probes used for this purpose shall provide complete coverage of sequences that could be inserted into the host plant, such as any parts of the vector or any carrier or foreign DNA remaining in the GM plant;
- the organization of the inserted genetic material at the insertion site and methods used for the characterization;
- in the case of deletion(s), size and function of the deleted region(s);
- sub-cellular location(s) of insert(s) (nucleus, chloroplasts, mitochondria or maintained in a non-integrated form) and methods for its determination;
- all sequence information including the location of premier used for detection and the nature of the flanking sequence;
- evaluation of the presence and functionality of novel chimeric *ORFs* (Open Reading Frames) on the complete sequence. If a chimeric ORF is detected that extends beyond its region, more of the flanking region shall be sequenced until the supposed end of the ORF is reached.

3.3.3.4 Information On The Expression Of The Insert

The expression of all the ORFs shall be analyzed. The methods used for the expression analysis shall be described and their sensitivity assessed. For ORFs intended to be expressed in the transgenic plant, chimaeric ORFs and all other genes on the insert, data shall be provided on the level, spatial and temporal specificity of the expression at the protein and/or transcript level.

Where tissue-specific promoters have been used, information may be requested on expression of target genes in other plant parts relevant for risk assessment. Evidence shall be provided to indicate that expression of the inserted gene(s) is as expected and stable in the tissues targeted.

The potential creation of newly expressed fusion proteins shall be investigated by bioinformatic analysis and the absence of any harmful fusion proteins demonstrated.

The properties of the protein that are expressed in the plant or the target proteins of which expression level has been altered shall be described. If there has been a DNA modification that affects the amino acid sequence of the expressed protein, the modified amino acid sequence shall be provided. Data on protein stability in the cell and the environment may be required.

3.3.3.5 Any Change To The Ability Of The GM Plant To Transfer Genetic Material To Other Organisms

The horizontal gene transfer is particularly important in foods derived from GM plants that retain the recombinant DNA in their final product. Due to homologous recombination, the risk of gene transfer and subsequent integration and expression may be enhanced by the presence of bacterial sequences within the GM plant insert DNA and thus the presence of such sequences shall be minimized. The inserted DNA shall be evaluated for possible enhancement of gene transfer potential (*e.g.*

presence of replication origins or genes/sequences that might enhance recombination).

3.3.5. TOXICOLOGY

The requirements of toxicological testing in the safety assessment of food/feed derived from GM plants must be considered on a case-by-case basis and will be determined by the outcome of the assessment of the differences identified between the GM product and its conventional counterpart, including available information on intended changes. Thus, the toxicological testing would not only include studies on newly expressed proteins but also the consequences of any genetic modification (*e.g.* gene silencing or over-expression of an endogenous gene).

In principle, the safety assessment must consider the presence of new proteins expressed as result of the genetic modification, the potential presence of other new constituents and/or possible changes in the level of natural constituents beyond normal variation. These potential deviations from the conventional counterparts may require different toxicological approaches and varying degrees of testing.

There may be circumstances, when the applicant considers that a decision on safety can be taken without conducting some of the tests recommended in this chapter and/or that other tests are more appropriate. In such cases the applicant must state the reasons for not submitting the required studies or for carrying out studies other than those mentioned below.

Those toxicological studies which are carried out shall be conducted using internationally agreed protocols. Studies shall be carried out according to the principles of Good Laboratory Practice (GLP).

3.3.5.1 Literature Review

The notifier shall perform a comprehensive literature review, discussing the absence of toxicity to humans and animals of the new substance

(protein or non-protein). This search shall be clearly referenced. A comprehensive search shall also be performed concerning the safety of the donor organism used.

3.3.5.2 Structural Homology Screening

The homology of the new substance and the known toxic components has to be screened e.g. by comparing the sequence of a protein with known protein toxins, using databases, predicted 3-D-Structure, an amino acid sequence in the region of the protein that are critical to toxicological properties. If the newly expressed substance is an enzyme, the characteristics and biological effects of that enzyme shall be described and considered. Database consultation and the use of computer-based amino acid search programs shall be clearly documented and verifiable.

3.3.5.3 Exposure Assessment

Estimation of intake of new substance shall be carried out per unit plant and food compound in order to derive the daily intake. This predictive exposure to the substance of interest shall be compared to the potential exposure to the real substance, produced by the donor organism.

3.3.5.4 Safety Assessment of Newly Expressed Protein and Non-Protein

Toxicological studies shall be conducted using internationally agreed state of the art protocol, and be carried out according to the principles of Good Laboratory Practice (GLP). If the substance of interest to be used in the toxicological test is produced through molecular biology technique in another organism (e.g. bacteria) than the plant, it has to be proven that the test substance is structurally, biochemically and functionally equivalent to the substance in the plant. Factors that shall be examined equivalence are: post – translational modification, full length of amino acid sequence, amino acid sequence/composition, molecular weight (using the most appropriate method), functional characteristics (immunorecognition in a Western blot assay and similar bioactivity).

3.3.5.5 Metabolic/Toxicokinetic Studies

A) Protein New substances

An *in vitro* digestibility assay simulated gastric and/or intestinal fluid is required. On a case-by-case basis, also an *ex vivo* gastric fluid test (e.g. cattle, pig and dog) or *in vivo* models may be required.

B) Non-protein New substance

For new non-protein substances (e.g. those exerting biocidal and pharmacological effects) toxicity shall be assessed on case-by-case basis depending on the identity and biological function of the substance in the plant and dietary exposure and according to the appropriate guideline and conventional toxicological approach (including metabolism studies, studies on toxicokinetics).

3.3.5.6 Acute toxicity

Acute oral toxicity of new substance (protein and non-protein)

Acute oral toxicity testing in laboratory rodent is required to confirm the lack of toxicity suggested by the literature reviews performed.

3.3.5.7 Genotoxicity

A) Protein new substances

In vitro mutagenicity test (bacteria mutagenicity test, chromosome aberration tests including cytogenicity tests in cultured mammalian cells) shall be performed on a case-by-case basis depending on the identity and biological function of the substance in the plant and dietary exposure.

B) Non - protein new substances

In vitro mutagenicity test (bacteria mutagenicity test, chromosome aberration tests including cytogenicity tests in cultured mammalian cells) is obligatory unless convincing evidence can be provided to deviate from standard procedures.

3.3.5.8 Repeated Dose Toxicity

Repeated dose toxicity studies shall be performed, unless reliable information can be provided which demonstrates the safety of the newly

expressed protein (including its mode of action) and that the protein is not structurally and functionally related to proteins which have the potential to adversely affect human or animal health.

Normally a 28-day oral toxicity study with the newly expressed protein in rodents shall be performed. Depending on the outcome of the 28-days toxicity study, additional targeted investigations may be required, including an analysis of immunotoxicity. If the applicant considers that a decision on safety can be taken without conducting a repeated dosing study or that other tests are more appropriate, the applicant must state the reasons for this.

3.3.5.9 Testing of the Whole GM Food/Feed

If the composition of the GM plant is modified substantially, or if there are any indications for the potential occurrence of unintended effects, based on the preceding molecular, compositional or phenotypic analysis, not only new constituents, but also the whole GM food/feed shall be tested. In such a case, the testing programme shall include at least a 90-day toxicity study in rodents. Special attention must be paid to the selection of doses and the avoidance of problems of nutritional imbalance.

At least two dose levels of the GM and parental test food shall be included in the diet. The highest dose level shall be the maximum achievable without causing nutritional imbalance, whilst the lowest level shall approximate the anticipated human intake. Stability of test diets and nutritional equivalence between control and test diets are other important aspects to consider.

In the case of complex genetic modifications involving the transfer of multiple genes, the potential risk(s) of possible interactions between the expressed proteins, new metabolites and original plant constituents shall be assessed.

3.3.6 ASSESSMENT OF POSSIBLE ALLERGINICITY

3.3.6.1 Introduction

All newly expressed proteins in recombinant-DNA plants that could be present in the final food shall be assessed for their potential to cause allergic reactions. This shall include consideration of whether a newly expressed protein is one to which certain individuals may already be sensitive as well as whether a protein new to the food supply is likely to induce allergic reactions in some individuals. The endpoint of the assessment is a conclusion as to the likelihood of the protein being a food allergen.

3.3.6.2 Initial Assessment

3.3.6.2.1 Source of the protein

As part of the data supporting the safety of foods derived from recombinant-DNA plants, information shall describe any reports of allergenicity associated with the donor organism. Allergenic sources of genes would be defined as those organisms for which reasonable evidence of IgE mediated oral, respiratory or contact allergy is available. Knowledge of the source of the introduced protein allows the identification of tools and relevant data to be considered in the allergenicity assessment. These include: the availability of sera for screening purposes; documented type, severity and frequency of allergic reactions; structural characteristics and amino acid sequence; physicochemical and immunological properties (when available) of known allergenic proteins from that source.

3.3.6.2.2 Amino Acid Sequence Homology

The purpose of a sequence homology comparison is to assess the extent to which a newly expressed protein is similar in structure to a known allergen. This information may suggest whether that protein has an allergenic potential. Sequence homology searches comparing the structure of all newly expressed proteins with all known allergens shall be done. Searches shall be conducted using various algorithms such as FASTA or BLASTP to predict overall structural similarities. Strategies such as stepwise contiguous identical amino acid segment searches may also be performed for identifying sequences that may

represent linear epitopes. The size of the contiguous amino acid search shall be based on a scientifically justified rationale in order to minimize the potential for false negative or false positive results. Validated search and evaluation procedures shall be used in order to produce biologically meaningful results.

IgE cross-reactivity between the newly expressed protein and a known allergen shall be considered a possibility when there is more than 35% identity in a segment of 80 or more amino acids (FAO/WHO 2001). All the information resulting from the sequence homology comparison between the newly expressed protein and known allergens shall be reported to allow a case-by-case scientifically based evaluation.

Sequence homology searches have certain limitations. In particular, comparisons are limited to the sequences of known allergens in publicly available databases and the scientific literature. There are also limitations in the ability of such comparisons to detect non-contiguous epitopes capable of binding themselves specifically with IgE antibodies.

3.3.6.2.3 Pepsin Resistance

Resistance to pepsin digestion has been observed in several food allergens; thus a correlation exists between resistance to digestion by pepsin and allergenic potential. Therefore, the resistance of a protein to degradation in the presence of pepsin under appropriate conditions indicates that further analysis shall be conducted to determine the likelihood of the newly expressed protein being allergenic. The establishment of a consistent and well-validated pepsin degradation protocol may enhance the utility of this method. However, it shall be taken into account that a lack of resistance to pepsin does not exclude that the newly expressed protein can be a relevant allergen.

Although the pepsin resistance protocol is strongly recommended, it is recognized that other enzyme susceptibility protocols exist. Alternative protocols may be used where adequate justification is provided.

3.3.6.3 Specific serum screening

For those proteins that originate from a source known to be allergenic, or have sequence homology with a known allergen, testing in immunological assays shall be performed where sera are available. Sera from individuals with a clinically validated allergy to the source of the protein can be used to test the specific binding to IgE class antibodies of the protein in *in vitro* assays.

For proteins from sources not known to be allergenic, and which do not exhibit sequence homology to a known allergen, targeted serum screening may be considered where such tests are available. These methods shall be scientifically sound and may include targeted serum screening (i.e. the assessment of binding to IgE in sera of individuals with clinically validated allergic responses to broadly-related categories of foods); the development of international serum banks; use of animal models; and examination of newly expressed proteins for T-cell epitopes and structural motifs associated with allergens.

In the case of a newly expressed protein derived from a known allergenic source, a negative result in *in vitro* immunoassays may not be considered sufficient, but shall prompt additional testing, such as the possible use of skin test and *ex vivo* protocols. A positive result in such tests would indicate a potential allergen.

3.4 NUTRITIONAL ASSESSMENT OF GM FOOD/FEED

The development of GM foods may have the potential to improve the nutritional status of individuals and populations and provide products with enhanced functionality. GM foods also have the potential to introduce nutritional imbalances as

a result of both expected and unexpected alterations in nutrients and other food components. The introduction of these novel or non-traditional plants into the Ghanaian food supply requires an assessment of the nutritional consequences and implications for the entire population and/or specific subgroups (e.g. children) who may consume extreme amounts. The evaluation is needed in order to ensure that the nutritional status of consumers is not unduly jeopardized by:

- substitution of dietary components of known nutritive value with less nutritious varieties;
- distortion of nutrient intakes as a result of unusual levels of particular nutrients or the presence of anti-nutrients that could affect the nutritional value of the remainder of the diet.

The safety of genetically modified feeds shall also be assessed for both livestock feeding and human nutrition. The insert and its products have to be assessed for their ability to cause detrimental effects if transferred into animals or if their proteins accumulate in the end product (milk, eggs, meat, etc.) of animals that are fed on these novel feeds.

The nutritional assessment of GM foods/feeds shall consider:

(a) Nutrient composition;

(b) Biological efficacy of nutrient components in the foods;

(c) Assessment of dietary intake and nutritional impact.

3.4.1 Compositional Data and Methods

A proximate analysis of the matter as well as the sampling procedures, analysis methods and the precise statistical distribution of the results shall be clearly outlined.

3.4.1.1 Proximate analysis

The major and minor constituents of the food/feed shall be determined. Depending on the crop and/or derived feed/food product to be considered, several components may not be relevant. The checklist below presents non-exhaustive information on the parameters of food/feed nutrition and safety.

- Moisture
- Total fat
- Crude Fibre

- Total ash (soluble and insoluble)
- Other carbohydrates (Nitrogen-free extractives)
- Fibre(Neutral Detergent Fibre, Hemicellulose, Acid Detergent Fibre, Lignin, cellulose)
- Carbohydrates (Feeds)
    - Non-structural carbohydrates (NSC): sugars, starches, fructans, galactans, pectins etc.
    - Non-starch polysaccharides: NSC minus sugars and starches
- Carbohydrate (Foods)
    - Reducing sugars, Mono and disaccharides, starch and othe polysaccharides.
- Proteins
    - Non-protein nitrogen (NPN)
    - Amino acids: essential amino acids, Non-essential amino acids
- Mineral composition and trace elements
- Vitamins: Fat soluble and water soluble

3.4.1.2 Analytical Methods

Reference methods must be used and mentioned. Depending on the feed/food involved, appropriate and currently available methods shall be used.

3.4.2 Statistical and Sampling Aspects

The sampling methods must be explained and must take into account the requirements linked to the statistical analysis as well as the distribution of the components in the raw material. It is important to consider the variability of the raw material e.g. by taking into consideration the impact of the geographical origin, the climate, the agronomic practice, etc. enough samples are to be analyzed with the help of a sampling plan and the results are to be evaluated on a statistical basis. Plants used to obtain samples for compositional analysis shall be grown under conditions that represent normal practice for the crop plant.

3.4.3 Nutritional Aspects

If the genetically modified crop is expected to have an important role in the diet, then appropriate information on nutritional composition is needed. Key nutrients shall be determined and their place (value) within the human or animal diet be identified. Depending on the composition and the estimated consumption of the GM crop, it appears justified to limit the testing to the most relevant nutrients; examples of which are outlined below:

3.4.3.1 Animal Feeds

- **Grass and forage crops**: Energy, Protein, Fibre, Vitamins, Minerals, and trace elements.
- **Dried forage and straw:** Fibre, Minerals and trace.
- **Silage:** Energy, Protein, Fibre, Minerals and trace elements.
- **Roots and tubers and related by-products:** Energy, Minerals and trace elements.
- **Cereals and related by-products:** Energy, Minerals and trace elements.
- **Protein concentrate:** Proteins, Energy, Minerals and trace elements.
- **Vitamins and trace mineral premixes: V**itamins, Minerals and trace elements.

3.4.3.2 Foods

- **Cereals:** carbohydrates (simple and complex), dietary fibre, B-vitamins, minerals and trace elements, proteins and amino acids (if present).
- **Fruits and vegetables:** water soluble vitamins, dietary fibre, carbohydrates, minerals and trace elements.
- **Milk and milk-products:** Total protein content and specific amino acid composition, fatty acid composition, fat soluble vitamins, calcium and other relevant minerals and trace elements.
- **Meat, Poultry, Fish and Meat replacers:** Total fat and fatty acid composition, fat soluble vitamins, total proteins (for meat replacers also specific amino acids), fat soluble vitamins, vitamin B12, trans fatty acids.

- **Fats and oils:** Fatty acid composition, fat soluble vitamins, total fat, trans fatty acids
- **Extras:** Macro-nutrient composition

This list shall be considered as an example and not as an exhaustive list.

3.4.4 Nutritive Value

The nutritive value is obtained as a result of chemical composition and digestibility. It is determined mainly from energy and protein value.

3.4.5 Anti-nutrients and toxicants

Information is requested as to the presence of anti-nutrients. This applies particularly to the key anti-nutrients for the product. Naturally occurring toxins that are inherent in the plant shall be determined and data on the sensitivity of the crop towards the formation of mycotoxins, pathogenic microorganisms, biogenic amines and other toxic substances or organisms formed in the product have to be given, if relevant.

Examples include protease inhibitors, amylase inhibitors, lectins or hemagglutinins, cyanogens, glucosinolates, alkaloids, phytic acids, mycotoxins, phytotoxins, gossypols, saponins etc.

3.4.6 Nutrient Bioavailability

Fingerprinting of the product by such techniques as HPLC, GC-MS, and conventional analytical methods is recommended. Where the food from a genetically modified source is a source of important dietary nutrients, animal studies may be needed as evidence of nutritional adequacy.