

Intended and unintended effects of GMOs and the concept of substantial equivalence.

**Starts with review of the concepts,
Interplay between assessments ,
Example
SE**

Scientific basis

Guidance for risk assessment of food and feed from genetically modified plants

Scientific opinion of the EFSA Panel on Genetically Modified Organisms (GMO)

adopted on 14 April 2011

Efsa.europa.eu



Why we are interested in unintended effects? – *because they are unpredictable*

The unintended effects are evaluated by :
Molecular, Compositional og Environmental assessments in combination.

Scientific basis

Guidance on the agronomic and phenotypic characterisation of genetically modified plants

Scientific Opinion of the EFSA Panel on Genetically Modified Organisms (GMO)

Published 24 June 2015

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Regulatory basis

Commission Implementing Regulation (EU) No 503/2013 of 3 April 2013

on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006

***Regulatory binding document
Vs. Guidance document***

Principles

- *The risk assessment strategy for GM plants and derived food and feed seeks to deploy appropriate approaches to compare GM plants and derived food and feed with their respective **comparators**.*
- *The underlying assumption of this comparative approach is that traditionally cultivated crops have **gained a history of safe** use for consumers and/or domesticated animals.*

Principles for the Risk assessment

- *Molecular characterization*
 - **Structure and expression of the insert(s)**
 - **Stability of the intended traits**
 - **Characterization of newly expressed proteins**
- *Comparative analysis*
 - **Similarities and differences between the GMO and comparators**
- *Outcomes further structure the risk assessment*

Principles (Stacks)

- *GM plants containing a combination of transformation events obtained (in most cases) by conventional breeding*
- *Risk assessment aims at:*
 - **Establishing that the combination is stable**
 - **Identifying interactions between the events that may raise safety concerns.**

Intended and unintended effects

- *Intended effects are those that fulfil the original objectives of the genetic modification.*
- *Unintended effects are **consistent** differences between the GM plant and its comparator, which go beyond the intended effect(s) of the genetic modification.*

***Term consistent differences can be replaced by :
significant, reproducible, relevant....***

Literature :

Codex Alimentarius WHO 2009 Rome

Unintended effects

Can result from the random insertion of DNA sequences into the plant genome, which may:

Cause disruption or silencing of existing genes, activation of silenced genes, or modifications in the expression of existing genes.

UE may also result in the formation of new changed patterns of metabolites.

F.ex. , the expression of enzymes at high levels may give rise to secondary biochemical effects, or changes in the regulation of metabolic pathways and/or altered levels of metabolites.

Principles

- *Unintended effects are not per sé a safety concern. It is general phenomenon that **can also occur in conventional breeding**.*
- *The toxicological / nutritional impact of any relevant change in the GM plant and/or derived food and feed resulting from the genetic modification should be assessed.*
- *The intended as well as the unintended effect(s) of the genetic modification should not have adverse effects on human and animal health*

Unintended effects

- *Predictable unintended effects*
 - **based on current knowledge of plant biology and metabolic networks**
- *Unpredictable unintended effects*
- *Identification*
 - **Molecular characterization**
 - **Comparative analysis**

The methods employed to demonstrate the intended effect of the genetic modification are also useful for identifying unintended effects

Tool 1: Molecular characterisation

- *The molecular characterisation should provide data on the structure and expression of the insert(s), and on the stability of the intended trait(s).*
- *It should be specifically indicated whether the molecular characterisation of the genetic modification(s) raises safety concerns with regard to the interruption of endogenous genes.*
- *The molecular characterisation should specifically aim to identify whether the genetic modification(s) raise(s) any issues regarding the potential for producing new toxins or allergens.*

Molecular characterisation

- *The results of the molecular characterisation further drive the approaches in the comparative analysis. Example below:*
- *Stability of intended traits*
→ *Analysis of trait related products*
- *Interruption of endogenous genes*
→ *Analysis of related gene products*
- *Potential for producing new toxins or allergens*
→ *Analysis for toxins or allergens.*

F.ex. Altered substrate specificity triggers analysis of potential enzyme product

Tool 2: Comparative analysis

- *The comparative analysis of **compositional, agronomic as well as phenotypic characteristics** constitutes, together with the molecular characterisation, the starting point for the risk assessment of GM plants and derived food and feed.*
- *It aims to identify differences between the GM plants and derived food and feed and its comparator which should be further assessed with respect to potential impact on human and animal health.*

Comparative analysis



GM-plant

?

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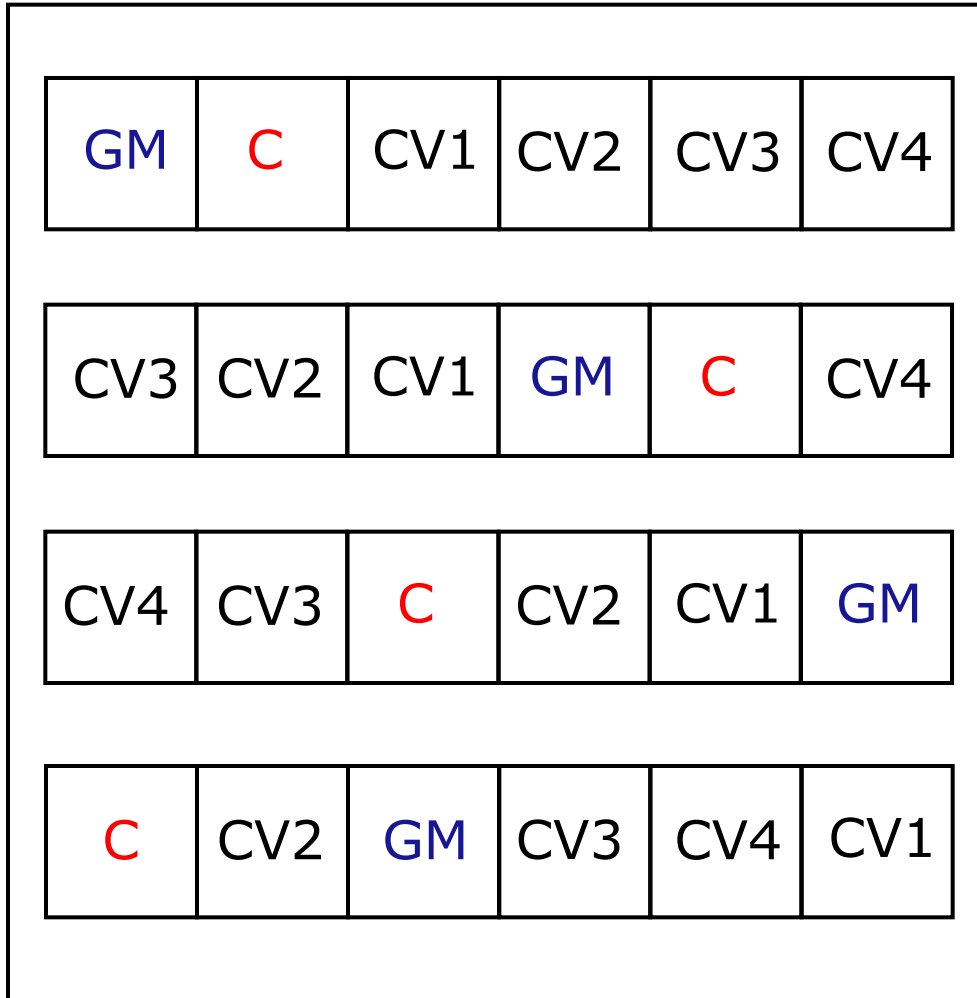


Non GM-plant

Comparative analysis

- **Field trials** used for production of material for the comparative analysis should be performed in order to determine differences and equivalences between the GM plant, its comparator and non-GM reference varieties.
- The objective is to determine whether the GM plant and/or derived food and feed is different from its comparator and/or equivalent to non-GM reference varieties with a history of safe use.

Typical applicants' experimental design for a compositional field trial



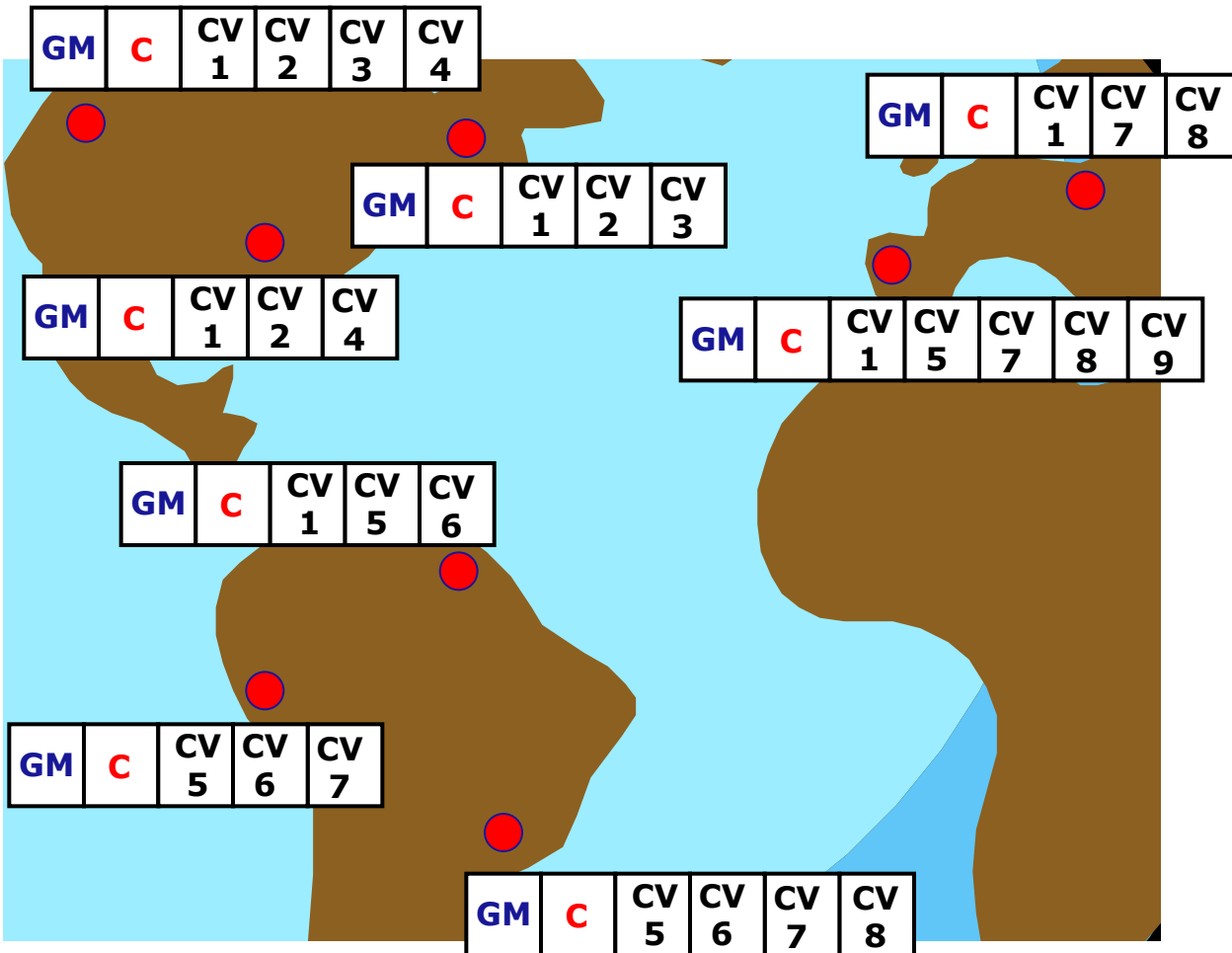
GM = GM plant

to be tested

C = Non-GM
comparator

CVs are different
commercial varieties

Experimental design for field trials – (ii) between sites



must be at least 8 sites, over one or more years

must be the same GM, non-GM comparator at each site

may be different commercial varieties at each site

must be at least 6 commercial varieties over all the sites

Comparative analysis

- *The statistical methods for the comparative analysis involves two approaches:*
 - **A test of difference**, to verify whether the GM plant is different from its comparator and might therefore be considered a hazard (potential risk)
Unintended effects are identified by the means of statistical difference tests .
 - **A test of equivalence** to verify whether the GM plant is equivalent or not to non-GM reference varieties with a history of safe use, apart from the introduced trait(s).
 - **Unintended effects** are stat. relevant *differences* between the GM plant and its comparator(apart from the introduced trait(s).
(biological vs statistical difference !)

Compositional analysis

- ***Analysis should be carried out on the raw agricultural commodity.***
- The compounds to be analysed should be selected in accordance with the OECD consensus documents on compositional considerations for new plant varieties (OECD- defines the spectrum of parameters to be measured always, important for the specific type of plant)
 - Proximates, key macro- and micro-nutrients, anti-nutritional compounds, natural toxins, and allergens.
 - other plant metabolites characteristic for the plant species.
 - **From an industry standard proximates include five constituents**

Ash

Moisture

Proteins

Fat

Carbohydrates (Calculation)

Compositional analysis

- *Depending on the intended effect of the genetic modification and the nutritional value and use of the plant, specific analyses may be required for an appropriate assessment*
 - **Fatty acid profile for oil-rich plants**
 - **Amino acid profile (individual protein amino acids and main non-protein amino acids) for plants used as an important protein source.**

Compositional analysis

- *The compounds selected for comparative compositional analysis are indicators of change for the total set of compositional characteristics*
- ***Differences** identified for these indicators may **trigger additional** comparative analysis of (metabolically) related compounds.*

This is case by case assessment !

Compositional analysis

- *The characteristics of the introduced trait may trigger **further analysis** of specific compounds including metabolites of potentially modified metabolic pathways.*
- *The same conditions apply for GM plants containing stacked events. Additional compounds may be selected for analysis depending upon the introduced traits, as appropriate.*

as described i OECD doc. The observed changes are only indicators of change

Compositional analysis/allergenicity

- *When the recipient of the introduced gene is known to be allergenic, any potential change in the allergenicity of the whole food derived from a GM plant should be tested by comparison of the allergen repertoire with that of its appropriate comparator(s).*

We have now tools in place to measure allergens concentration in plants

- *Preliminary important information on the **likelihood of an unintended alteration** of the overall allergenicity can be obtained by including relevant identified endogenous allergens in the comparative compositional analysis.*

Tool 3 : Agronomic and phenotypic characteristics

- *The comparative analysis of the GM plant should address also aspects of the biology of the plant, in the form of agronomic and phenotypic traits.*
 - **e.g. yield, plant morphology, growth performance, flowering time, response to plant pathogens and insect pests, sensitivity to abiotic stress.**
- *The protocols for field trials to study these characteristics should follow the specifications described for the compositional analysis.*

Agronomic and phenotypic characteristics

- *Differences in agronomic traits between the GM plant and the comparator apart from the **intended effect** of the genetic modification may provide indications for **unintended effects** which should be further elucidated by means of molecular characterisation and compositional analysis*
- ***N.B.: Phenotypic analysis is also used in the early stages of the breeding process for selecting successful transformants (event sorting)***

Tool 4. Effects of processing

- *It should be assessed whether or not the processing technologies applied **are likely to modify** the characteristics of the GM end-products compared with their comparators*
- *A genetic modification targeting metabolic pathways may result in changes in the concentration of plant constituents and lead to the production of new metabolites. Processed products derived from such GM plants may require specific approaches for their risk assessment.*
- *F.ex. If you find out that oil content is higher due to gen modification you also further assess it in processing*

Comparative analysis - Conclusions

- *The conclusion of the comparative analysis should clearly state whether:*
 - **compositional characteristics of the GM plant and derived food and feed are different to those of its comparator**
 - **agronomic and phenotypic characteristics of the GM plant are different to those of its comparator**
 - **further assessment is needed for those characteristics showing differences**
 - **there are indications of interactions between the combined events in the case of GM plants containing stacked events.**

Practical examples – Case studies

- *Identification of potential unintended effects*
- *Example taken from recently adopted EFSA opinions or publicly available summaries of applications under Regulation 1829/2003*
 - **Herbicide tolerant GM soybean 356043**
Efsa.europa.eu - opinion...

Herbicide tolerant soybean 356043

- *The 356043 soybean has been genetically modified for herbicide tolerance.*
- *This was achieved by the introduction of the *gat4601* and the *Gycine max-hra* (*gm-hra*) coding sequences surrounded by their necessary regulatory components.*

Herbicide tolerant soybean 356043

- *gat4601* is an optimized form of the glyphosate acetyltransferase (*gat*) coding sequence from *Bacillus licheniformis* that confers tolerance to glyphosate- and glyphosate-ammonium based herbicides.
- *gm-hra* is an optimized form of the endogenous acetolactate synthase (*als*) coding sequence from soybean (*Glycine max*), that confers tolerance to ALS-inhibiting herbicides, such as chlorimuron, thifensulfuron or sulfonylureas.

Herbicide tolerant soybean 356043

- *Molecular characterization*
 - **In tests for substrate specificity, the newly expressed GAT4601 protein was shown to acetylate aspartic acid and glutamic acid. The protein was found to have a very low affinity for serine, threonine and glycine.**
 - **One of the specific amino acid changes introduced into the Glycine max-ALS enzyme to form the Glycine max-HRA enzyme (i.e. replacement of tryptophan 560 by leucine), is expected to increase the 2-ketobutyrate pool available for odd chain fatty acid biosynthesis due to decreased affinity to that intermediate.**

Herbicide tolerant soybean 356043

- *Agronomic characterization*
 - **The 356043 soybean and its conventional counterpart were grown under the same agronomic conditions. In addition plots were included where 356043 soybean was treated with glyphosate herbicides and/or ALS inhibiting herbicides**
 - **356043 soybean was shown to be **agronomically** not different from its conventional counterpart with the exception of the newly introduced traits.**

Herbicide tolerant soybean 356043

- *Compositional analysis*
 - **The levels of the acetylated amino acids N-acetylaspartate (NAA) and N-acetylglutamate (NAG) were measured in seeds of 356043 soybean, its conventional counterpart and commercial soybean varieties. The mean values for NAA and NAG in 356043 soybean were different from those of its conventional counterpart and markedly outside natural ranges determined for commercial soybean varieties. This effect was observed independently of the herbicide treatment regime.**
 - **Conclusion: pool of acetylated amino acids was bigger..... ?**

Herbicide tolerant soybean 356043

Table 3: Levels [mg/kg dry weight] of N-acetylaspartate (NAA) and N-acetylglutamate (NAG) in seeds from 356043 soybean untreated or treated with glyphosate and ALS-inhibiting herbicides (i.e. target herbicides) compared to seeds from control soybean (South American locations, 2005-2006)

Analyte		Control soybean Jack untreated with target herbicides	356043 soybean untreated with target herbicides	356043 soybean treated with target herbicides	Reference varieties
NAA	Mean	1.92	653	681	0 - 2.27
	Range	1.10 - 3.67	490 - 870	502 - 994	
NAG	Mean	2.34	18.3	18.1	0 - 3.17
	Range	1.42 - 3.35	9.86 - 43.2	8.27 - 31.8	

unintended effect at all locations.

Differences seen in acetylated amino acids which were not at OECD list, therefore EFSA

looked at odd chain fatty acids . Question: Why we do include target herbicide ?

Herbicide tolerant soybean 356043

- *Compositional analysis*
 - **Consistent statistically significant compositional differences between 356043 soybean and its conventional counterpart were found for the odd chain fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acid, independently of the herbicide treatment regime. Levels determined for 356043 soybean were around two to three times higher than those observed for the conventional counterpart and outside the ranges observed for other commercial soybean varieties**

Herbicide tolerant soybean 356043

unintended effect at all locations

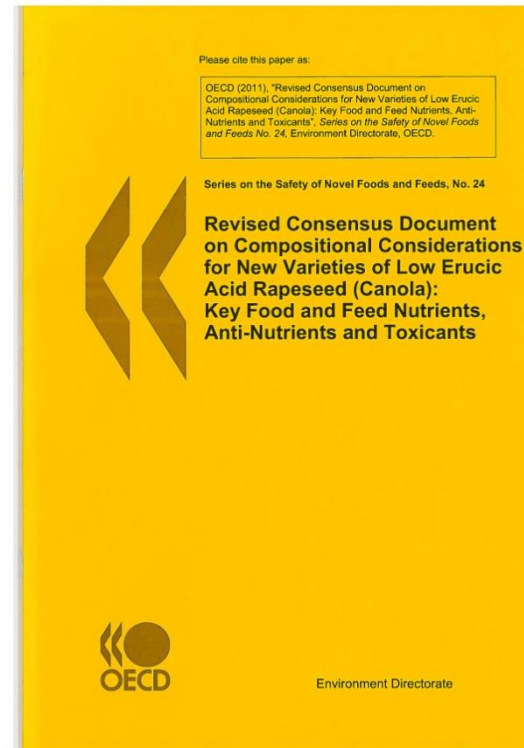
Table 2: Levels [% of total fatty acids] of heptadecanoic acid (C17:0), heptadecenoic acid (C17:1) and heptadecadienoic acid (C17:2) in seeds from 356043 soybean untreated or treated with glyphosate and ALS-inhibiting herbicides (i.e. target herbicides) compared to those in seeds from the conventional counterpart Jack (North American locations, 2006)

Analyte		Control soybean Jack untreated with target herbicides	356043 soybean untreated with target herbicides	356043 soybean treated with target herbicides	Reference varieties
C17:0	Mean	0.129	0.326	0.330	0.085 -
	Range	0.105 - 0.304	0.207 - 0.408	0.152 - 0.423	0.146
C17:1	Mean	0.063	0.179	0.183	0.073 -
	Range	0.049 - 0.136	0.117 - 0.240	0.067 - 0.248	0.087
C17:2	Mean	0.056	0.150	0.153	0 - 0.068
	Range	0.045 - 0.121	0.099 - 0.203	0.061 - 0.211	

Herbicide tolerant soybean 356043

- Assessment of unintended effects
 - **Odd chain fatty acids are present in the diet and the intake of small amounts of these fatty acids via food or feed is not expected to produce adverse effects.**
 - **NAA and NAG are normal constituents in the mammalian metabolism and the estimated increases in their intake are considered low when related to the normal intake of L-aspartic acid and L-glutamic acid.**
 - **Further toxicological, allergenicity and nutritional analysis provided no indications of adverse effects.**
 - **No safety issue per se – based on **exposure assessment which was done !** (if there is a concern the managers shall label it)**

OECD Consensus Document Food/Feed 1993: on substantial equivalence/SE/



Substantial Equivalence OECD 1993

The difficulties of applying of risk assessment to the whole foods meant that the alternative approach was required for the safety assessment of GM foods.

**This led to the concept of
SUBSTANTIAL EQUIVALENCE**

"the concept of substantial equivalence (SE) embodies the idea that existing organisms used as food or as a source of food can be used as the basis for comparison when assessing the safety of the human consumption of a food or food component that has been modified or is new".

Comparisons are made on case by case basis

Substantial Equivalence OECD 1993

“If a new food or food component is found to be substantially equivalent to an existing food or food component, it can be treated in the same manner with respect to safety.

No additional safety concerns would be expected.”

SE at different levels:

- **Chemical/compositional**
- **Agronomical**
- **Phenotypical**
- **Genotypical (transcriptomics)**
- **Toxicological**

Terms for comparison

- **GMO vs conventional counterpart (e.g. isogene line)**
- **Grown under identical conditions**
- **Product from a GMO to similar product**
- **Natural variations should be taken into account**

Results (FAO/WHO 1996)

3 outcomes of substantial equivalence:

- Substantially equivalent
- Substantially equivalence apart from certain defined differences
- Not Substantially equivalent.
- **“Not substantially equivalent ..”does not necessarily mean it is unsafe and not all such products will necessarily require extensive testing”.**

Next step:

- If only defined differences: Focus only on those
- Otherwise: should be evaluated on the basis of its composition.

Critics of Substantial Equivalence Concept WHO 2000 Consultation:

- Not properly defined- based on mistaken perception that the determination of SE was the end point of safety assessment, rather than the starting point. SE does not characterize hazard.
- Not a scientific concept
- Focussing only on chemical targeted analysis.
- Animal test is needed.
- Substantial equivalence is not accurate term . It is substituted now by:

“COMPARATIVE APPROACH” because SE for some people implies **safety**

Safety can only be determined when the results of all aspects under comparison are integrated .

Codex Alimentarius 2009 Rome

The concept of SE is a key step in the safety assessment process .

It is starting point that is used to structure the safety assessment

It is not a safety assessment in itself

The safety assessment carried out in this way does not imply absolute safety of the new product; rather , it focuses on assessing the safety of any identified differences so that the safety assessment of the new product can be considered
relative to its conventional counterpart

Thank you

Safety aspects of genetically modified foods of plant origin WHO/FAO 2000

- Comparative approach: This concept embodies a science-based approach in which a genetically modified food is compared to its existing, appropriate counterpart.
- The approach **is not intended to establish absolute safety**, which is an unattainable goal for any food. Rather, the goal of this approach is to ensure that the food, and any substances that have been introduced into the food as a result of genetic modification, is as safe as its traditional counterpart.
- Substantial equivalence is not an accurate term - now it is rather used term : **Comparative approach.**

OECD Consensus Document Food/Feed

Sugar Beet	Barley	Sweet Potato
Potato	Alfalfa	Papaya
Maize	Sunflower	Sugarcane
Wheat	Tomato	Low Erucic Acid Rapeseed
Rice	Cassava	Soybean
Cotton	Grain Sorghum	

Hazard identification

First step in the risk assessment of GMOs

- *Identification of biological, chemical, and physical agents capable of causing adverse health effects*
- *Identification of differences and/or lack of equivalences between the GM plant and its comparator*
- *Determines the additional studies required to assess the possible impact on human and animal health.*

