The Safety of Food and Feed Derived from GE Crops
In the United States, the regulation of food and feed derived from GE crops is based on product characteristics as opposed to process-based regulations used in the European Union.

Rather than the method of production; genetic engineering in this case, comparison of the features of the new GE crop and its traditional counterpart is the core of the product safety evaluation.

This approach has been determined by the WHO, the OECD, and the FAO and is termed ‘substantial equivalence’. It is based on the safe history of the use of the parent crop used to generate the GE crop under question.
Safety assessment of foods derived from GE crops in the future

Progress in this field is likely to occur as a result of characteristics of new GE crops currently being produced and as novel test methods become available as a result of scientific advancements.
Food safety—What needs to be regulated?

- Food additives
- Food labeling
- Dietary supplements
- Novel and GE foods
- Food security and protection of food supplies
Food Safety Systems—Institutions

• **OECD: Organization for Economic Cooperation and Development**
  – Promotes policies for highest sustainable economic development in member states
  – Establishes guidelines for chemical testing, toxic chemicals, pesticides, and biotechnology

• **Food and Agriculture Organization (FAO) of the United Nations**
  – Leads international efforts to ensure sufficient nutrition for all

• **World Health Organization (WHO) of the United Nations**
  – Provides scientific advice on matters related to food safety through its Food Safety Department
FAO/WHO Codex Alimentarius Commission

Founded in 1963 by a joint initiative of the FAO and the WHO, the Codex Alimentarius Commission

- Formulates and harmonizes food standards and ensures global implementation
- Develops food standards, guidelines, and related texts such as codes of practice under the Joint FAO/WHO Food Standards Programme
- Generates guidelines to protect the health of consumers and ensures fair trade practices in food trade, and
- Promotes coordination of all food standards work undertaken by international governmental and non-governmental organizations

The Codex Alimentarius Commission established an Intergovernmental Task Force on Foods Derived from Biotechnology in 1999 to evaluate the health and nutritional implications of such foods. The task force performs all of the functions listed above in relation to safety assessment of foods derived from genetically engineered organism based on the input of independent scientific expert consultations.
The Evolution of Food Safety Systems

The Codex Alimentarius Commission has issued (since 1963)

- 237 Food standards for commodities
- 41 Codes/Hygiene or technological practice
- 25 Guidelines for contaminants
- 185 Evaluations on pesticides
- 1,005 Evaluations on food additives
- 54 Evaluations on veterinary drugs
- 3,504 Documents/Limits pesticide residues

So far 5 expert consultation reports regarding safety of foods derived from genetically engineered organisms (including microorganisms, plants and animals) have also been issued.
Food Safety in the U.S.

The Food and Drug Administration (FDA) is responsible for the regulation of meat and food products and takes its authority under the following acts:

- Food, Drug, and Cosmetic Act (FDCA)
- Food Additives Amendment
- Dietary Supplement Health and Education Act (DSHEA)

The FDCA is directly relevant to the safe administration of foods derived from biotechnology. The last two acts listed above provide insight for the evaluation of biotechnology foods.
What Exactly We Ingest When We Eat Food: An example: Common Food X

The Codex Committee had 19 sessions to determine the standards regarding the matter

- 1981 – The standards were adopted
- 2001 – Draft revision
- 2003 – Final revised standards
  - Recommended methods of analysis and sampling
  - % of total weight of the basic ingredients in the finished product
  - Definitions
  - Labeling
  - Amounts of food additives
Final Standards for Food X

Acidity regulators – 17
Glazing agents – 5
Flavoring agents – 3
Emulsifiers – 8
Antioxidants – 6
Colors – 2
Sweeteners – 11
Bulking agent – 1
Processing aid – 1

Food X: Chocolate

~100 kg/day has to be consumed for 2 years to reproduce these effects in humans

Butylate Hydroxyanisole
- Chronic exposure – gall bladder, endocrine, lungs, thorax, respiration, tumors
- Mutagen – DNA inhibition, unscheduled DNA synthesis, DNA damage
- Chronic exposure – reproductive damage
- Prolonged repeated exposure can cause allergies in sensitized individuals
  - 200 mg/kg

Hexane
- Flammable
- Delayed target organ effect
- Peripheral nervous system
- Kidney
- Testes-tumors
- Reproductive effects
- Potentially carcinogenic
  - 1 mg/kg

10X more of Acceptable Daily Intake of carrots (~ 1 lb) is more achievable to consume in a day
What is there that is not poison?

All things are poison and nothing is without poison.

Solely the dose determines that a thing is not a poison.

Paracelcius (1493-1541)
General Principles of Risk Analysis

Risk is associated with hazard & exposure

First Step: Hazard Identification
- Formaldehyde causes cancer
- Cholera toxin causes severe diarrhea

Second Step: Hazard Characterization
- Quantitative and qualitative assessment of the nature of the hazard
- Dose-response relationship
- Usually animals are administered 3 doses: very small to doses that exceed multiple orders of what would be expected to determine NOAEL=(No Observed Adverse Effect Level)
- Margin of safety determination:
- To account for interspecies and intra-species variation, NOAEL is divided by 100 (uncertainty factor)
Exposure Assessment

- Determine the amount and distribution of the hazardous substance and routes and locations that the population can come into contact.
- In the case of food safety studies, food dietary intake information is needed.
- Acceptable daily intake (ADI) is determined – usually with lifetime studies with rodents.
Safety Assessments of Foods

- Food toxicology is unique
- Complex—1000s of macromolecules, micronutrients, anti-nutrients
- Ever-changing properties – Environment – Genetic rearrangement occurring in the plant
- For processed foods – Additives and chemicals migrating from the package
- Common food items – Presume their safety based on familiarity and history of use
  – Neurotoxic glycoalkaloids present in potatoes

Therefore FDCA states that – Safety cannot be proved absolutely

Safety assessment seeks a level of reasonable certainty that harm will not occur (as long as they are free of contaminants)
Concern Level, Tolerance Levels

Are required for the following

✓ Pesticide residues
✓ Drugs used in food producing animals
✓ Heavy metals
✓ Food-borne molds and mycotoxins
✓ Bacterial toxins
✓ Substances produced by cooking
Safety Assessment of Foods Derived from GE Crops

- Presumption of safety = Comparators
  Usually the traditionally bred parent crop
- Comparative assessment = Substantial Equivalence (FAO/WHO, 1991)
  - Agronomical and morphological characteristics
  - Chemical composition
    - Macro and micronutrients
    - Key toxins and anti-nutrients

Are there any significant changes?
Do they pose a hazard to human health?
Hazard Identification & Characterization of GE Crops

1. The parent crop (the comparator) – hazards?

2. The transformation and inserted DNA

3. Gene product – toxic/allergenic?

4. Unintended changes
   – Compositional changes
   – Assess any adverse impact
     ▪ Allergy/toxicity/nutritional alterations
Toxicity Testing Methods

Many of the regulatory requirements for chemicals such as food additives and pesticides were first established during the 70s. These led to the development of a battery of tests to assess the safety of chemicals in foods.

Most often, the results from three approaches are combined:

1. Structure/function relationship – toxicity/allergenicity
2. In vitro assays – enzymes, receptors, cell lines
3. In vivo animal studies

In order to monitor the performance of the product and the side effects, post-market surveillance can also be incorporated for certain products.

4. Post-market monitoring
   - Early warning
   - Facilitates product recall
   - Absence of adverse health effects
   - Determining consumption patterns – implications and applications relevant to food toxicology to help determine estimated daily intake (EDI)
Up to this point we have briefly examined food safety systems and food safety assessment and have introduced the general principles of risk assessment. We have also looked at basic toxicology testing methods that have applications in the food safety assessment of foods derived from genetically engineered crops.

In the next section of this module, we will introduce the safety assessment of foods derived from GE crops in detail by using a similar format to that presented by König et al, in 2004, in the *Food and Chemical Toxicology Journal*. 
Test Methods to Assess the Safety of Foods Derived from GE Crops

Hazard Identification/Characterization

Parent Crop
- Phenotype
- Chemical
- Composition

Transformation
- Donor organism
- DNA construct
- Consequences of DNA insertion

Gene product(s)
- Proteins and metabolites
- Toxic potential
- Allergenic potential

GE crop
- Equivalence to parent crop

+ Exposure Assessment ➔ Safety Assessment

Figure modified from König et al, 2004
Step 1 — Parent Crop

Parent crop
- Origin, genotype, morphological and agronomic features
- Other related traditional and wild varieties and species
- Geographical distribution
- History of safe use
- Compositional analysis

No new toxins
Anti-nutrients
Allergenic compounds
Bioactive compounds

OECD Consensus Documents

Figure modified from König et al, 2004
Step 2 — Donor Organism and Transformation

Parent Crop

Donor organism
- Taxonomy
- Allergen/toxic/pathogenic
- Compositional information
- History of safe use/exposure
- Function of rDNAs used in the transformation process—used DNA should not be related to any adverse properties of the donor

Transformation

DNA construct, transformation & insertion
- Vector DNA, components, source of the components, function in the source organism, organisms used to amplify
- A vector map with restriction sites
- Nucleotide sequence of the vector
- The method of gene delivery
  - Agrobacterium
  - Gun delivery
- Characterize introduced DNA sequences
  - PCR
  - Southern blot—copy # - Xs - instability
  - Ends of the inserted sequence—possibility of fusion proteins
- Characterize insertion site
  - Insertion junction
  - Disruption of major endogenous genes
  - Fusion proteins

Figure modified from König et al, 2004
Step 3 — Gene Products

Recombinant proteins/metabolites
- Protein-safety concern?
- Previous exposure/novel protein
- Structure, sequence, biochemical properties
  - Equivalent to the version produced in the source
    - MW
    - Aa sequence
    - Post-translational modification
    - Immuno-equivalance

- Mode of action
- Toxicity
- Allergenicity
  - Is the source an allergen/is the protein allergen?
  - Does the recombinant protein induce de novo sensitization?
  - Cross-reactivity with IgE induced by known allergens

Gene product(s)
- Proteins and metabolites
- Toxic potential
- Allergenic potential

GE crop
- Equivalence to parent crop

Figure modified from König et al, 2004
Step 4 — GM Crop

Finally the GE crop itself is subjected to tests to ensure that it is as safe and as nutritious as its traditional counterpart.

GE crop

- Phenotypic and agronomic features
  - Alterations: metabolic perturbations/pleitropic effects due to the modification
- Compositional analysis
  - Macro- and micro-nutrients, endogenous toxins and anti-nutrients
  - From different geographies
  - Helps design the animal diet

Figure modified from König et al, 2004
Step 4 — GE Crop

An example:
- **Roundup Ready soybeans**
  - Soybeans naturally contain certain levels of anti-nutrients; trypsin inhibitor, lectins and isoflavones
  - Protein, oil, fiber, carbohydrates, moisture content, amino acid and fat composition in seeds and toasted soybean meal compared with conventional counterparts
  - Trypsin inhibitor levels were 11-26% higher in GE soybeans in defatted non-toasted soybean meal (not consumed-starting material)
  - In defatted, toasted soy meal trypsin inhibitor values were not different than the comparator
  - Feeding studies in rats, chickens, catfish, dairy cattle confirmed no nutritional value differences

Figure modified from König et al, 2004
Step 4 — GE Crop

GE crop

- Animal studies (FAO/WHO, 2000)
  - Recommends dietary sub-chronic rat study
  - Broiler, dairy cattle, beef cattle, sheep, and swine
  - Uncertainties regarding equivalence
  - Foods are very complex
  - Can be administered at low multiples of the average human intake
  - Dietary imbalance – false positive in terms of adverse effect
  - The use of biomarkers suggested (adaptive versus toxic)

Figure modified from König et al, 2004
Test Methods to Assess the Safety of Foods Derived from GE Crops

As risk is correlated with levels and frequency of exposure to a certain hazard, safety assessment of food derived from GE crops can be completed with exposure assessment.

- **Hazard Identification/Characterization**
  - **Parent Crop**
    - Phenotype
    - Chemical
    - Composition
  - **Transformation**
    - Donor organism
    - DNA construct
    - Consequences of DNA insertion
  - **Gene product(s)**
    - Proteins and metabolites
    - Toxic potential
    - Allergenic potential
  - **GE crop**
    - Equivalence to parent crop

+ Exposure Assessment → Safety Assessment

Figure modified from König et al, 2004
Exposure Assessment

- Food supply information
- Household expenditure
- Food consumption surveys
- Import statistics

- Recombinant proteins in transgenic plants: 0.01-0.1% of total protein content (Betz et al, 2000)
- Estimated daily intake (EDI) for humans: 0.017-0.07 mg/kg/day (König et al, 2004)
- NOAEL with acute toxicity tests >100 mg/kg/day (Chassy et al, 2002)

Even if people consumed ~1,400X that of the EDI, there would not be a safety concern.
Exposure Assessment

- GE seeds may be commingled with conventional ones
- Food ingredients derived from commodity crops are in many different products
- Food processing might alter ratios, may cause degradation

Therefore, current exposure assessment approach does not take these degradation and overestimation into account to achieve the highest level of safety.
Toxicity Testing Methods

As described so far toxicity testing methods are used with slight modifications to assess safety of food derived from GE crops

1. Structure/function relationship – toxicity/allergenicity
   - Common structural features, databases

2. In vitro assays – enzymes, receptors, cell lines
   - Simulated gastric digestion

3. In vivo animal studies

4. Post-market monitoring
   - Several companies for certain products
     - Early warning
     - Facilitates product recall
     - Absence of adverse health effects
     - Determining consumption patterns – implications and applications relevant to food toxicology as it might help to determine estimated daily intake (EDI) of a given
In the future?

• Existing methodologies are considered sufficient for safety assessment of GE crops
• First generation of GE crops; herbicide tolerant or insect resistant
• Next generation of GE crops; more complex – nutritionally enhanced or resistant to abiotic stress
• New methodologies for safety assessment?
• Most likely
In the Future?

Advances in Molecular Biology

Genomics

Characterization of the Parent Crop
Whole genome projects produced vast amounts of information. With more advanced bioinformatics tools, functions of individual genes will be more predictable. In addition, advancements in omics technologies will improve compositional analysis.

Characterization of the Transformation Event
Quicker sequencing and better characterization of the insertion site will enable potential changes to important endogenous genes and also formation of fusion proteins.

More Effective Transformation Methods and Site Directed Mutagenesis
Use less amount of DNA
Increase efficiency

Eliminating Selectable Markers
1. DNA microinjection
2. Homologous recombination
3. Co-transformation followed by segregation
4. Recombinase-mediated Excision
   No selectable marker = simplified safety assessment
In the Future?

**Protein Allergenicity**

- Improve understanding of allergy at molecular and cell level

**Protein Structure and Function**
- Design better proteins with no allergenic proteins

**Protein Stability – Simulated Gastric Fluid Test**
- A correlation between stability of a protein to digestion and its ability to cause allergies. However, this correlation is not absolute as partial digestion might make hidden epitopes available. Therefore the process by which digestion affects allergenic potential is being investigated. The results are likely to increase understanding of allergy

**Cell-based Models**
- Models developed based on mast cells could allow addressing cross-reactivity. New models are required to study sensitizing potential of proteins in vitro

**Animal Models**
- Some animal models are promising however none of them have been validated yet. Studies are ongoing to identify alternatives to evaluation of allergies by antibody induction. Effect of food matrix on sensitizing potential of the allergens also need to be investigated.
Identification and Assessment of Unintended Effects

Profiling methods such as omics, magnetic resonance and liquid chromatography) will enable more detailed compositional analysis which will in turn enable identification of both intended and unintended changes.

Models to Test Safety and Nutritional Properties

Animal studies have been particularly challenging due to difficulties involved in designing a nutritionally balanced diet for the subjects, proving the source of negative effects if they occur, not being able to obtain large doses of pure protein for acute studies from the plants and absence of validated animal models for allergy. Profiling methods mentioned earlier have been suggested to have potential to enable identification of markers for more sensitive endpoints to gain more information from animal studies.

Core: Established Methods of GE Crop Safety

Available toxicological test methods adopted for safety assessment of food derived from GE crops (developed by expert consultation) have been sufficient for evaluation of first generation transgenic crops. For safety assessment of GE crops with more complex traits (nutritionally enhanced, abiotic resistance) advances in science is likely to affect two key areas in the future.

Safety Testing

In the Future?
Now and In The Future

- Codex Alimentarius Commission, 2003
- NAS, 1987

Conclusion: Potential risks that foods derived from GE crops are not different than those of new varieties produced with conventional breeding

- Substantial equivalence
- Case-by-case analysis tailored for the GE crop under question
- No adverse effects so far
- Future? – Advances in molecular biology, biochemistry, allergy science, nutrition, and toxicology
Resources

http://www.who.int/foodsafety/biotech/en/

http://www.fao.org/UNFAO/about/index_en.html

http://www.cfsan.fda.gov/list.html

http://www.foodsafety.gov/~fsg/biotech.html
**Food & Fuels Glossary**

**Novel foods:** ‘Foods resulting from a process not previously used for food. Products that are new to the diet in a given population. Foods that have been modified by genetic manipulation, also known as genetically modified foods, GM foods, genetically engineered foods or biotechnology-derived foods’. (modified from Health Canada; [http://www.hc-sc.gc.ca/fn-an/gmf-agm/index_e.html](http://www.hc-sc.gc.ca/fn-an/gmf-agm/index_e.html))

**GE foods:** Genetically-engineered foods. Foods derived from genetically modified crops. Also called GM foods.

**Anti-nutrients:** ‘Substances that act in direct competition with or otherwise inhibit or interfere with the use or absorption of a nutrient’ (Nutritional and food safety assessment of foods and feeds nutritionally improved through biotechnology. Comprehensive Reviews in Food Science and Food Safety, 2004:3:38-104). For example, trypsin inhibitors in soybean interfere with digestion of proteins. Trypsin inhibitors are inactivated upon heating.

**Pleiotropic effect:** A single gene influences more than one characteristic of the phenotype.
Food additive: ‘Any substance not normally consumed as a food by itself and not normally as a typical ingredient of food, whether or not it has nutritive value. The intentional addition of which to a food is for a technological (including organoleptic) purpose in the manufacture, processing, preparation, treatment, packing, transport or holding of such food results, or may be expected to result, (directly or indirectly), in it or its byproducts becoming a component of or otherwise affecting the characteristics of such foods’ (Nutritional and food safety assessment of foods and feeds nutritionally improved through biotechnology. Comprehensive Reviews in Food Science and Food Safety, 2004:3:38-104).

Organoleptic: ‘Able to perceive a sensory stimulus such as taste’ (Nutritional and food safety assessment of foods and feeds nutritionally improved through biotechnology. Comprehensive Reviews in Food Science and Food Safety, 2004:3:38-104).

Dietary supplement: Congress defined the term "dietary supplement" in the Dietary Supplement Health and Education Act (DSHEA) of 1994. A dietary supplement is a product taken by mouth that contains a "dietary ingredient" intended to supplement the diet. The "dietary ingredients" in these products may include: vitamins, minerals, herbs or other botanicals, amino acids, and substances such as enzymes, organ tissues, glandulars, and metabolites. Dietary supplements can also be extracts or concentrates, and may be found in many forms such as tablets, capsules, softgels, gelcaps, liquids, or powders. They can also be in other forms, such as a bar, but if they are, information on their label must not represent the product as a conventional food or a sole item of a meal or diet. Whatever their form may be, DSHEA places dietary supplements in a special category under the general umbrella of "foods," not drugs, and requires that every supplement be labeled a dietary supplement (FDA-CFSAN – Food and Drug Administration, Center for Food Safety and Applied Nutrition - http://www.cfsan.fda.gov/~dms/ga-sup5.html).
Food & Fuels Glossary (cont’d)

**Allergenic compound:** A substance that causes allergy.

**Toxic compound:** A substance that cause damage to living organisms by interfering with their metabolism or accumulating in their tissues or sub-cellular compartments.

**Bioactive compound:** In the context of plants and food, a bioactive compound refers to a substance with extranutritional functions when produced in plants in small quantities. Examples of bioactive compounds are antioxidants, flavones and phenolic compounds.

**Taxonomy:** Science of categorization of living organisms.

**rDNA (Recombinant DNA):** ‘A DNA molecule formed by joining DNA segments from different sources (not necessarily different organisms). This may also include DNA synthesized in the laboratory’ (Nutritional and food safety assessment of foods and feeds nutritionally improved through biotechnology. Comprehensive Reviews in Food Science and Food Safety, 2004:3:38-104).

**Endogenous gene:** A gene which is original, unmodified component of the genome of a given organism.

**Fusion protein:** A protein created by joining two genes together. Fusion proteins may occur naturally or can be created in the laboratory for research (National Cancer Institute - http://www.cancer.gov/Templates/db_alpha.aspx?CdrID=44591).
**PCR (Polymerase Chain Reaction):** ‘A molecular biology technique through which specific DNA segments are amplified selectively. The process mimics *in vitro* the natural process of DNA replication occurring in all cellular organisms, where the DNA molecules of a cell are duplicated prior to cell division. The original DNA molecules serve as templates to build daughter molecules of identical sequence’ (Nutritional and food safety assessment of foods and feeds nutritionally improved through biotechnology. Comprehensive Reviews in Food Science and Food Safety, 2004:3:38-104).

**Agrobacterium:** A bacterium that causes crown-gall disease in plants. This bacterium has the ability to transfer segments of its own genes to plant’s genome and use plant's metabolic machinery for its own metabolic needs. This property of *Agrobacterium* is used in plant biotechnology to transfer desired genes into genomes of target crops (Agrobacterium mediated transformation). *Agrobacterium* strains used in plant transformation are ‘disarmed’, meaning that the genes responsible for their disease causing ability have been removed.

**Gun delivery:** A method of gene delivery used in genetic engineering where the genes to be delivered are coated on gold particles which act as bullets and send onto the cells or tissue to be transformed with at a very high speed enabling the entry of the genes to the cells and become incorporated in their genomes. May also be referred to as ballistic.

**Vector DNA:** A piece of DNA used as carrier to deliver genes to desired organisms. It contains both the gene of interest and other pieces of DNA that act as regulators. Different components of the vector may come from different organisms, the same organism or they can be synthetically created by scientists *in vitro.*
Restriction site: Specific sequence of DNA which are recognized by proteins that introduces breakage at these regions of DNA. The knowledge about these sites enables scientists to manipulate DNA precisely by cutting in those regions and introducing the desired elements there.


Novel protein: In the context of genetically engineered crops, a novel protein is a protein that was not previously part of the diet. They may be produced in GE crops by transformation with genes protein products of which were not part of diet earlier or they may be products of synthetic genes. See novel food.

Post-translational modification: A process through which protein molecules are biochemically modified within a cell following their synthesis. A protein may undergo a complex series of modifications in different cellular compartments before its final functional form is produced.

Immuno-equivalence: Having similar functions in relation to immunological properties. For example, ability to bind to a certain specific antibody or induce similar kinds and amounts of antibody.

De novo sensitization: First exposure to a substance that induces immune activation so that subsequent exposures to the agent result in an allergic response.

IgE: A class of antibody primarily involved in allergic hypersensitivity reactions.
Macro-nutrient: ‘In humans and animals, a substance that is required in relatively large amounts for healthy growth and development, and belongs to one of three groups: carbohydrates, fats, and proteins’ (Nutritional and food safety assessment of foods and feeds nutritionally improved through biotechnology. Comprehensive Reviews in Food Science and Food Safety, 2004:3:38-104).

Micro-nutrient: ‘In humans and animals, a substance, such as a vitamin or trace element, essential for healthy growth and development but required only in minute amounts’ (Nutritional and food safety assessment of foods and feeds nutritionally improved...