



# The Safety of Food and Feed Derived from GE Crops

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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.

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# Content

- ✓ Introduction to food safety
- ✓ Principles of risk analysis
- ✓ Current established methods for safety assessment of foods derived from GE crops
  - In relation to general principles of risk analysis and food toxicology
  - Novel approaches required
- ✓ 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

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## Food safety— What needs to be regulated?

- Food additives
- Food labeling
- Dietary supplements
- Novel and GE foods
- Food security and protection of food supplies

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## **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.

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# 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.**

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# 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.

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## 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

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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

## Final Standards for Food X



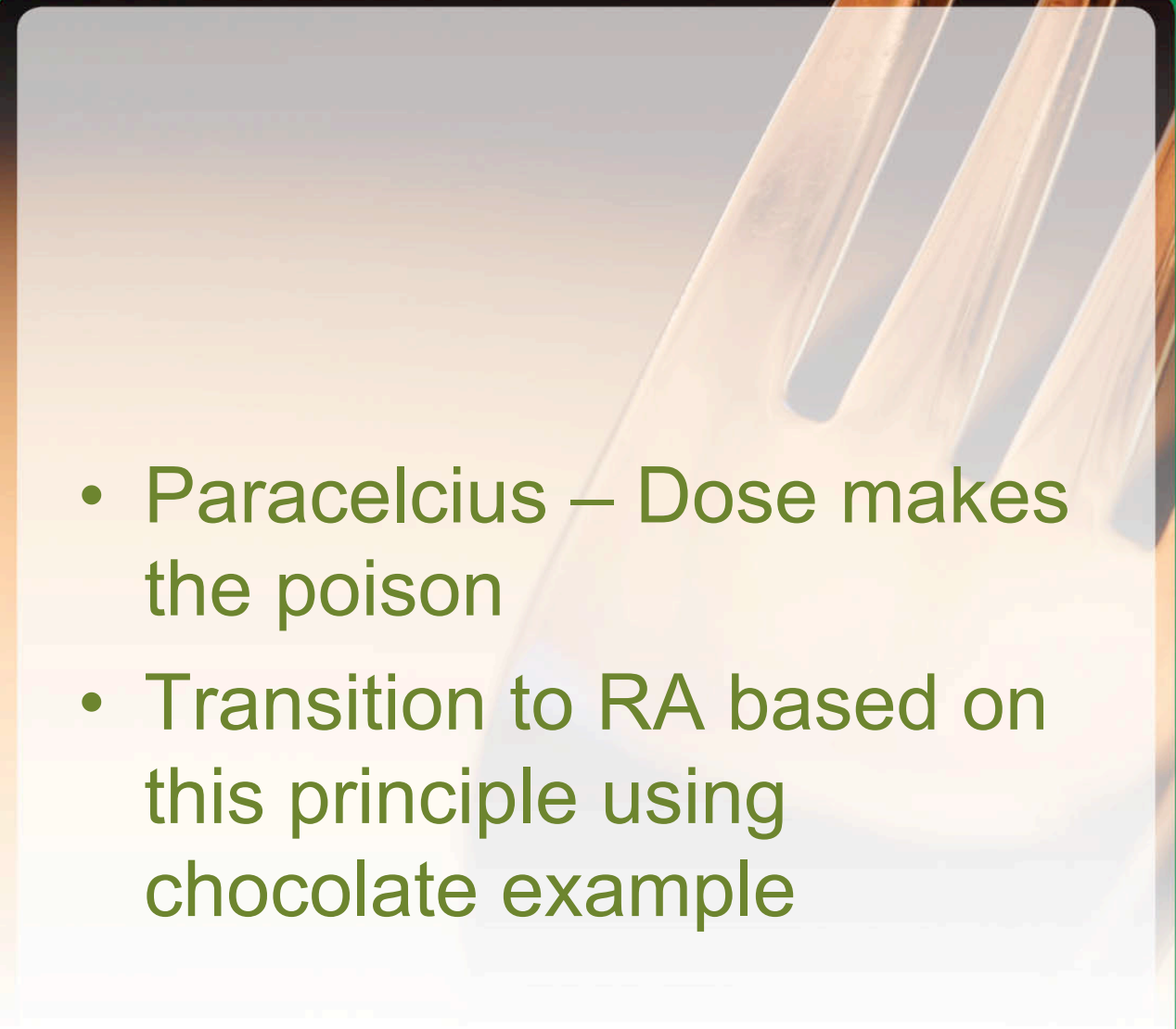
### Butylate Hydroxyanisole

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

10X more of  
Acceptable Daily Intake  
(~ 1 lb) is more achievable  
to consume in a day

### Hexane

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

- 
- Paracelcius – Dose makes the poison
  - Transition to RA based on this principle using chocolate example

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# 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)

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# 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

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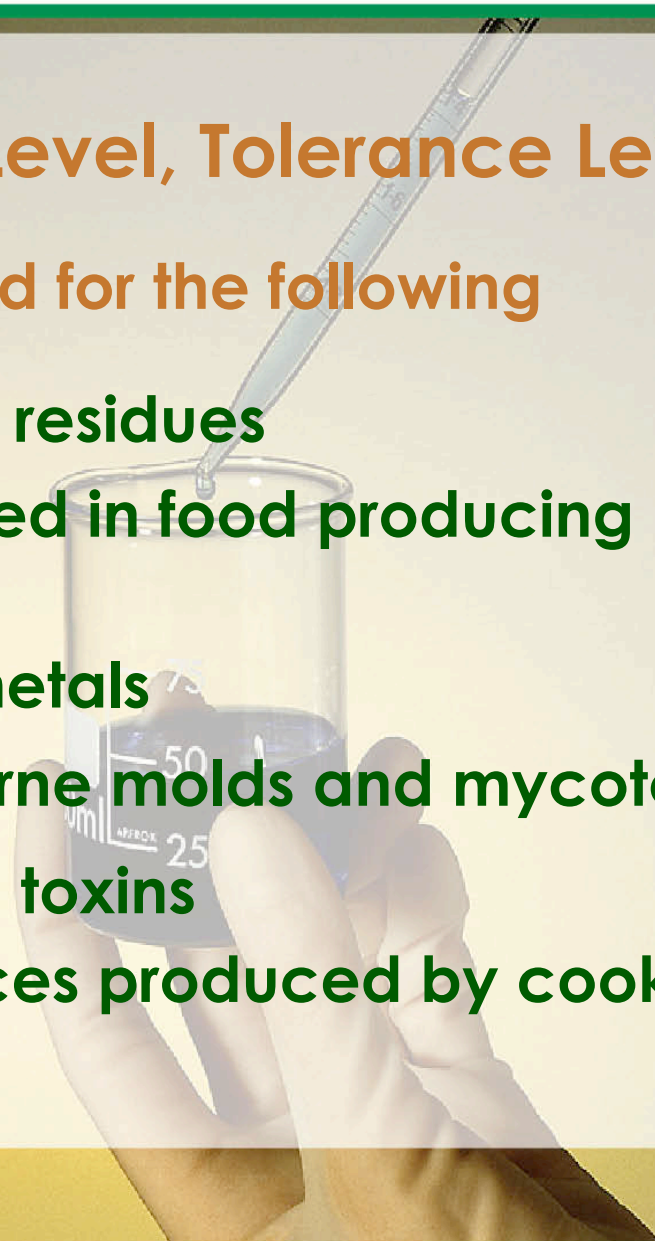
# Safety Assessments of Foods

- 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 can not be proved absolutely*
- Safety assessment seeks a level of  
*reasonable certainty that harm will not occur*  
(as long as they are free of contaminants)

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## 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

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# 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?**

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## 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

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# 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)

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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*.

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# Test Methods to Assess the Safety of Foods Derived from GE Crops

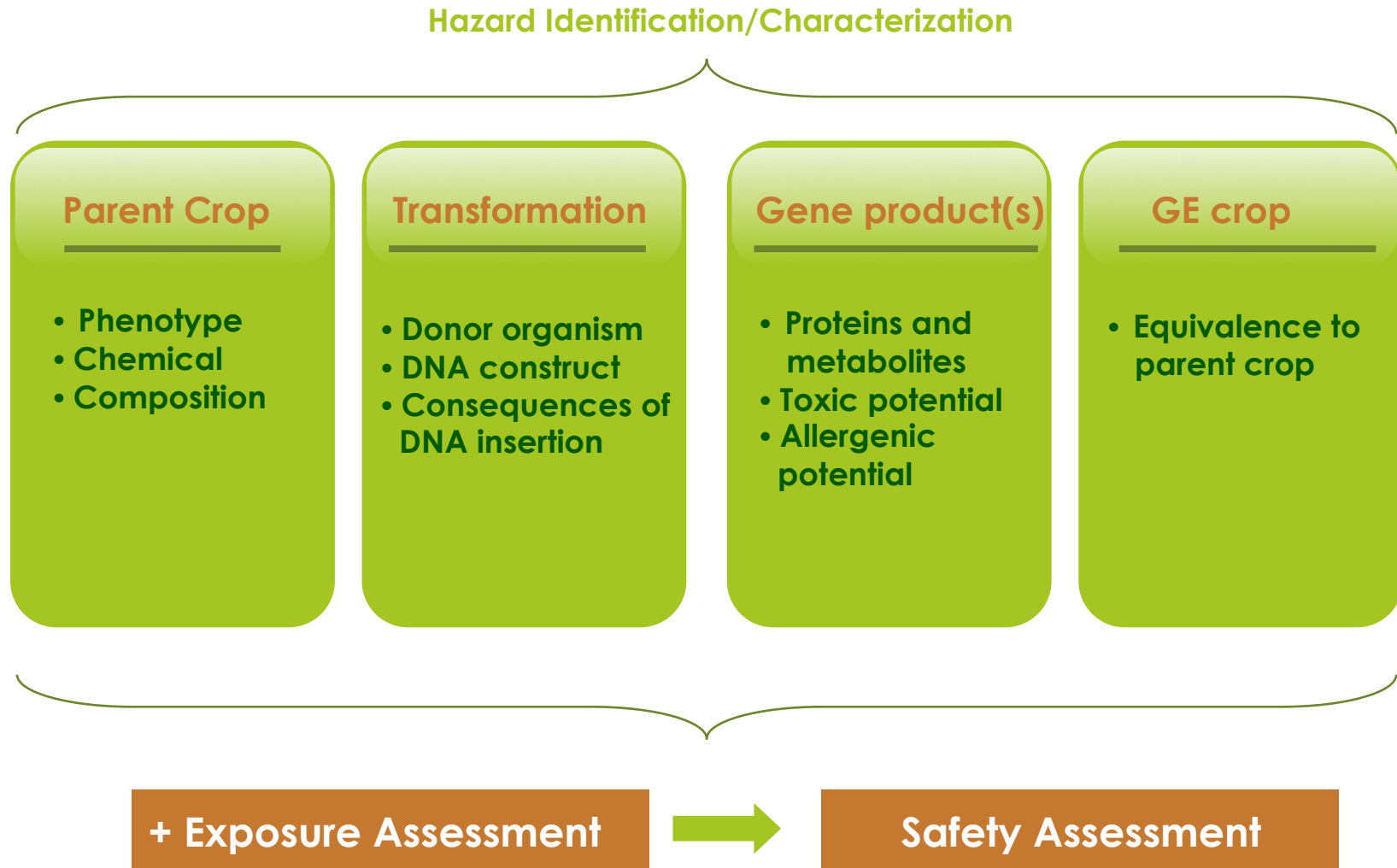


Figure modified from König et al, 2004



# Step 1 — Parent Crop

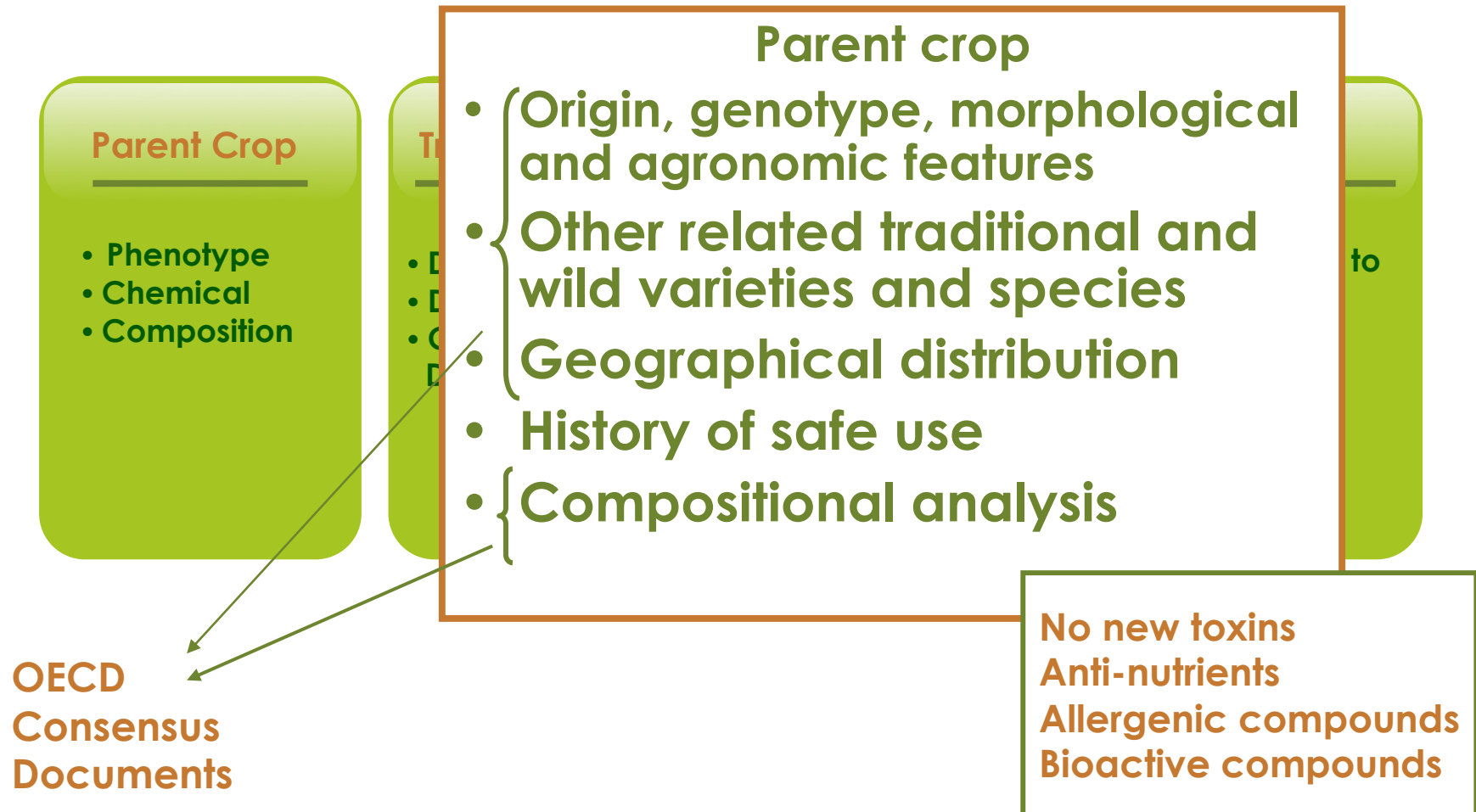


Figure modified from König et al, 2004

## Step 2 — Donor Organism and Transformation

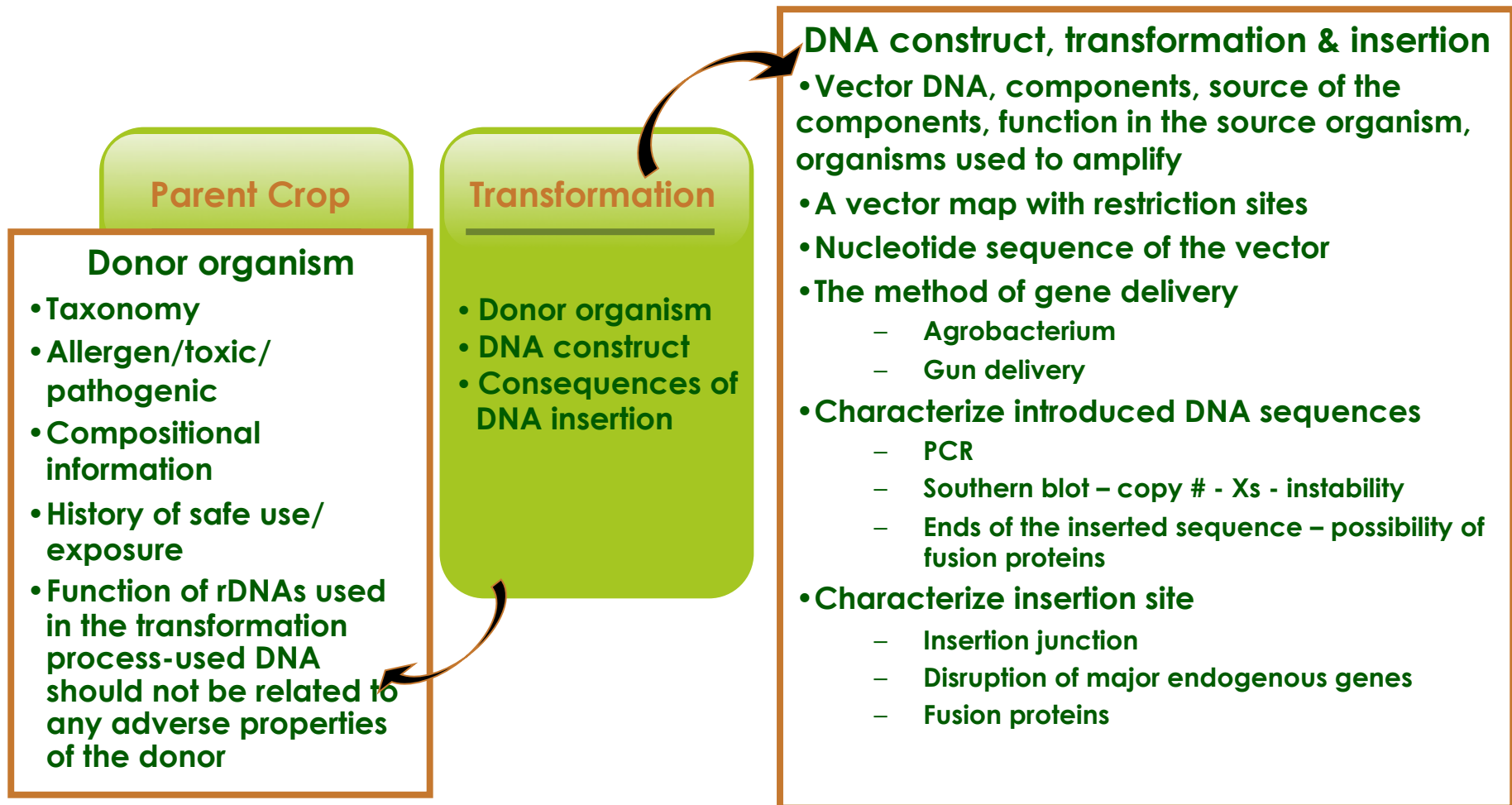


Figure modified from König et al, 2004

## 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-equivalence
- 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
  - FAO/WHO(2001), Codex Alimentarius (2003)

### Gene product(s)

- Proteins and metabolites
- Toxic potential
- Allergenic potential

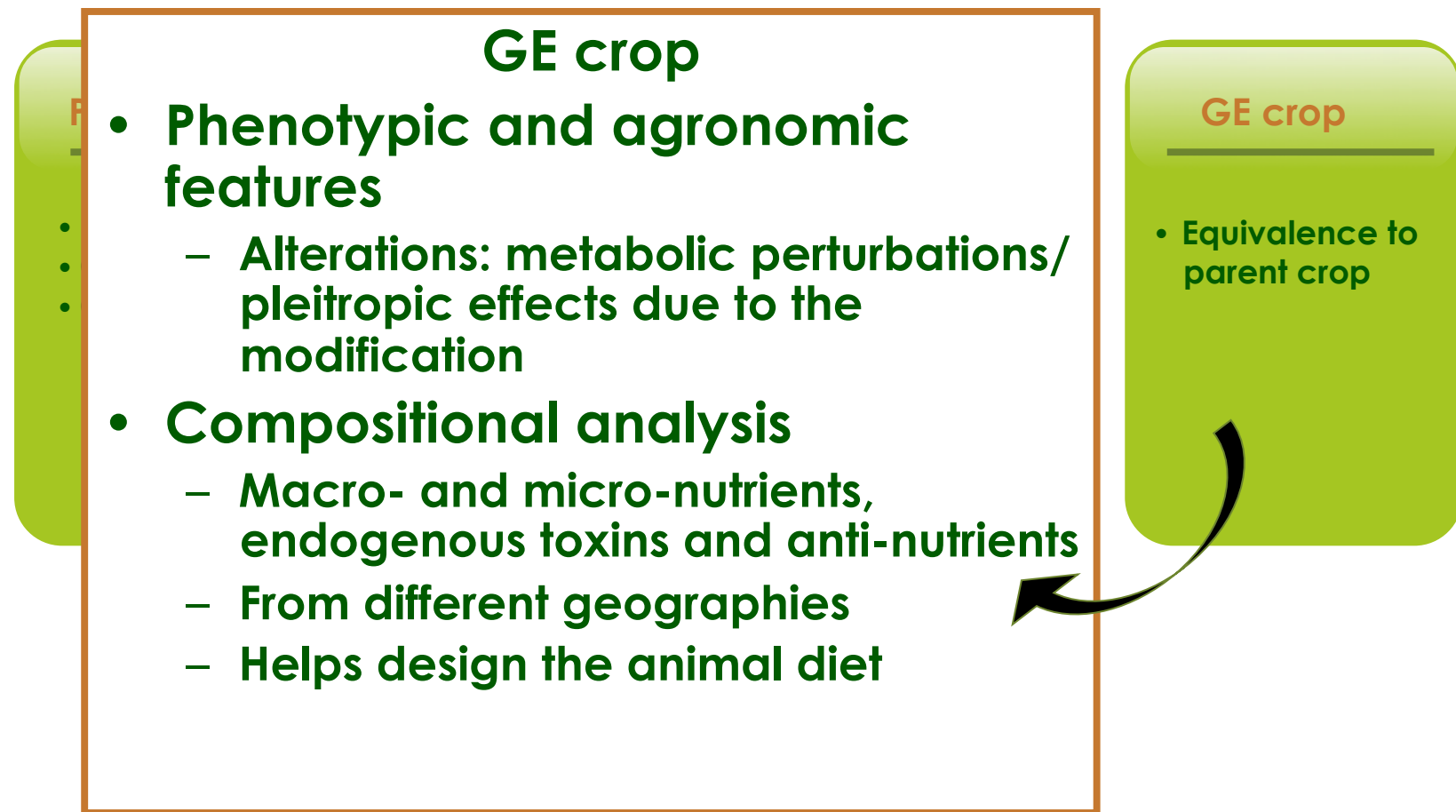
### GE crop

- 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.



## Step 4 — GE Crop

### An example:

#### – Roundup Ready soybeans

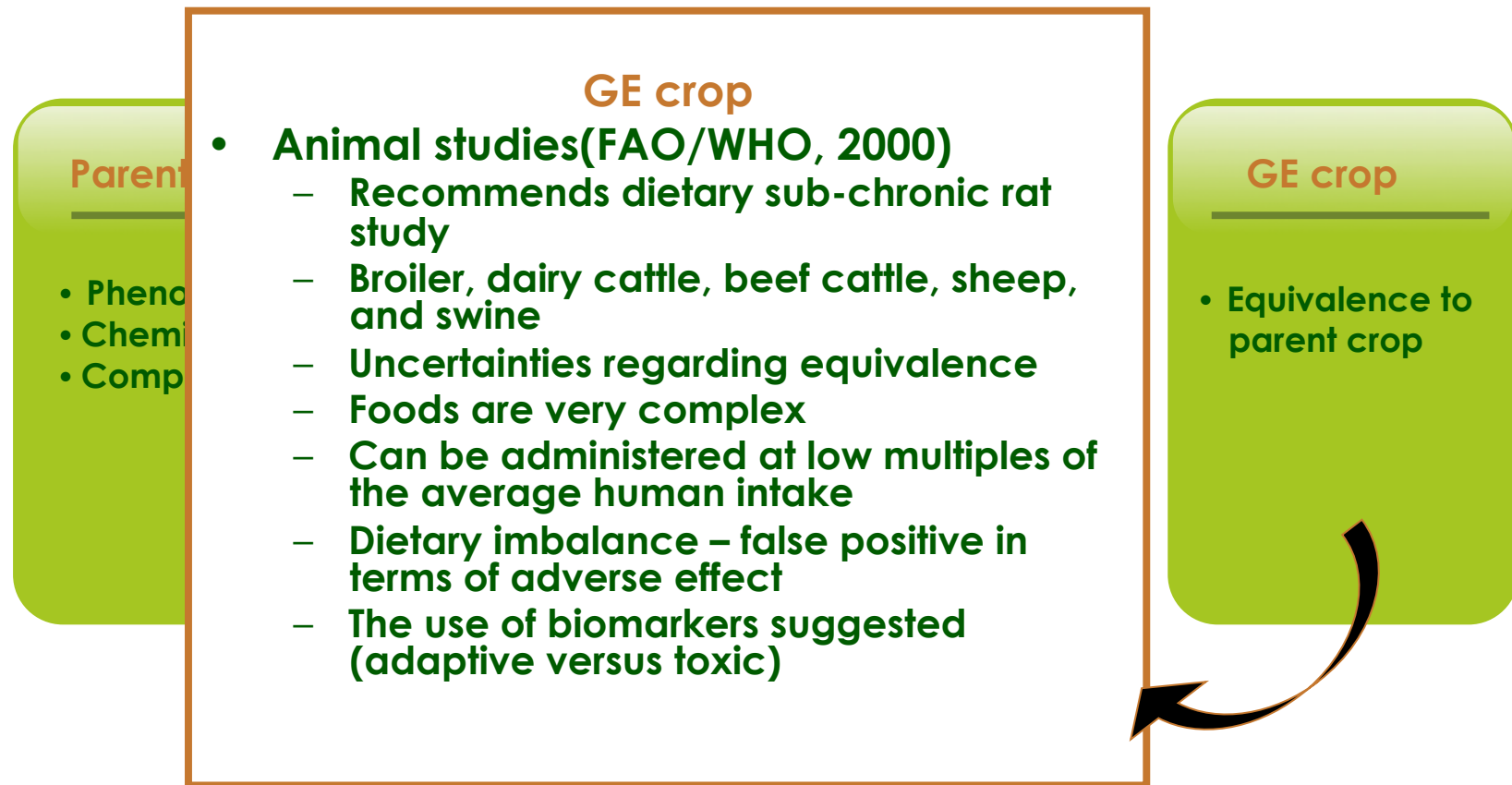
- Soybeans naturally contain certain levels of anti-nutrients; trypsin inhibitor, lectins and isoflavones
- Protein, oil, fiber, carbohydrates, moisture content, amino acid and fat composition in seeds and toasted soybean meal compared with conventional counterparts
- Trypsin inhibitor levels were 11-26% 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

#### GE crop

- Equivalence to parent crop



## Step 4 —GE Crop



# 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

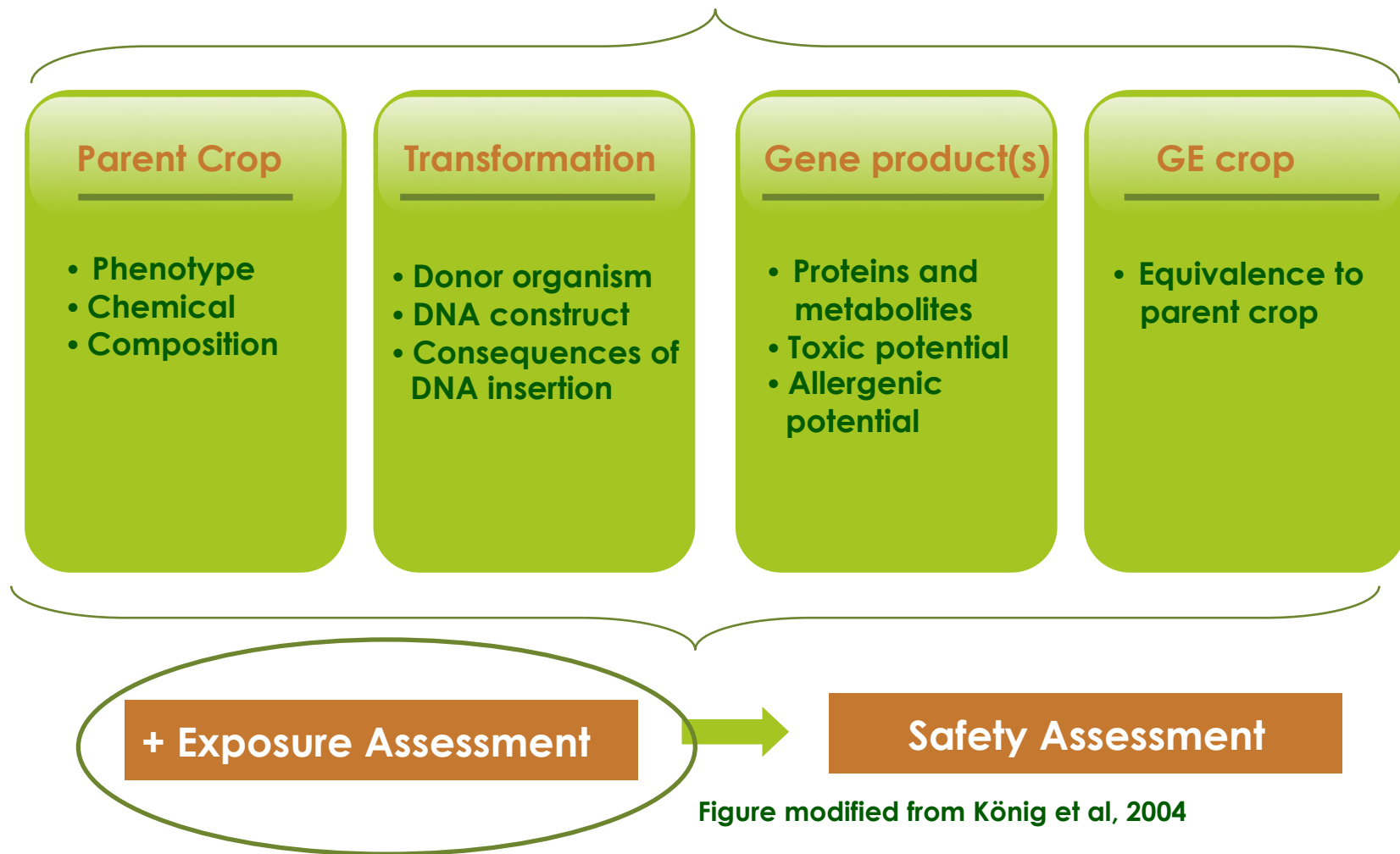


Figure modified from König et al, 2004

# 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: 0.017-0.07mg/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.

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## 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

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# 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
  - Allergenicity (FAO/WHO 2001, Codex Alimentarius Commission 2003)
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

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# 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

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# 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.

# In the Future?

## Safety Testing

### 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.

### 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

## Now and In The Future

- **FAO/WHO, 1991, 1996, 2000, 2001**
- **Codex Alimentarius Commission, 2003**
- **NAS, 1987**
- **NRC, 1989, OECD, 1993, 1996, 1998, 2002**
- **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**

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# Resources

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

[http://www.fao.org/UNFAO/about/index\\_en.html](http://www.fao.org/UNFAO/about/index_en.html)

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

<http://www.foodsafety.gov/~fsg/biotech.html>

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