Risk assessment of GM plants and derived food and feed

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Issues

- Strategies for Risk Assessment of GM Plants and derived Food and Feed

- Specific Food/Feed Safety Aspects:
  - **Compositional Analysis**
  - **Animal Feeding Trials of Whole GM Foods/Feed**

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GUIDANCE DOCUMENT OF THE SCIENTIFIC PANEL ON GENETICALLY MODIFIED ORGANISMS FOR THE RISK ASSESSMENT OF GENETICALLY MODIFIED PLANTS AND DERIVED FOOD AND FEED

(Question No EFSA-Q-2003-005)

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International Food Safety Strategies for Foods Derived from Modern Biotechnology

- OECD Group of National Experts on Safety in Biotechnology, 1993, 1994, 1996
- OECD Task Force on the Safety of Novel Foods and Feed, 1998-present
- FAO/WHO Expert Consultations, 1991, 1996, 2000, 2001, 2003
- CODEX Task Force on Foods Derived from Biotechnology, 1999-2004
- European Commission Directives and Regulations, 1996-present
- ENTRANSFOOD, the EU Thematic Network on the Safety Assessment of Genetically Modified Food Crops, 2000-2003
- European Food Safety Authority, Guidance Document GMO Panel

Codex Principles for Risk Analysis and Guidelines for Safety Assessment of Foods Derived from Modern Biotechnology 2003

- Principles for the Risk Analysis of Foods Derived from Modern Biotechnology (CAC/GL 44 -2003)

- Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants (CAC/GL 45 -2003)

- Guideline for the Conduct of Food Safety Assessment of Foods Produced Using Recombinant-DNA Microorganisms (CAC/GL 46 -2003)

Http://www.codexalimentarius.net

Sequential Steps in the Risk Assessment of GMOs

- **HAZARD IDENTIFICATION**

  characteristics which may cause adverse effects
- **HAZARD CHARACTERIZATION**

  potential consequences for man and the environment
- **EXPOSURE ASSESSMENT**

  likelihood of occurrence/exposure
- **TOTAL RISK CHARACTERIZATION**

  evaluation of risk(s) posed by each identified characteristic

Key Elements for the Assessment of GMOs

- Characterization of donor and host organism
- Molecular characterization of the genetic modification event:
  - **methods**
  - **inserted genes**
  - **gene expression**
- Analysis of agronomical and compositional properties
- Toxicity/allergenicity/ nutritional testing
- Environmental risk assessment
- Environmental monitoring/surveillance

Comparative Safety Assessment Approach for GMOs

Underlying assumption:

- **Traditionally cultivated crops have gained a history of safe use for the environment/consumer/animals**

- **These crops can therefore serve as a *baseline* for the environmental and food/feed safety assessment of GM crops**

Comparative Safety Assessment Approach for GMOs

1. Identification of differences between the GM and non-GM crop
2. Assessment of the identified differences regarding

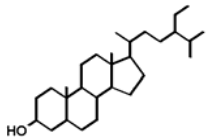
   environmental/food/feed safety/nutritional impact
   - Concept of Familiarity
   - Concept of Substantial Equivalence or Comparative Safety Assessment
3. No absolute safety assessment in itself

Risk-Analysis Strategies for Transgenic Foods I

Phase 1

Phase 2

Traceing of differences between the GM food and the conventional product (Concept of Substantial Equivalence)



Introduced genes

(New) proteins

(New) metabolites and toxins

Toxicity / nutritional investigations

Gene transfer

Degradation Toxicity Allergenicity

Toxicity

Whole foods

Risk-Analysis Strategies for Transgenic Foods II

Phase 3

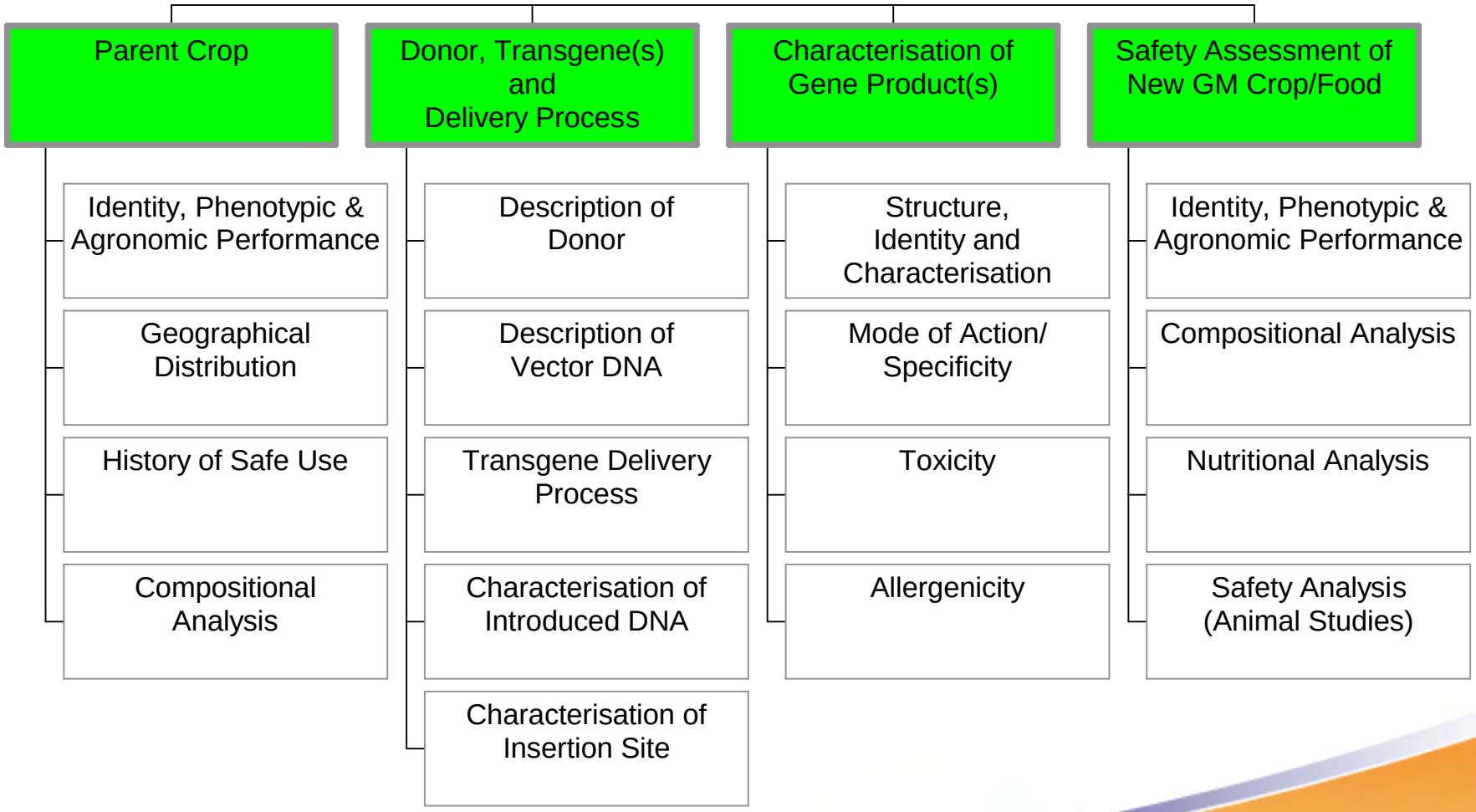
Intake studies

- **Role of the product in the diet**
- **Intake of the product by the consumer**

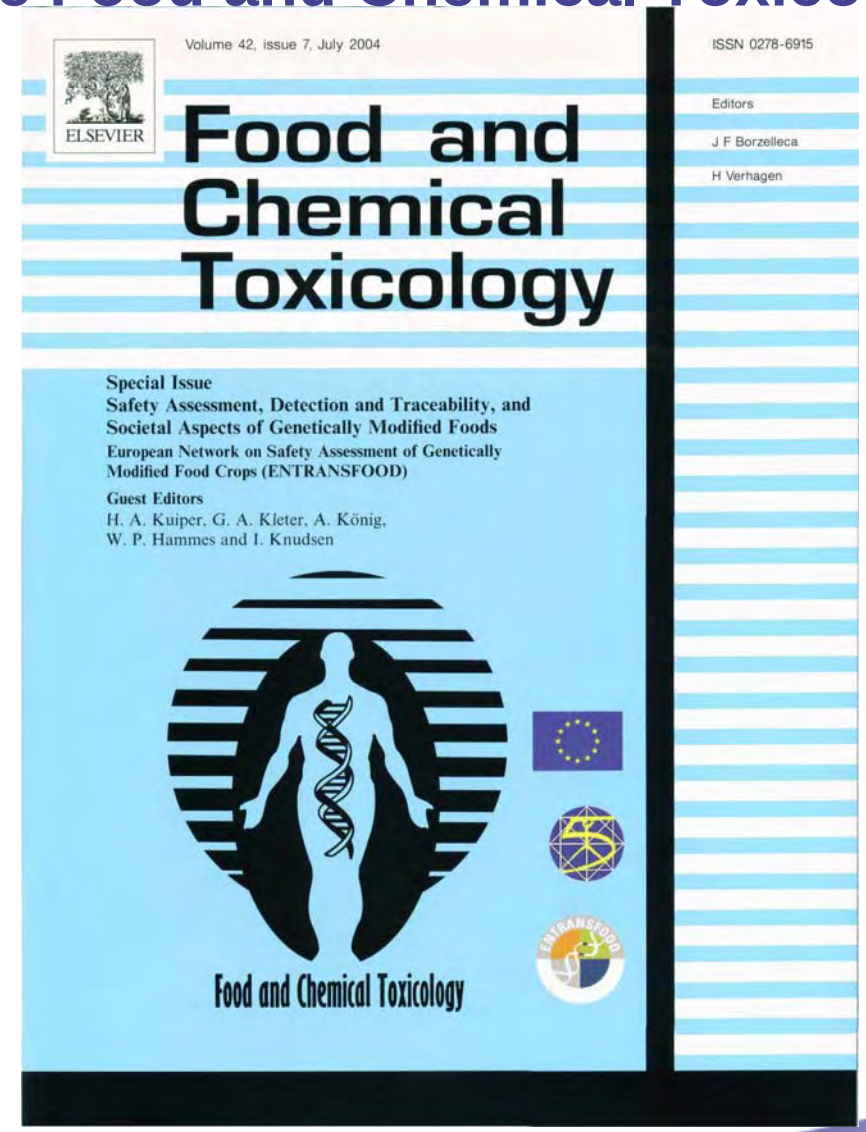
Phase 4

Risk assessment of transgenic foods

Integrated Approach to the Hazard Assessment of a New GM Variety (ENTRANSFOOD)



Special Issue Food and Chemical Toxicology



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Comparative Comprehensive Risk Assessment

- All evidence from the molecular, agronomical, compositional, toxicological/nutritional and environmental impact characterization should be taken into account.
- Science evolves continuously and therefore there is permanent need for further method development

ISSUES

- COMPOSITIONAL ANALYSIS
  - **SELECTION OF COMPOUNDS (OECD)**
  - **NATURAL VARIATIONS (OECD, ILSI, Reviewed Literature)**
  - **ANALYSIS: STATISTICS AND BIOLOGICAL RELEVANCE**

- ANIMAL FEEDING TRIALS WITH GM FOODS/FEED
  - **PURPOSE OF THE STUDY**
  - **PREDICTIVE FOR REPEATED DOSE TOXICITY**
  - **SAFETY MARGINS**
  - **NATURAL VARIATIONS**
  - **STATISTICAL ANALYSIS AND BIOLOGICAL RELEVANCE**
  - **MON 863 FEEDING TRIAL**

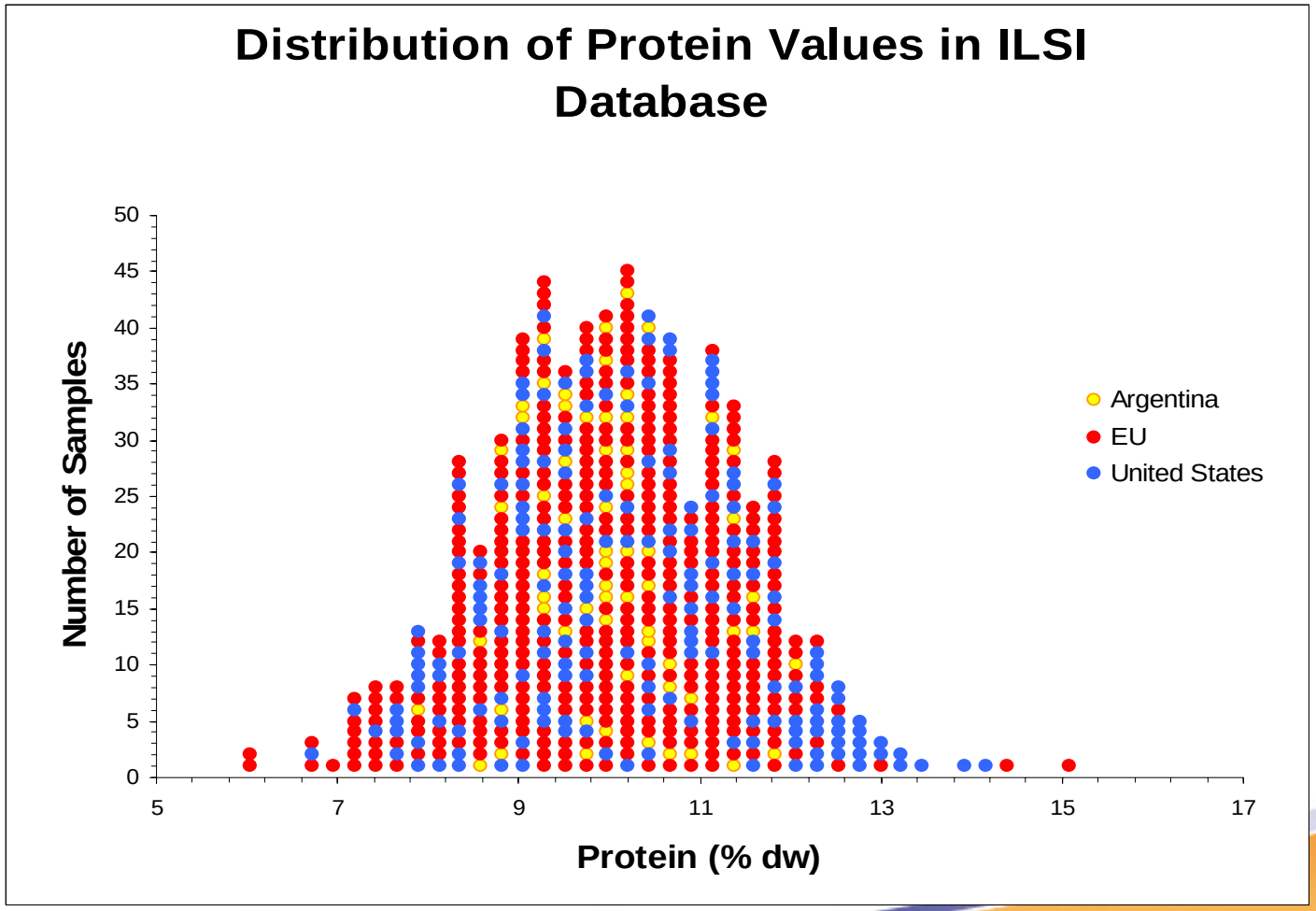
COMPOSITIONAL ANALYSIS

- Choice of the comparator:
  - **Non-GM isogenic variety**
  - **Non-GM lines of comparable genetic background**
  - **Controls produced by back-crossing**
- Key macro- and micro nutrients anti-nutritional compounds, natural toxins
  - **Crop specific**
  - **Trait specific (herbicide tolerance- aromatic amino acid synthesis)**
- OECD Consensus Documents for selection of compounds

Analysis of Compositional Data

- Large number of analyses of different compounds (Control vs GM line) yields always some significant differences expected by chance alone
- *Systematic* differences in components should be identified and assessed (locations, seasons) against background levels and variations
- Data on natural variations:
  - **Literature data**
  - **OECD Consensus Documents**
  - **ILSI database**
- Specific attention for values that fall outside normal ranges of variation

Distribution of Maize Protein Values in ILSI Database



Animal Feeding Trials with Whole GM Foods/Feed

- **Foods are extremely complex matrices with many biologically active compounds, that may cause adverse reactions**
- **Very few foods have been subject to toxicological studies, yet they are accepted as being safe**
- **Very little known about long term effects of any food**
  - *Wide genetic variability*
  - *Changing diets over time.*

When Animal Feeding Trials With GM Foods/Feed?

- Profound changes in the composition of the GM plant
- Indications for potential unexpected effects (molecular characterization, agronomic, compositional analysis)
- 90-days study in rodents recommended
- Reassurance study
- Protocols (OECD) for low molecular weight chemicals testing should be adapted for testing whole foods.

EFSA Guidance Document
GM Plants and derived Food/Feed

Difficulties with Animal Feeding Trials with GM Foods/Feed

- Natural bulkiness of food,
- Effects on satiety
- Need to maintain nutritional balance
- Limit of dietary administration (5%) in order to prevent dietary imbalance
- Matrix effects
- Semi-synthetic diets can be prepared with inclusion levels as high as 60% or more.

3-Month Study in Rodents Predictive for Long term Effects?

- US NTP: Studies of industrial and agro chemicals indicated that for 70% of the compounds evaluated toxicological findings in the 2 year rodent test were also seen in or predicted by the 3 month subchronic test (British Toxicology Society, 1994)
- Review of other data sources including monographs of JECFA, covering 613 substances, indicated that “in many cases, the lowest and most conservative NOEL for a substance came from a subchronic study” (Munro et al.,1996)
- Similar observations in dog studies (Box and Spielman, 2005)

Safety margins

- Uncertainty Factors are normally applied to allow for inter and intra-species variations in sensitivity and specificity, adding further Margins of Safety (MOS) for consumers.

- Estimation of the average daily intake by humans of a given whole food, and comparison with that consumed by rats in the subchronic 90-day feeding study, indicates the MOS for consumers.

Safety margins

Maize

- 90 day rat subchronic studies with GM maize in the diet at 33 % (w/w) or more, represent a NOAEL.
- Averaged over the whole study a rat typically consumes 25 g maize/kg bw/day.
- An EU estimated intake for humans is 17g/person /day, corresponding to 0.24g maize/kg bodyweight /day
- This provides at least a margin of safety (MOS) of a 100 fold

Animal Feeding Trials with Whole GM Foods/Feed

- A Working Group of the GMO Panel explores possibilities on how to further develop in vitro and in vivo tests to characterize the toxicological and nutritional properties of whole foods/feed

90-Days Animal Feeding Trial with MON 863 Maize

- 90-days studies with rats, 20 males and 20 females per test group
- MON 863 maize, at 11% and 33% inclusion level
- Non-transgenic isogenic control, and 6 commercial lines
- Total of 400 animals
- Feed consumption, bodyweight, clinical parameters, organ weights, histopathology according to OECD guidelines
- Standard toxicological testing procedures (OECD)

90-Days Animal Feeding Trial with MON 863 Maize

- Lymphocyte counts slightly increased in males (33% test group), no changes in other leucocyte counts and differences fall within variations of reference control data
- Reticulocyte counts in females(33% test group) statistically significantly lower than in controls and reference lines, within the range of control and reference groups
- No other changes in haematological parameters

90-Days Animal Feeding Trial with MON 863 Maize

- Kidney weights of males (33% test group) were stat. significantly lower, within the range of the reference control groups
- Pathology analysis showed a lower incidence of mineralised kidney tubules in female rats (33% test group)
- In male animals a higher incidence in focal chronic inflammation and tubular regeneration was observed

Analysis of Results

- Data were analysed statistically using ANOVA and Student t-tests)

- Various contrasts were tested for differences:
  - **GM test groups (high dose/low dose) and the non-GM control groups (high dose/low dose)**
  - **GM test groups and reference lines**

GMO Panel Evaluation

- The Panel has analyzed all t-test results provided by the applicant that compare GM maize with its non-GM counterpart
- Panel has considered all statistically significant differences and the biological relevance of these differences taking the biological variation into account
- Internationally accepted approach taken when analyzing results of toxicological studies
- The Panel did not notice relevant differences between the GM and non-GM maize which could have an adverse health impact on humans or animals

Criticisms on Followed Procedures

- Analysis of the data as performed by the applicant permits at best a conclusion on the possible existence of *differences* between the GM and non-GM product, while the claim is that the products are equivalent
- Absence of evidence for a difference is not an evidence of absence:
  - *there may not be a difference,*
  - *test procedure was unsuitable,*
  - *test was not carried out carefully enough*

Proposals for Alternative Statistical Analysis

- Equivalence study may be applied to address the question of substantial equivalence of GM food/feed compared to their conventional counterparts:

- Multi-variate statistical analysis may be applied

Comments

- Testing for potential *differences* between the GM and non-GM counterpart and assessment of identified differences is the cornerstone of the safety assessment of GM foods.
- Sensitive analytical and toxicological methods are used to identify potential differences in large arrays of test parameters
- Equivalency testing has gained extensive experience with testing of pharmaceuticals with well-defined preset test criteria
- Information on variation in food components and on background variation in biological endpoints of animal studies is essential and should be build in statistical analysis models

Comments

- The potential application of equivalence testing, multi-variate statistical analysis and adaptation for food safety assessment purposes is further explored during a Self Tasking Activity of the GMO Panel

Self task activities of the GMO Panel

- Biosafety of antibiotic resistance marker genes
- Post-market environmental monitoring of GM crops
- The use of animal feeding trials for the safety evaluation of whole GM foods/feed
- Update the approaches for allergenicity assessment of GMOs
- Strategies for statistical analysis in comparative analysis and animal studies
- Assessment of GM plants used as production platform for non-food/feed products
- Assessment of GM plants with enhanced nutritional properties (*not started*)