Risk Assessment of GM Plants and Derived Food and Feed as Applied by the EFSA GMO Panel

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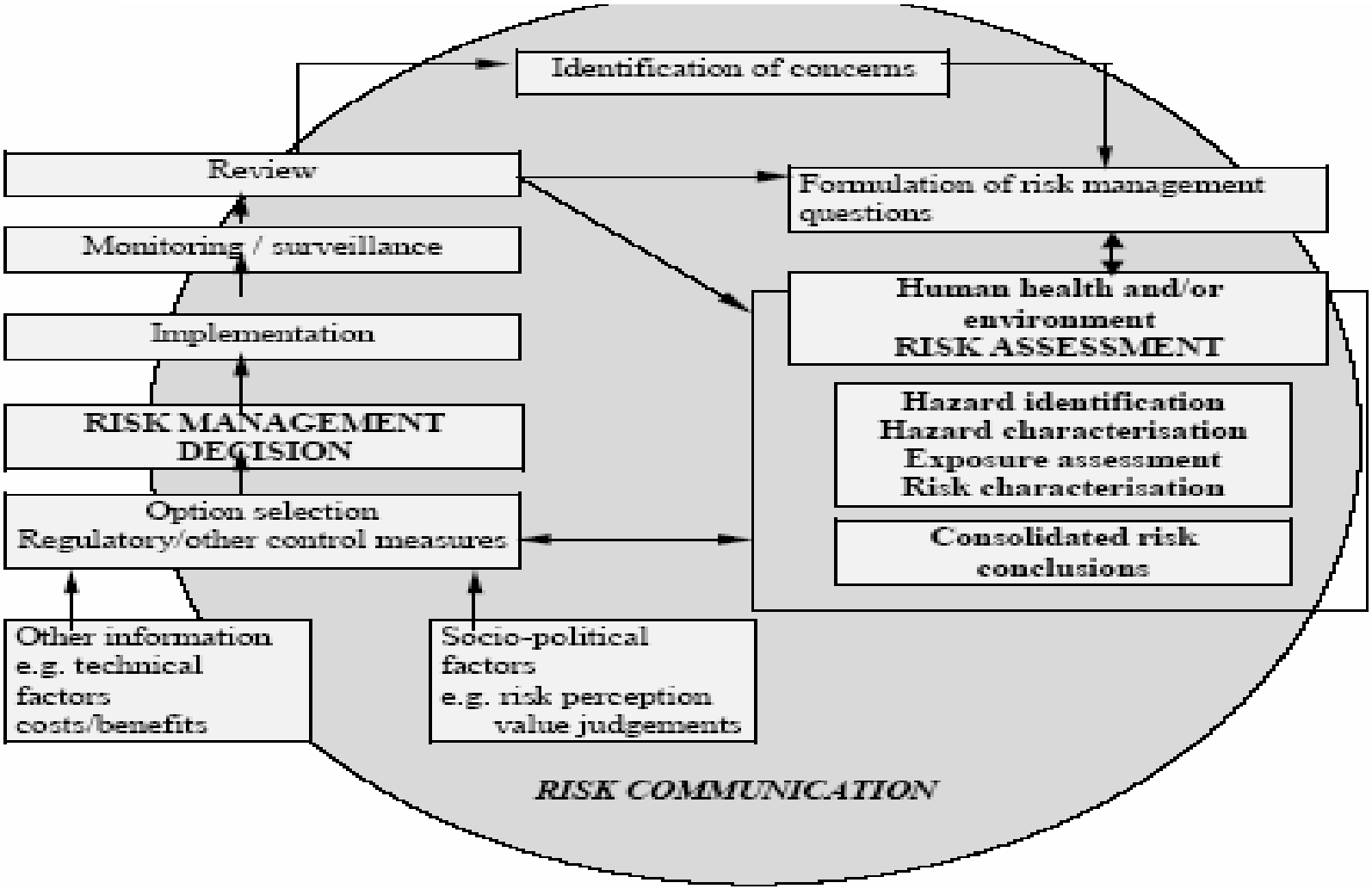
Issues

- Structure Risk Analysis Framework
- EFSA GMO Panel Guidance Document for the Risk Assessment of GM Plants and derived Food and Feed
- Uncertainty Analysis and Long Term Effects
- Developments in risk assessment

The Risk Cycle: Components of Risk Analysis,



EU Scientific Steering Committee Report, 2000, 2003

International Food Safety Strategies for Foods Derived from Modern Biotechnology

- OECD Group of National Experts on Safety in Biotechnology, 1993- 1996
- OECD Task Force on the Safety of Novel Foods and Feed, 1998-present
- FAO/WHO Expert Consultations, 1991- 2003
- CODEX Task Force on Foods Derived from Biotechnology, 1999- 2005
- CODEX Principles for Risk Analysis and Guidelines for Safety Assessment of Foods Derived from Modern Biotechnology 2003
- 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

Comparative Safety Assessment Approach for GMOs

Underlying assumption:

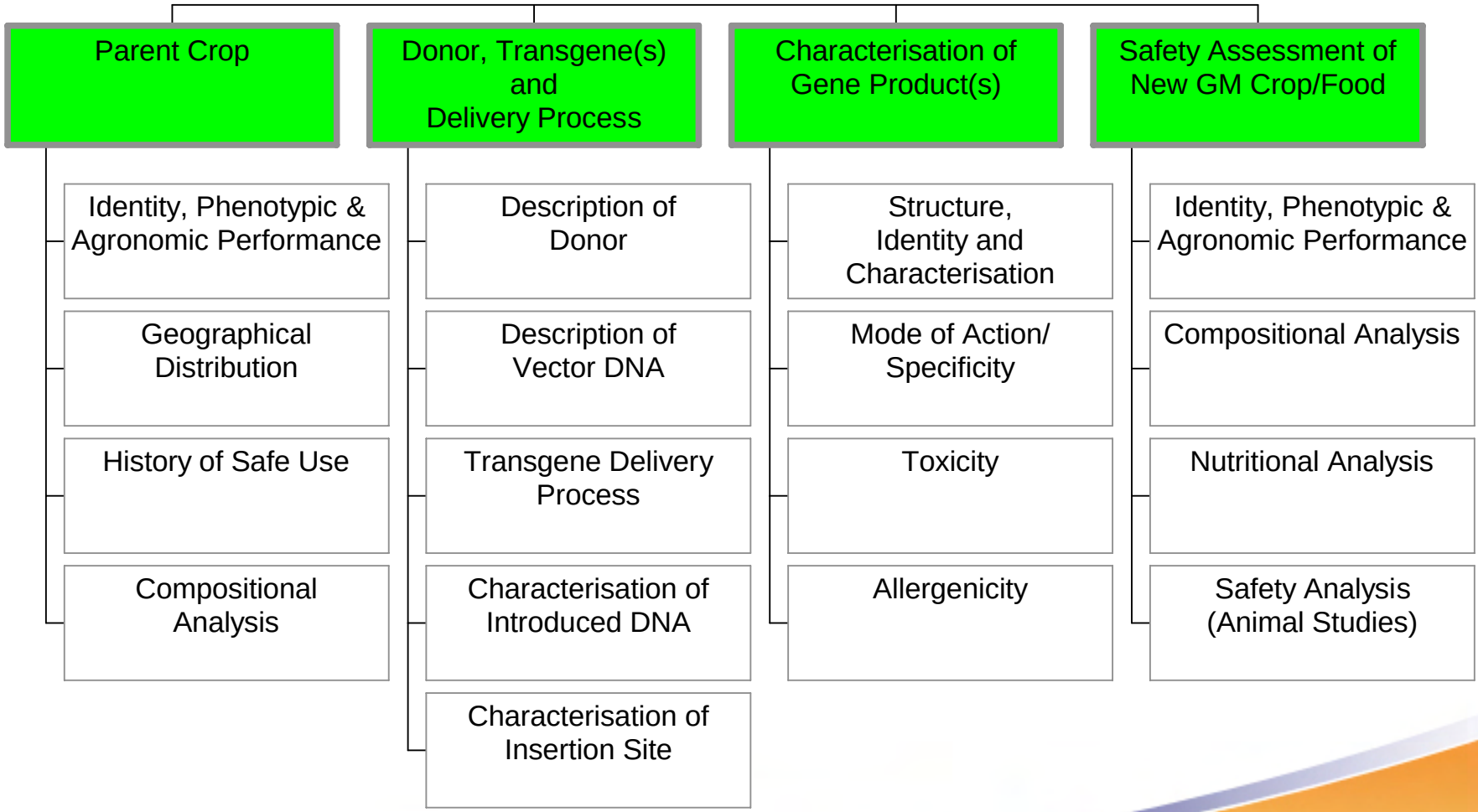
- **Traditionally cultivated crops have gained a history of safe use for the environment, consumers and animals**

- **These crops can serve as a *baseline* for the environmental and food/feed safety assessment**
  - Concept of Familiarity
  - Concept of Substantial Equivalence or Comparative Safety Assessment

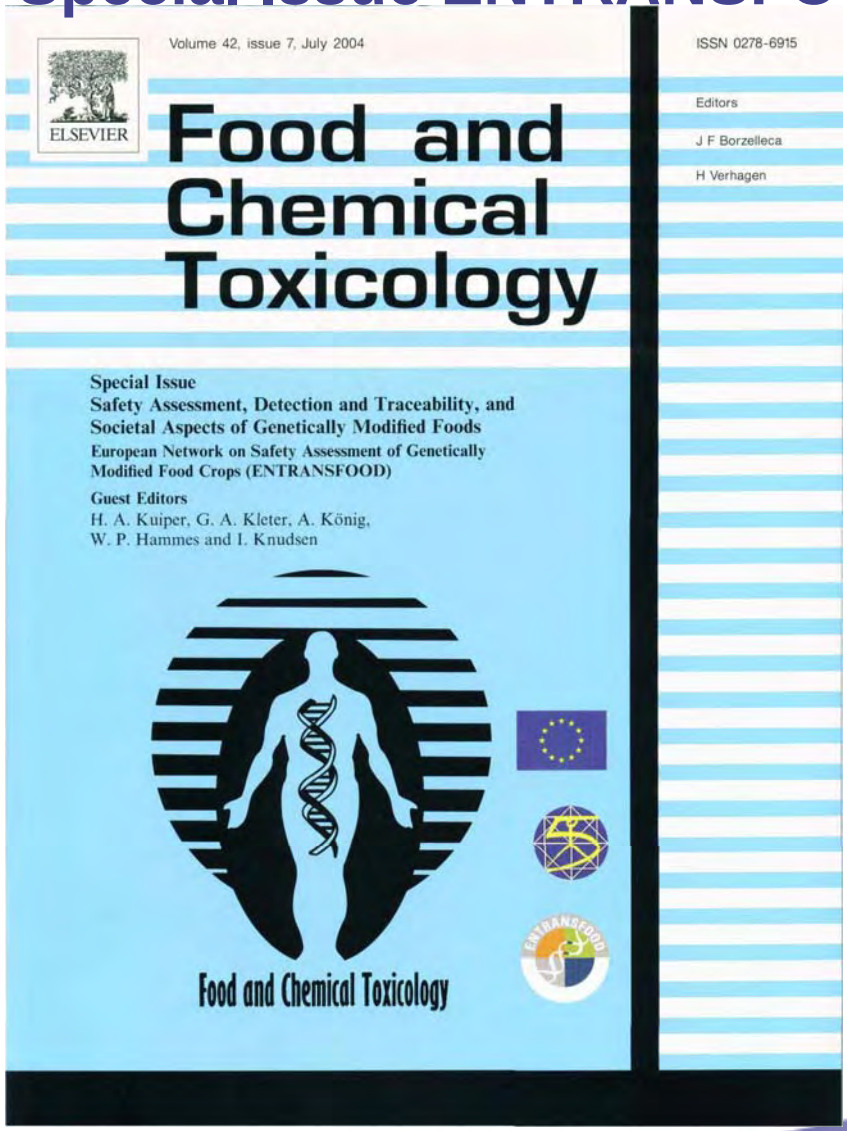
Comparative Safety Assessment Approach for GMOs

- Identification of *differences* between the GM and non-GM crop
- Assessment of the identified differences regarding environmental/food/feed safety/nutritional impact
- No absolute safety assessment in itself

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



Special Issue ENTRANSFOOD



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European Food Safety Authority

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)

Adopted on 24 September 2004

Final, edited version of 8 November 2004

The EFSA Journal (2004) 99, 1-93

EFSA GMO PANEL Guidance Document

- The document indicates necessary data to underpin the safety of GM Plants and derived food/feed
- The document indicates various types of studies and protocols (EU, OECD, GLP)
- Case-by case approach; not a cook book
- Assessment based on applicant's data, and other data (e.g. literature, EU project database)
- Focus on assessment of possible adverse effects upon short and long-term exposure
- Uncertainty analysis is core business in risk assessment

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
- Post-market monitoring
- Environmental risk assessment
- Environmental monitoring/surveillance

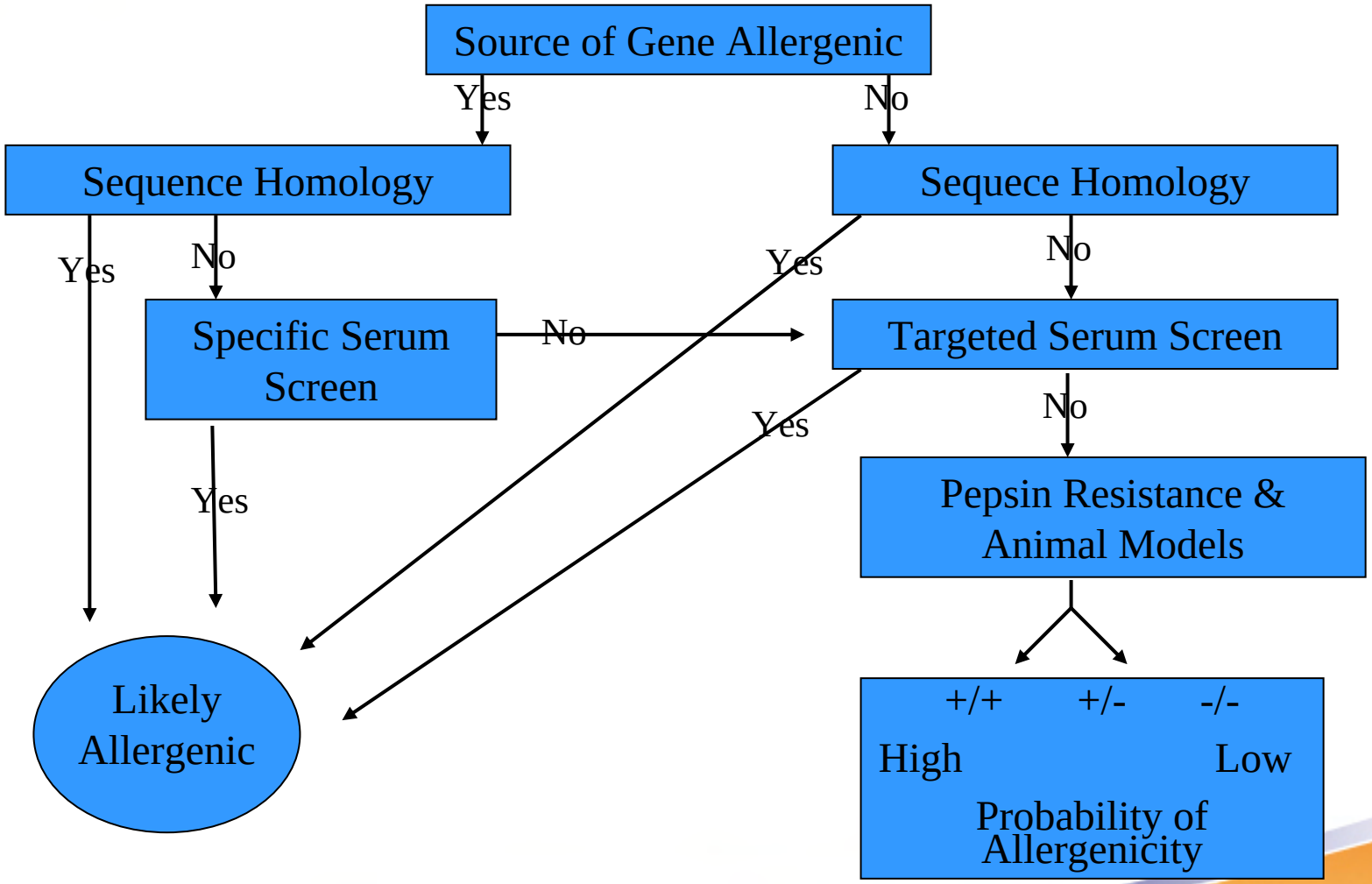
Toxicological studies of GM Food/Feed

- Toxicity testing of newly expressed proteins
  - Protein equivalency, homology, stability etc
  - Repeated dose toxicity (28 days) and if needed additional studies (immunotoxicity)
- Toxicity testing of constituents other than proteins
  - Core set studies on metabolism/kinetics, genotoxicity, (sub)chronic toxicity, reproduction and developmental toxicity, carcinogenicity
- Testing of whole GM food/feed
- Case-by case

Assessment of Allergenicity

- Approach outlined by the Codex *ad hoc* Task Force on Foods derived from Biotechnology, 2003
- No single test can reliably predict an allergenic response
- Weight of evidence approach
- Further development of bioinformatic approaches and experimental models is ongoing

Assessment of the Allergenic Potential of Foods Derived from Biotechnology FAO-WHO 2001



Environmental Risk Assessment (E.R.A.) Elements

- Persistence and invasiveness
- Selective advantage or disadvantage
- Potential for gene transfer
- Interactions between the GM plant and target organisms
- Interactions of the GM plant with non-target organisms
- Effects on human/animal health
- Effects on biogeochemical processes
- Impacts of the specific cultivation, management and harvesting techniques
- Potential interactions with the abiotic environment
- Monitoring and Surveillance

Tiered Approach to Risk Assessment

- **Tier 1. Laboratory Experiments** > **Hazard identification**, Impacts at first trophic level, direct non targets. Data on maximum or high dose tests in order to catch potentially all possible effects.

- **Tier 2. Growth Room/Glasshouse** > Interactions, 2nd trophic level, indirect non targets.

- **Tier 3. Field Experiments** > **Exposure** at range of trophic levels, indirect/agronomic effects

- **Tier 4. Monitoring** > long term/large scale impacts

Opinion of the GMO Panel on Post Market Environmental Monitoring (PMEM)

- To confirm assumptions made during the environmental risk assessment
- To identify occurrence of adverse effects of the GMO or its use on human health or the environment which were not anticipated in the environmental risk assessment
  - **Case-specific monitoring**
  - **General surveillance**
- Three Stakeholders Workshops and a Public Consultation on the EFSA Web

European Commission

Community Research

A Review of Results

EC-sponsored Research on Safety of Genetically Modified Organisms

Programme	Number of Projects
Fifth Framework Programme (1998-2002 only)	
• Cell Factory Key Action	5
• Food, Nutrition and Health Key Action	5
Fourth Framework Programme (1994-1998)	
• Biotechnology (II)	18
• Agriculture and Fisheries (FAIR)	2
• Standards, Measurements and Testing	1
• International Co-operation	5
Third Framework Programme (1990-1994)	
• Biotechnology (I)	9
• Agriculture and Agro-Industry (AIR)	2
Second Framework Programme (1987-1991)	
• Biotechnology (BRIDGE)	14
• Food-linked Agro-Industrial Research (FLAIR)	1
First Framework Programme (1984-1987)	
• Biotechnology Action Programme (BAP)	19
Total Number of Projects	**81**
	€ 70 Million
	400 teams

QUALITY OF LIFE AND MANAGEMENT OF LIVING RESOURCES

EUR 19884

Risk Assessment must be Comprehensive

- *All evidence* from molecular, agronomical, compositional, toxicological/nutritional and environmental impact data should be taken into account for final conclusions on risk assessment

- Science evolves continuously and therefore there is permanent need for further method development

Chapter IV Guidance Document
Risk Characterisation

" The quantitative or semi-quantitative estimate including attendant uncertainties, of the probability of occurrence and severity of adverse effects in a given population under defined conditions based on hazard identification, hazard characterisation and exposure assessment" (SSC, 2000)

Uncertainty Analysis is a Key Element in the Risk Assessment of GM Plant derived Food/Feed

- Completeness and quality of the molecular, compositional and toxicological/nutritional data set
- Adequacy of performance of experiments, data collection and processing and statistical analysis
- Possible occurrence of unintended alterations in the composition (new proteins/metabolites)
- Safety/nutritional impact of newly formed products

Uncertainty Analysis is a Key Element in the Assessment of GM Plant derived Food/Feed

- **Uncertainties in assumptions in toxicological, allergenicity or nutritional analysis**
  - *Strength data provided on the allergenic potential of newly expressed proteins/GM crop*
  - *Extrapolation of data from animals to humans*
  - *Delayed onset of effects (short term versus chronic exposure)*
  - *Need for specific toxicological data (reproductive and developmental toxicity)*
  - *Risks associated with variations in susceptibility in populations*
  - *Evaluation of intake estimations*
- **Necessity for post-market monitoring?**

Uncertainty Analysis is a Key Element in the Environmental Risk Assessment of GMOs

- Soundness of predictions on stability of introduced and expressed traits under representative environmental conditions
- Extrapolation of data from environmental laboratory studies to complex ecosystems
- Predictability of potential adverse environmental effects in the long term

Assumptions in the E.R.A. of GMOs

- Most crop plants are annuals, some biennials and few perennials. They are not generally species in natural ecosystems.
- Most crops have been developed to grow in managed environments and there is little competition to weeds
- Crossable wild plants are highly evolved and adapted to certain niches. They are variable with a constant flux in populations and genotypes (e.g. in Genus *Beta* and *Brassica*).
- Introduction of a novel gene is unlikely to have a similar effect on all populations in all niches.

Long Term Effect(s) Assessment

- Long term food feed safety
- Long term environmental safety

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
- Sentinel/Reassurance study

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 Pilot study of 40 chemicals:
  - **70% of the studies indicated all toxicological findings in the 2 year rodent test were also seen in 3 month subchronic tests (non-tumour findings) (British Toxicology Society, 1994).**
  - **Majority of *new* findings were associated with histopathological changes in liver and/or kidney, which are commonly subject of acute toxic injury**
- 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:90 days studies are sufficient for identification of toxicological effects (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

Long term E.R.A. of GMO's: Worst Case Scenarios

- Cultivation of Bt maize (MON810) three years continuously at the same field addressing Bt protein accumulation and long-term effects on non-target organisms:
  - **no effect detectable e.g. for the most Cry1Ab sensitive lepidoperan species known (e.g. *Plutella xylostella*) feeding on weed leaves inside the Bt maize plots (to be published)**

Possible Long Term Adverse Effects of GM Plants: Monitoring

- 6-year-field evaluation of Bt maize in Spain (Event 176,Cry1Ab) (Matilde Eizaguirre et al., Transgenic Research (2006) 15:1–12
- Scope of the study: the potential of the corn borers *Sesamia nonagrioides* and *Ostrinia nubilalis* to evolve resistance to Bt and effects on non-target organisms.
- Resistance to Bt in target corn borers in Spain has *not* developed as of 2005, after 7 years of growing Bt maize
- Transgenic maize did not have a negative impact on non-target pests: more aphids and leafhoppers but similar numbers of cutworms and wireworms in Bt versus non-Bt fields;
- No difference in the numbers of the most relevant predators in fields containing transgenic or no transgenic maize

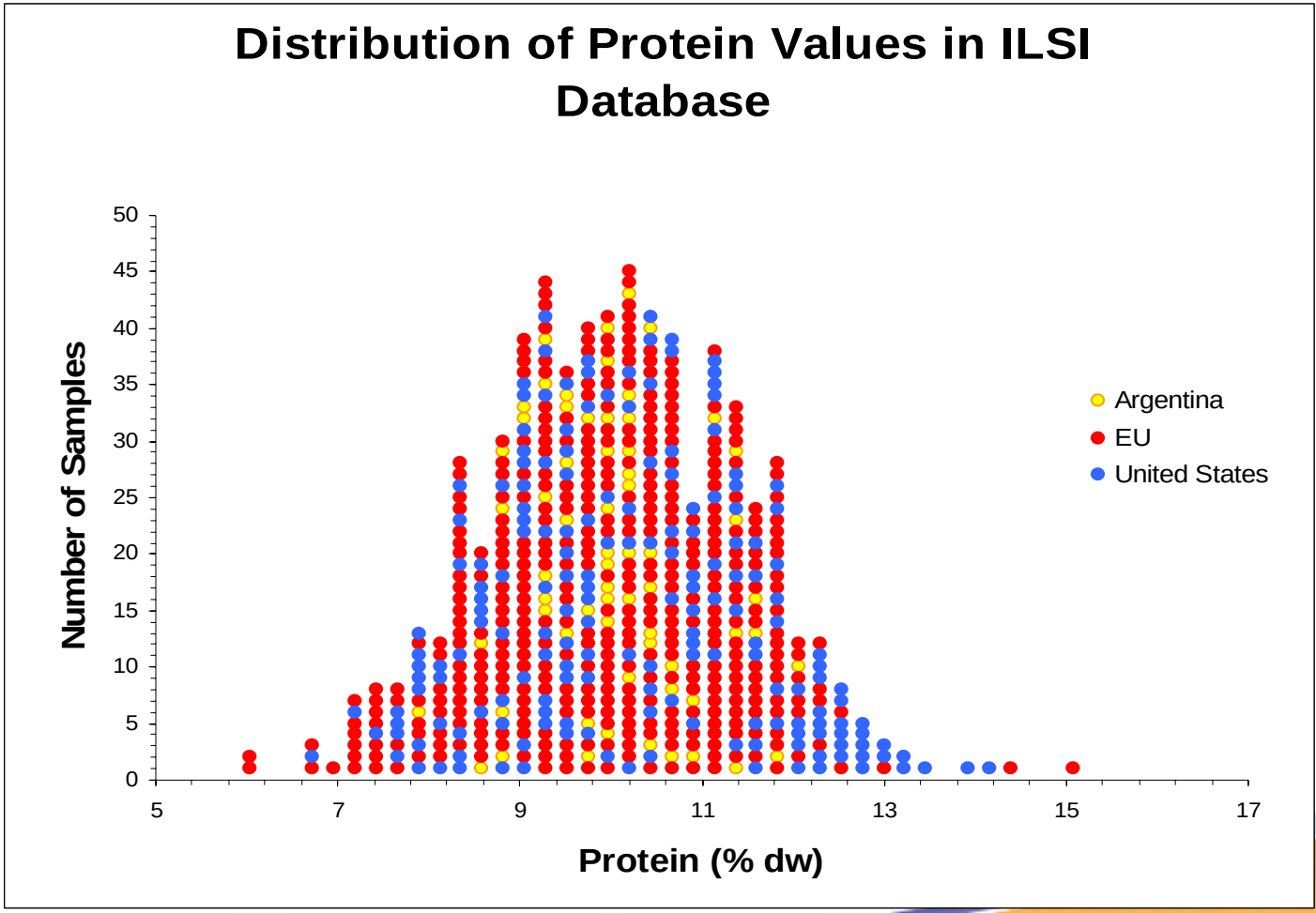
COMPOSITIONAL ANALYSIS

- 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



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, but 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

GMO Panel Evaluation

- All t-test results provided by the applicant were analysed that compare GM maize with its non-GM counterpart
- All statistically significant differences were considered and the biological relevance of these differences taking the biological variation into account
- Internationally accepted approach taken when analyzing results of toxicological studies
- No relevant differences between the GM and non-GM maize were noted which could have an adverse health impact on humans or animals

Interaction with national Expert Body

- Consultation with the CGB
- Second Opinion by two independent consulting veterinary pathologists on kidney lesions:
  - **Spontaneous kidney disease, progressive chronic nephropathy, occurring in most rat strains**
  - **All microscopic changes wer of minimal or mild severity and not treatment related**

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*)

CONCLUSIONS

- Internationally Agreed and Robust Framework for Risk Assessment of GM Plants and derived Food/feed
- GMO Panel Guidance Document provides guidance and refers to existing protocols
- Case-by case approach
- All available evidence is taken into account during risk assessment
- Uncertainty analysis and evaluation of long term effects are key elements in the risk assessment