Scientific perspectives on regulating the consumer safety of GM Food

Professor Mike Gasson
Head of Food Safety Science
Institute of Food Research
Norwich, UK.
UK Safety Committee
ACNFP - Advisory Committee
on Novel Foods and Processes

Wide range of scientific expertise
Genetic Modification
Nutrition
Microbiology
Toxicology
Food Technology
Consumer Representative
Ethicist
EC NOVEL FOODS REGULATION 258/97

EU-wide pre-market approval system for novel foods and food ingredients including those containing or derived from genetically modified organisms.
SAFETY ASSESSMENT STRATEGY

- Integrated and step wise
- Case by case
- Uses decision trees
- Uses a series of structured questions
LIMITATIONS OF CONVENTIONAL TOXICOLOGICAL TESTING FOR WHOLE FOODS

- Complexity makes the interpretation of any observed effect difficult.
- Bulk limits the quantity that can be fed to experimental animals making it impossible to set acceptable safe intake levels.
LIMITATIONS OF CONVENTIONAL TOXICOLOGICAL TESTING FOR WHOLE FOODS

- Whole foods contribute to nutrition making it impossible to differentiate between any nutritional or toxic effects.
- These limitations were experimentally proven during the safety evaluation of food irradiation and mycoprotein (Quorn).
LIMITATIONS OF CONVENTIONAL TOXICOLOGICAL TESTING FOR WHOLE FOODS

➢ The sacrifice of experimental animals where useful data are unlikely to be obtained cannot be justified.
SUBSTANTIAL EQUIVALENCE

FAO/WHO 1991 Geneva
OECD 1993 Paris
FAO/WHO 1996 Rome

FAO – Food and Agriculture Organisation of the United Nations.
OECD – Organisation for Economic Co-operation and Development.
WHO – World Health Organisation.
SUBSTANTIAL EQUIVALENCE

- Recognises the fact that GM derivatives are based on food materials with a history of safe consumption.

- Aims to establish that a GM derivative is as safe as its conventional counterpart.

- It is not intended to establish absolute safety.
SUBSTANTIAL EQUIVALENCE

➢ Uses a comparative approach designed to reveal intended and unintended differences between a GM derivative and its conventional counterpart. These differences become a focus for further safety evaluation.

➢ Agronomic, genetic and chemical aspects are compared with a special focus on known toxins, allergens and antinutrients.
SUBSTANTIAL EQUIVALENCE

- It is not a safety evaluation in itself and it does not identify hazard.
- Acts to structure safety evaluation relative to a conventional counterpart.
SAFETY ASSESSMENT OF EXPRESSED TRAITS

- Possible toxicity.
- Possible physiological effect.
- Possible allergenicity.
ASSESSMENT OF ALLERGENICITY

- Allergens are heat resistant.
- Allergens resist digestion by GI tract enzymes.
- Known allergens can be recognised on the basis of their amino acid sequence, facilitating the use of protein database searching.
GM PLANT DEVELOPMENT

First generation
Improved agronomic characteristics

Second generation
Improved functional characteristics
SECOND GENERATION GM PLANTS

- Improved seed storage proteins.
- Improved starch content (waxy starch and novel carbohydrate).
- Improved oil quality.
- Fortify micronutrients and antioxidants.
- Remove allergens.
SAFETY ASSESSMENT

Unintended secondary effects due to disruption of host DNA and gene expression or perturbation of metabolism.

Involves assessment of:

- Critical macro- and micro-nutrients.
- Antinutrients.
- Toxicants.
- Allergens.
- Physiologically active substances.
POSSIBLE IMPROVEMENT OF SUBSTANTIAL EQUIVALENCE

- Present evaluation is based on a selected sample of composition parameters.

- Molecular profiling may facilitate a more complete, holistic comparative analysis.
  
  DNA microarrays
  Proteomics
  Metabolic profiling
Metabolic Profiling

- Chemical fingerprints
- Fractionation by solvent extraction
- NMR or GC-MS
Molecular Profiling

• The context of data interpretation is vital

• Gene expression is in a state of flux
  • Differences are normal
Molecular Profiling

GM foods need to be evaluated against a background that accommodates the range of variation due to conventional breeding and normal flux in gene expression and chemical composition.
SAFETY ASSESSMENT

- The transformation process.
- Characterisation of the introduced DNA.
- Stability.
- Antibiotic Resistance Marker Genes.
- Potential for Gene Transfer.
TRANSFORMATION TECHNIQUES

- *Agrobacterium* binary vector
- Plant cell protoplasts
- Microparticle bombardment
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- *Agrobacterium* binary vector
- Plant cell protoplasts
- Microparticle bombardment
Monsanto GM soya

• Recent more detailed molecular analysis revealed additional DNA insert.

• Microparticle bombardment is associated with extensive DNA rearrangement that contributes unintended effects.
• Host range extension of *Agrobacterium* binary vector system will reduce dependence on microparticle bombardment.

• Some companies have a policy of using *Agrobacterium* in place of bombardment.
Safety evaluation of \textit{nptII} gene as a plant marker

Comprehensive argument put forward by Calgene accepted by US FDA and others.

Calgene Inc. (1990)
FDA Docket Number 90A-00416
Safety of *nptII* gene

- Limited importance of kanamycin and neomycin in medicine.
- Antibiotic resistance already widespread thus gene transfer from GM food of no practical consequence.
- Transfer event unlikely to take place.
Avoiding the use of antibiotic resistance marker genes

- Separate integration of antibiotic Resistance marker and trait gene(s) followed by Mendelian segregation.

- By chance in GM soya (biolistic).

- Designed Agrobacterium binary vector

Avoiding the use of antibiotic resistance marker genes

- Removal of marker genes following primary transformation and transgene selection.

- Exploitation of site specific recombination e.g. the cre / lox system.

Alternative selection marker genes

Norvatis ‘Positech’ system

Selection for growth on mannose

Phosphomannose isomerase gene
Antibiotic resistance genes with retained bacterial promoter.

*bla* - ampicillin
*aad* - spectinomycin, streptomycin
*nptIII* - kanamycin, neomycin, amikacin

Confer resistance to antibiotics with greater use in clinical medicine.
Present in plant transformants from both biolistic and *Agrobacterium* transformation.
DNA DEGRADATION

- DNA may remain available for transformation in the oral cavity.
- DNA is degraded very rapidly further down the GI tract.
Plant pathogenic *Erwinia chrysanthemi* and transgenic tomato carrying pBR322.

Plant to bacterium transfer was not detected.

*In vitro* analysis estimated transfer potential to be below $5.8 \times 10^{-14}$ in an experiment with 0.9g of potato and $6.4 \times 10^8$ bacteria.

Naturally competent *Acinetobacter* and a marker rescue strategy.

Plant selection marker derived from *nptII*.

Recipient bacteria carried homologue of *nptII* under bacterial promoter control.
Homologous recombination restored an active \textit{nptII} gene allowing recovery of kanamycin resistant transformants at a frequency of 0.9 x 10^{-4} per \textit{nptII} gene without the need for autonomous replication. In the absence of homology transformation was below the 1.3 x 10^{-13} detection limit.
SAFETY ASSESSMENT OF CONVENTIONALLY BRED PLANTS

- Conventional plant breeding can involve treatments such as mutagenesis or induced polyploidy through colchicines.

- These treatments are more likely to cause unintended changes in gene expression than GM technology.
SAFETY ASSESSMENT OF CONVENTIONALLY BRED PLANTS

- Introduction of new varieties of existing crop plants rarely results in adverse effects in humans.

- Tests are undertaken for some known components of safety relevance e.g. alkaloids in potato and cucurbiticin in squash and zucchini.