

Fachtagung zum Diskurs Grüne Gentechnik des BMVEL

”Was ist Sache in der Grünen Gentechnik”

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***Food Safety Assessment Strategies
On Genetically Modified Food Crops***

By

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EU Legislation GM Organisms

- Directive 90/219
contained use of GMOs, such as microorganisms in industry
amended by dir. 98/81: self cloned microorganisms exempted
- Directive 2001/18
field testing, environmental- and market release, animal feed
applications
part A: general provisions, part B: field testing and
environmental release, part C: market release
- Novel Food Regulation 258/97
market release of novel foods, including GMOs

International Food Safety Strategies for Foods Derived from Modern Biotechnology

- International Food Biotechnology Council (1990)
- 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
- FAO/WHO Task Force on Foods Derived from Biotechnology, 1999-present
- EU, 1996-present
- ILSI, 1996

***Food Safety Assessment Strategies
On Genetically Modified Food Crops:
Collecting Information***

- Parent crop
- Donor and transformation process(es)
- Characterization of introduced protein(s) and/or secondary metabolite(s)
- Information on the GM crop/food including the new inherent gene product(s) and eventual unintended change(s)

*Food Safety Assessment Strategies
On Genetically Modified Food Crops:
1. Parent Crop*

- Phenotypic/agronomic performance
- Geographical distribution
- History of safe use
- Compositional analysis

Concept of Substantial Equivalence

OECD, 1993

- Existing traditional foods are considered to be safe, through their long history of use
- Traditional foods can serve as a basis for the safety assessment of GM foods, since most of these foods are obtained from them (concept of *Substantial Equivalence*)

Natural toxicants in selected crop plants I (Data compiled from Holm et al. 1998)

Cassava	Linamarin	Tubers, total cyanogenic glycosides content (preliminary limit 10 mg/kg JECFA)	*
	Lotaustralin		
	Proteinase inhibitors	Tubers	
Potato	α -chaconine	Tubers, Total glycoalkaloid content (limit at 200 mg/kg in old varieties and 100 mg/kg in new varieties Nordic Working Group)	*
	α -solanine		
Rapeseed	Glucosinolates	9 different compounds	
	Erucic acid	(limit 5% for food consumption in DK)	*
	Saponins		
	S-methyl-L-cysteine sulfoxide	(toxic amino acid)	
Chicory	Lectins		
	Lactucin	Leaves, root	
	Lactucopicrin	Leaves, root	
	8-deoxylactucin	Leaves, root	

*Toxic components in plants with recommended highest level for food consumption

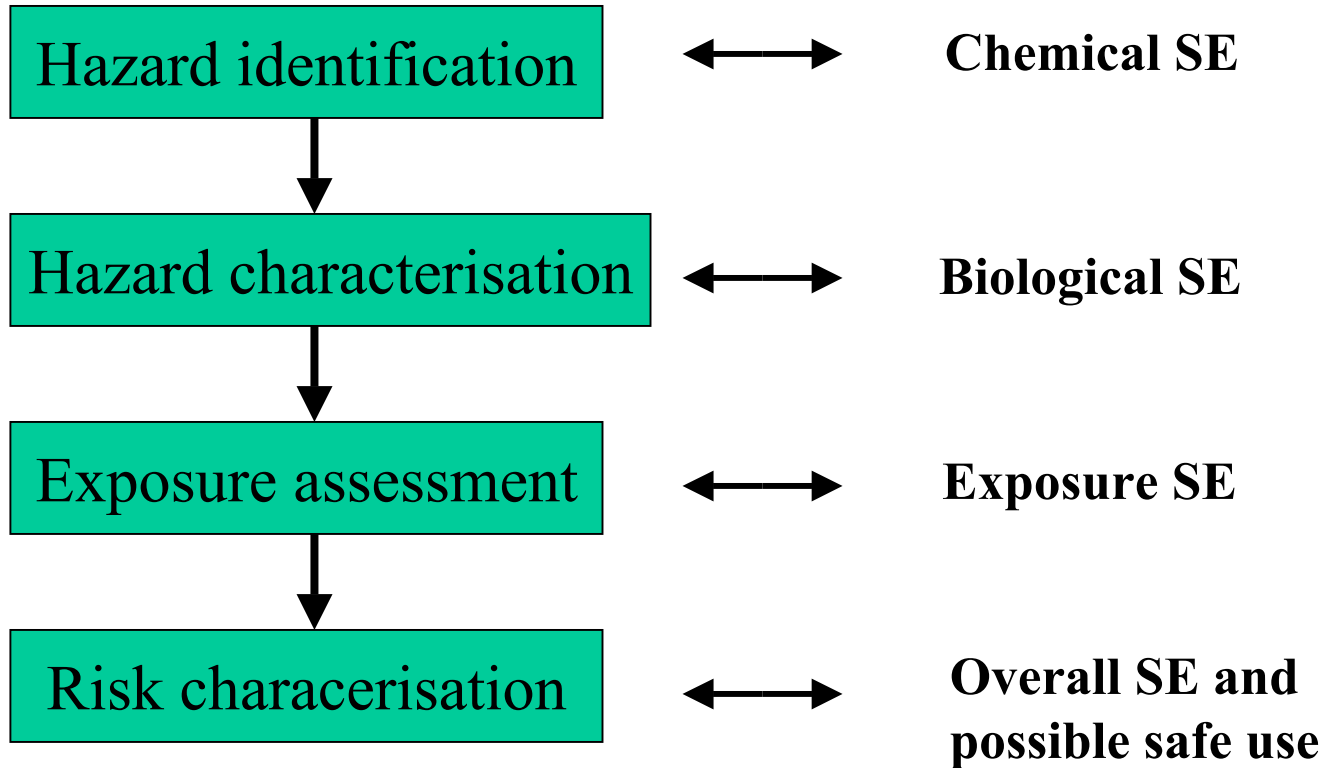
Natural toxicants in selected crop plants II (Data compiled from Holm et al. 1998)

Crop plant	Toxic constituent	Comments:	*
Wheat	Dhurrin	Young vegetative plant parts	
	Lectins		
	Proteinase inhibitors	Grain	
Rice	Trypsin inhibitor	Grain	
Maize	Cyanogenic glycoside	Young vegetative plant parts	
	Trypsin inhibitor	Grain, Cob	
Barley	Epiheterodendrin	Young vegetative plant parts	
	Trypsin inhibitor	Grain	
Soybean	Saponins		
	Lectins		
	Coumestrol		
	Daidzein	Seeds	
	Genistein	Seeds	
	Proteinase inhibitors		
Sorghum	Dhurrin	Cyanogenic glycosides in unripe plants	

*The Use of **Substantial Equivalence**
In Risk Assessment of Genetically Modified Food Plants*

Risk assessment

Substantial Equivalence (SE)



***Food Safety Assessment Strategies
On Genetically Modified Food Crops:
2. Donor and Transformation Process***

- Identity of gene(s)
- Source(s) of gene(s)
- History of use/functionality of insert(s)
- Gene construct(s), including flanking sequences
- Insertion process
- rDNA characterization
- Number of insertion(s) and the(ir) site(s)

*Food Safety Assessment Strategies
On Genetically Modified Food Crops:
3. Characterization of Introduced Gene Products*

- Chemical identity of protein(s) and/or secondary metabolite(s)
- Molecular characterization, including tertiary structures
- Mode(s) of action, specificities
- Toxicological information on the trait(s)
- Information on allergenicity

Five examples of GM plants and toxicological tests performed.

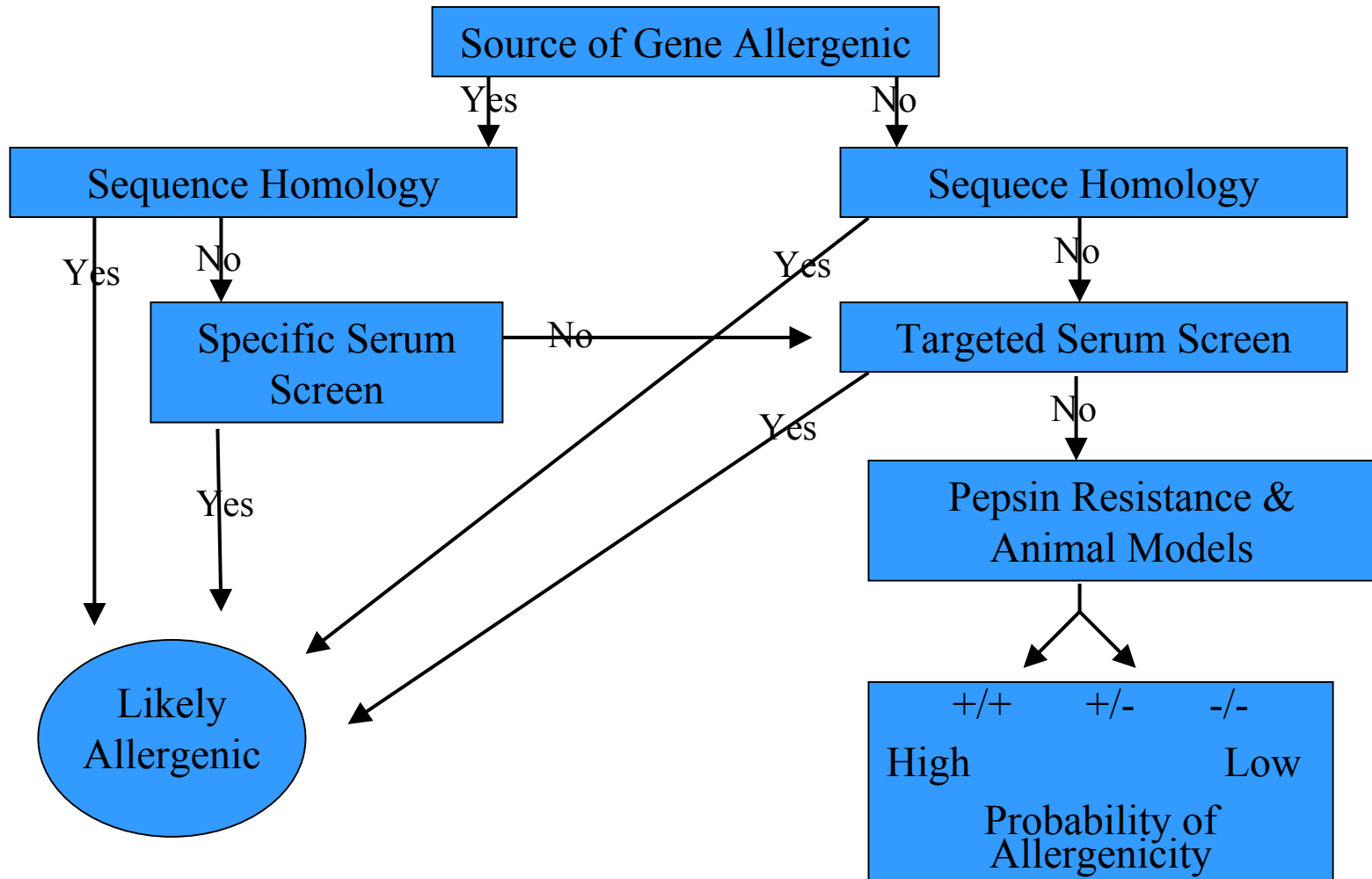
Plant	Gene	Protein test in animal. One dose unless specified	Source B=Bacteria P= Plant	Digestibility Of protein	Animal test of plant material	Search in database for homologous
Maize Bt11	PAT	Animal: mouse Dose: 5050 mg/kg Days: 14	B			X
	CryIA(b)	Animal: mouse Dose: 4000 mg/kg Days: 14	B (E.coli)			X
Maize 176	CryIA(b)	Animal: mouse Dose: 5050 mg/kg Days: 14	P	X	38 days broilers	X
	CryIA(b)	Animal: mouse Dose: 5050 mg/kg Days: 14	B (Bacillus)	X		
	PAT	Animal: mouse Dose: 5050 mg/kg Days: 14	B	X		
Maize GA21	MEPSPS	Animal: mouse Dose: 50 mg/kg Days: 13	B B	X X	38 days broilers	X
Soya	CP4-EPSPS	Animal: mouse Dose: 572 mg/kg Days: ?	B (E.coli)	X	6 week broiler 4 week cow 4 week rat 10 week catfish 5 day quail	
Bt-Tomato ¹	CryIA(b)	Animal: mouse Dose: 0,45 mg/kg daily Days: 28 Animal: rabbit Dose: 0,06 mg/kg Days: 31	B (E.coli)		90 day rat	
²	NPTII	Animal: mouse Dose: 5000 mg/kg Days: 8-9	B (E.coli)			

SAFOTEST 28 days rat study

- Determination of acute toxicity of pure PHA-E lectin in rats
- Determination of 3 dosages of pure PHA-E for the 28 day study
- Spiking of the different dosages of pure PHA-E lectin to parent rice
- Measuring sensitive end points for toxicity in vivo and in vitro
- Identification of the *Lowest-Observed-Adverse-Effect-Level (LOAEL)* for PHA-E

FAO/WHO 2001 Decision Tree

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



Animal Models

- Brown Norway rat, Mouse
- Different routes of administration, oral/ip
- Ranking studies with strong and weak food allergens
- Animal models do not reflect all aspects of IgE-mediated food allergies in humans
- Used for additional assessment

***Food Safety Assessment Strategies
On Genetically Modified Food Crops:
4. Information on the GM crop/food***

- Proximate data and data on key nutrients and key toxicants
- Data on phenotypic and agronomic performance
- Genomic, proteomic and metabolomic data
- Data on biological safety
- Nutritional data in animals/humans
- Intake data/expected usage

Recommended minimum lists for compositional analysis of specific crop plants I (Nordic Council 1998).

	Cotton	Oilseed rape	Maize	Soybean	Tomato
<i>Proximate analysis</i>					
Protein	X	X	X	X	X
Fat	X	X	X	X	X
Ash	X	X	X	X	X
Moisture	X	X	X	X	X
Carbohydrates					
Fibre	X	X	X	X	X
Starch			X		
Fatty acid profile	8^a	8^a	5^a	5^a	
Amino acid profile			16^b	16^b	

Recommended minimum lists for compositional analysis of specific crop plants II (Nordic Council 1998).

Key toxicants/antinutrients/ allergenic substances	Cotton	Oilseed rape	Maize	Soybean	Tomato
Total gossypol	x				
Free gossypol	x				
Sterculic acid	x				
Malvalic acid	x				
Dihydrosterculic acid	x				
Glucosinolates		4 ^c			
Erucic acid (C22:1)		x			
Phytic acid				x	
Soy lectin				x	
Glycinin				x	
β-conglycinin				x	
Trypsin inhibitor (Kunitz)				x	
Diadzein				x	
Genistein				x	
Genistin				x	
α-tomatine					x

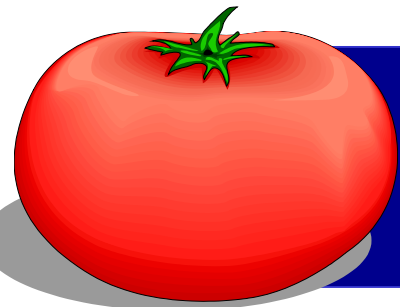
Recommended minimum lists for compositional analysis of specific crop plants III (Nordic Council 1998).

Key nutrients (vitamins, minerals)	Cotton	Oilseed rape	Maize	Soybean	Tomato
Sodium					x
Potassium					x
Calcium					x
Magnesium					x
Phosphorus					x
Iron					x
Vitamin A					x
Vitamin B ₁			x		x
Vitamin B ₂			x		x
Vitamin B ₆					x
Vitamin C					x
Folic acid			x		x
Tocopherols	x	x	x		

Unintended Effects in GMO's

Random integration of transgenes

- ⇒ insertional mutagenesis
- ⇒ disruption of endogenous gene functions
 - gene activation / inactivation
 - production of new proteins
- ⇒ changes in
 - enzymes
 - metabolites
 - phenotype



Analysis of (Un)Intended Effects

- Plant
- Tissue
- DNA
- mRNA
- Proteins
- **Metabolites**



- Phenotypic alterations
- Phenotypic alterations
- DNA analysis
- Genomics
- Proteomics
- **Metabolomics**

Long-Term Effects

- A 90-days study is the minimum requirement to test the safety of repeated consumption of a food
- Additional studies may be considered on a case-by-case basis (proliferative changes observed in a 90-days study)
- Highest dose levels should not cause nutritional imbalance and lowest levels should be comparable to anticipated human intake

SAFOTEST 90 day rat study

- Control group: Non-GM rice, 60% in the diet
- GM rice with PHA-E lectin inherent, 60%
- GM rice with PHA-E lectin inherent, 60%, spiked with pure PHA-E lectin (LOAEL)

Risk Assessment Process for GM Crops

