Animal Toxicity and Livestock Feeding Studies- Need and Approaches

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Safety assessment of GM food

Conventional food

- Plants are part of human food/animal feed
- History of substantial human/animal exposure
- GRAS

GM food

- In contrast GM foods has the genetic material derived from organisms that have not previously been present in the human diet to any great extent

- The corresponding gene products are considered to be novel with respect to human consumption

Focus of GM feed safety assessment

Intended effect Vs Unintended effect

Intended effect of genetic modification

- Insertion of target gene; Expression products of target gene;
- Assess the safety of the expressed protein which is not part of the conventional plant

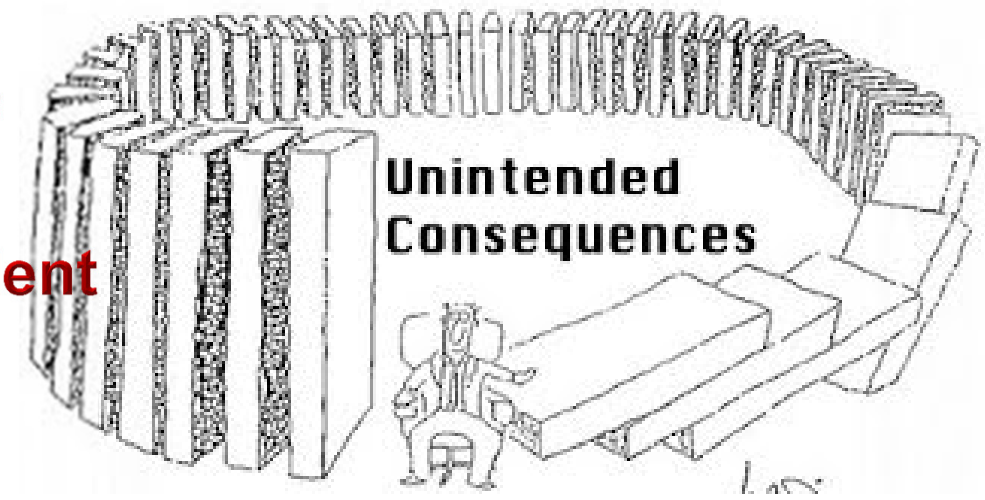
Focus of GM feed safety assessment

Unintended effect

"consistent differences between the GM plant and its appropriate control lines, which go beyond the primary expected effect(s) of introducing the target gene(s)"

- **Genetic re-arrangements or disruptions of metabolic pathways in the recipient plant through gene insertion.**

- **alterations in metabolic pathways, increased levels of endogenous toxins or allergens, or lower levels of essential nutrients, or expression of previously silent genes encoding toxins or allergens.**



Animal toxicity and livestock feeding studies

To find out whether the GM food is

'As Safe as' and 'As nutritious as'

its non-GM counter part (comparator)

The comparator provides the baseline for the food/feed safety assessment

Tests suggested by RCGM

- Acute Oral Safety Limit Study In Rats and Mice
- Sub-chronic Feeding Study In Rodents
- Protein Thermal Stability
- Pepsin Digestibility Assay
- Livestock Feeding Study

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Acute Oral Safety Limit Study In Rats and Mice

- Assessing the potential effects of the expression product(s) of the inserted gene(s)
- 14 day single dose acute toxicity study by oral route
- If treatment-related mortality, morbidity or clinical symptoms result, then further study may have to be considered for ascertaining the cause of toxicity

Acute Oral Safety Limit Study In Rats and Mice

- **Limit dose of 2000 mg/kg**
- **The potential human dietary intake of functionally active Cry protein from Bt maize could range from 0.008 to 2 µg/kg body weight/day (Hammond and Koch, 2012).**

Acute Oral Safety Limit Study In Rats and Mice

Test substance	NOAEL[a]	Reference
Cry protein		
Cry1Ab	4000 mg/kg	Betz et al. (2000)

A 70-kg-body weight human adult would need to consume > 900,000 kg of grain in one day to attain the same acute dosage (4000 mg/kg) of Cry1Ab protein given to mice which produced no adverse effects (Hammond and Cockburn, 2008).

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Sub-chronic feeding study in rodents
(90 day feeding study)

- **Performed when compositional equivalence cannot be established**
- **Provides information on the possible health hazards likely to arise from repeated exposure over a prolonged period of time**
- **Provides information on the major toxic effects, including possible target organs, and the possibility of cumulative effects.**
- **Assessment of potential to cause neurotoxic, immunological or reproductive organ effects, which may warrant further in-depth investigation.**
- **Goal is to determine if unintended differences occurred during production of a GM resulting in adverse effects**

Sub-chronic feeding study in rodents

Test article	Dose/dietary level	Study type and test animal	Reference
Bt crop			
Bt tomato	10% in diet[a]	90-day rat	Noteborn et al. (1995)
Bt/HT[b] maize(ECB[c]/RR[d])	11/33% in diet[a]	90-day rat	EFSA (2005d)
Bt/HT maize(CRW[e]/RR)	11/33% in diet[a]	90-day rat	EFSA (2005e)
Bt/HT maize(ECB/CRW/RR)	11/33% in diet[a]	90-day rat	EFSA (2005b)
Bt maize(ECB/CRW)	11/33% in diet[a]	90-day rat	EFSA (2005a)
Bt maize (ECB)	11/33% in diet[a]	90-day rat	Hammond et al. (2006b)
Bt maize (CRW)	11/33% in diet[a]	90-day rat	Hammond et al. (2006a)
Bt maize (ECB)	11/13% in diet[a]	90-day rat	MacKenzie et al. (2007)
Bt cotton	10% in diet[a]	90-day rat	Dryzga et al. (2007)
Bt rice	60% in diet[a]	90-day rat	Schrøder et al. (2007)
Bt/HT maize(CRW/Gluf[f])	35% in diet[a]	90-day rat	Malley et al. (2007)
Bt/HT maize(CRW/RR)	11/33% in diet[a]	90-day rat	Healy et al. (2008)
Bt maize (CRW)	50/70% in diet[a]	90-day rat	He et al. (2008)
Bt/HT maize(ECB/CRW)	34% in diet[a]	90-day rat	Appenzeller et al. (2009)
Bt rice (Cry1Ab/Cry1Ac)	60% in diet[a]	90-day rat	Wang et al. (2013)
Bt rice (Cry1Ac)	73–82% in diet[a]	78-week rat	Zhang et al. (2014)
Multigenerational studies			
Bt maize (ECB)	68% in diet[a]	5-generation rat	Haryu et al. (2009)
Bt maize (ECB)	20% in diet[a]	3-generation rat reproduction	Kiliç and Akay (2008)

[a]*Percent (w/w) maize, rice, or cottonseed meal added to the diet.*
[b]*HT, herbicide tolerant.*
[c]*ECB, European corn borer.*
[d]*RR, Roundup Ready® (tolerant to glyphosate herbicide).*
[e]*CRW, corn rootworm.*
[f]*Gluf, glufosinate (tolerant to glufosinate herbicide).*

Sub-chronic feeding study in rodents

Chronic Dietary Exposure Assessment (Hammond and Cockburn, 2008)

- **The average corn consumption in the UK for adults is ~16 g corn/ person/day 70 kg body wt/person = 0.23 g/kg**
- **The average adult dietary intake of Cry1Ab protein would be: 0.23 g/kg/day 0.3 mg/g corn = 0.07 mg/kg for an adult (0.00007 mg/kg)**
- **The average rat dietary intake of Cry1Ab protein in a 90-day feeding study is 25 g corn/kg BW 0.3 mg/g corn = 7.5 mg/kg**
- **The margin of safety for chronic dietary exposure to Cry1Ab protein is 7.5 mg/kg divided by 0.07 mg/kg = 107 X**

The 90 day sub-chronic study reflects eating >100x human dose of GM whole grain (1.6 kg/day) for 90 continuous days

Sub-chronic feeding study in rodents

EFSA GMO panel, 2008

- **Feeding trial results of many GM plants (Maize, potatoes, rice, soybeans and tomatoes) on mice or rats**
- **Traits for herbicide tolerance and/or insect resistance**
- **Majority of these experiments did not indicate clinical effects or histo-pathological abnormalities in organs or tissues of exposed animal**

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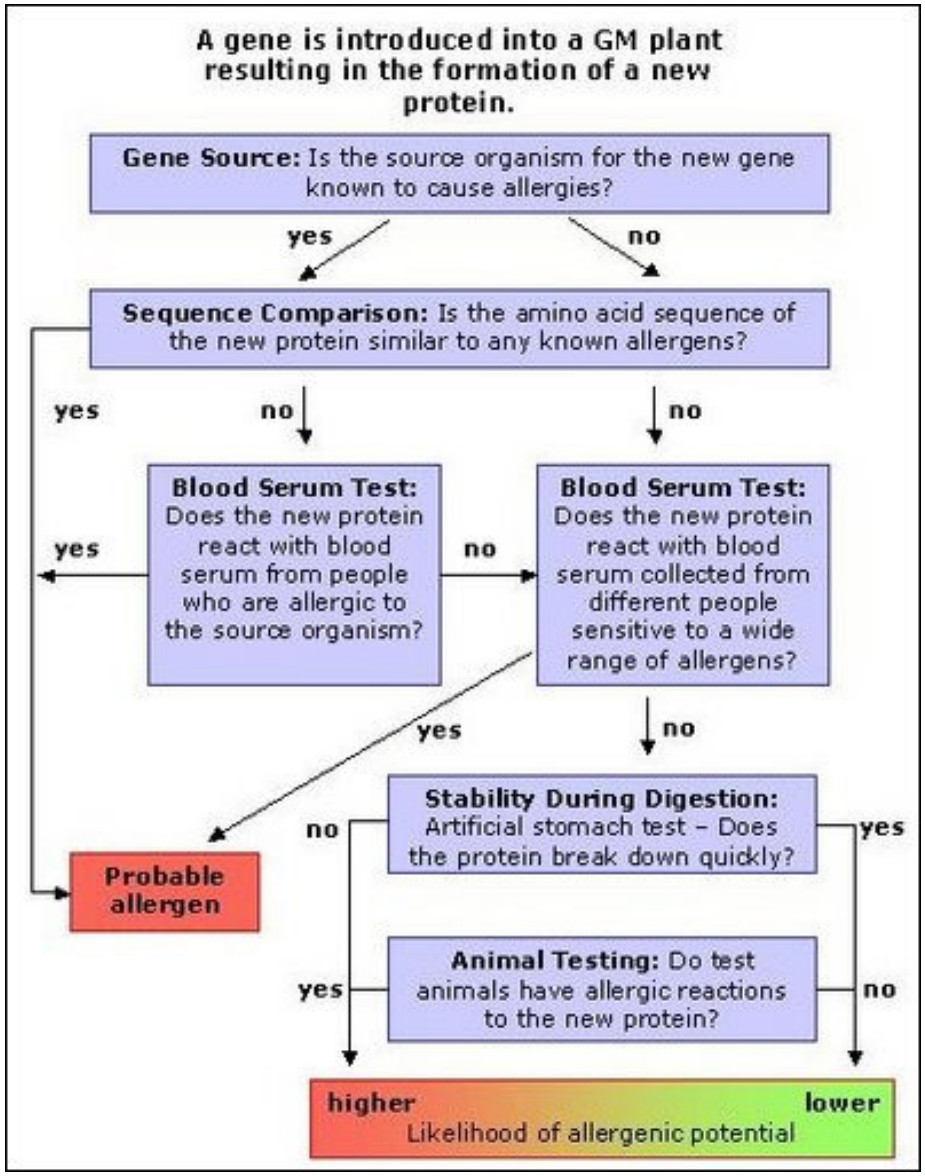
Safety Assessment for Allergenicity

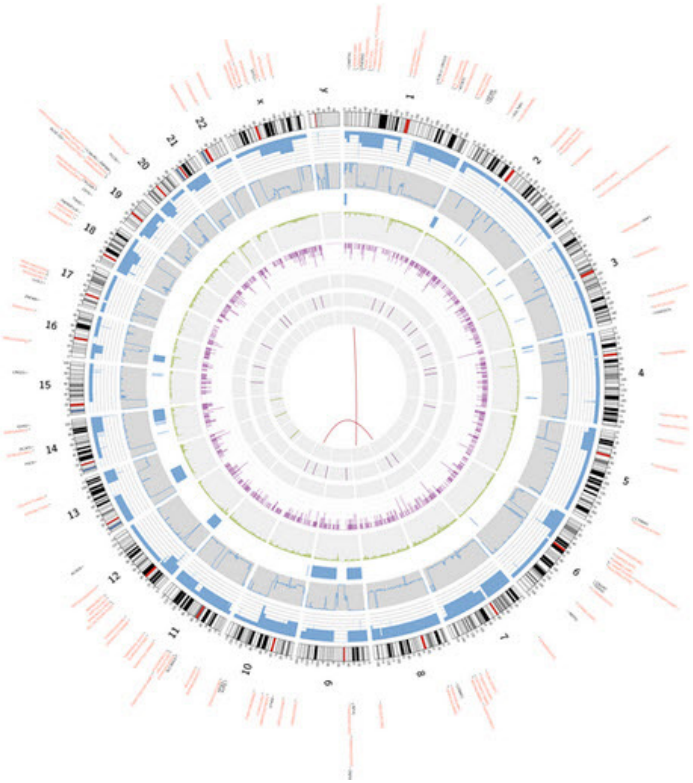
(Weight of evidence)



Looking ahead ….

- How do we design the acute toxicity studies for the GE plants with no protein product (for eg. RNAi based plants: potential for off-target regulation in mammals)?

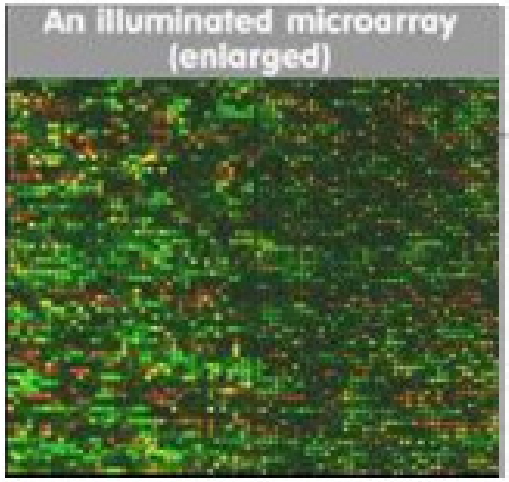
- Can NGS be used to sequence the complete genome of GE plant? Can it be deduced with parental line?



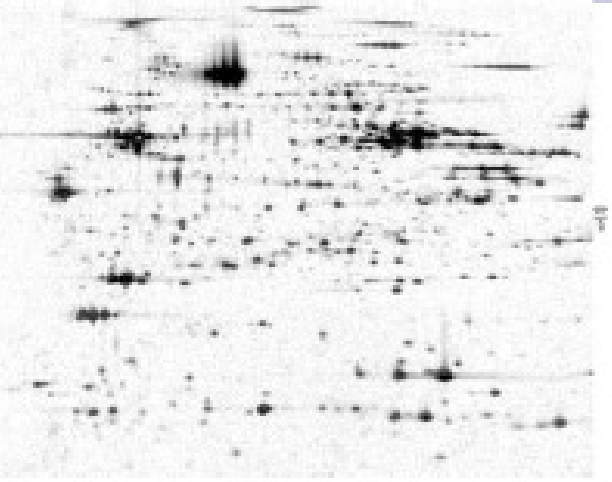
Looking ahead ….

Can omics approach supplement compositional analysis?

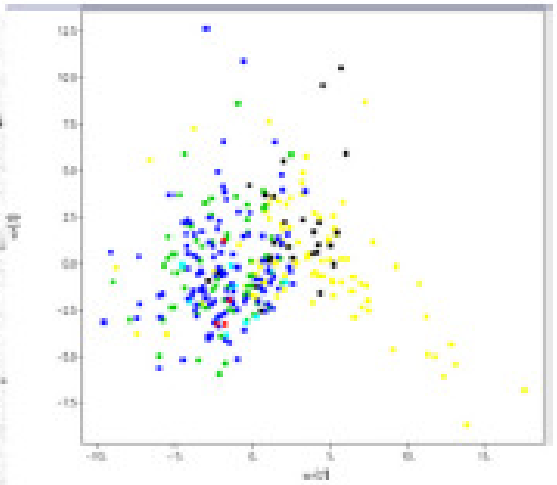
Transcriptomics



Proteomics



Metabolomics



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Nutritional equivalence

- **Nutrition and nutritional value of food and feed are major determinants of human and animal well-being**
- **Nutritional quality and equivalence of GM food should be ensured**
- **It is important to demonstrate that a food derived from GM plants is not only as safe but also has the same nutritional values/characteristics as the conventional comparator**

Compositional analysis

- **Compositional analysis is the cornerstone of nutritional assessment**
- **Numerous reports available comparing the composition of GM plants modified for herbicide tolerance and insect resistance to their near isogenic counterparts**
- **Indicate compositional equivalence except for the inserted traits**
- **Even if it is statistically different, well within the ranges of commercial varieties**

Need for animal feeding studies

Compositional analysis does not provide information on

1. Digestibility

2. Bio-availablity

Published feeding studies with food producing animals fed with feedstuffs from GM plants with input traits in comparison with near isogenic plants (summarized by Flachowsky et al., 2005a)

Animal species/ Categories	No of experiments	Nutritional assessment
Ruminants		No significant differences in composition
Dairy cows	23	No significant differences in digestibility of nutrients, animal health, animal performances, composition and quality
Beef cattle	14	of foods of animal origin between feed from near isogenic or GM plants
Others	10	
Pigs	21	
Poultry		
Laying hens	3	
Broilers	28	
Others (fish, rabbits, *etc.*)	5	

Animal feeding studies

For plants that have been genetically modified through the insertion of one or more genes the reported studies indicates,

- **Once compositional equivalence has been established then nutritional equivalence can be assumed in poultry, beef cattle, dairy cows, pigs, etc.**

- **Further animal feeding studies are adding little to their nutritional assessment**

Recommendations on animal numbers to be used in feeding trials

Recommendations from the "Best practices for the conduct of animal studies to evaluate crops genetically modified for input traits (GM plants of the first generation)"

Animal (species/ categories)	Number of animals (assumed coefficient of variation 4–5%)	Duration of experiments	Composition of diets[a]	Measurements
Poultry for meat production	10–12 pens per treatment with 9–12 birds per pen	5 weeks or more	Balanced diets	Feed intake, weight gain, feed conversion
Poultry for egg production	12–15 replicates per treatment with 3–9 layers per pen	18–40 weeks of age, at least three 28-day phases	Balanced diets	Feed intake, egg production, feed conversion, egg quality
Pigs	6–9 replicates per treatment with 4 or more pigs per replicate	Piglets (7–12 kg), 4–6 weeks Growers (15–25 kg), 6–8 weeks	Balanced diets	Feed intake, weight gain, feed conversion, carcass quality
Growing and finishing ruminants	6–10 replicates per treatment with 6 or more cattle per replicate	90–120 days	Balanced diets	Feed intake, gain, feed conversion, carcass data
Lactating cows	12–16 cows per treatment	Latin square: 28 day periods	Balanced diets	Feed intake, milk production and composition
	28 cows per treatment	Randomized block		body weight, body condition score (BCS), cell counts in milk, animal health composition

Extracted from ILSI (2003).

[a] Feed from GM plants should be included in high portions in diets and compared with near isogenic counterparts.

GM crops with increased or modified nutritional characteristics

1. **GM food with increased nutritional precursor (eg. Increased Beta carotene which is a precursor for Vitamin A)**

2. **Increase in the content of nutrients such as amino acids**

3. **Increased digestibility**

Livestock feeding trial should verify the claims of the increased or changed nutritional properties of the GM crops

1. **Bioavailability or conversion of nutrient precursors into nutrients (e.g. b-carotene)**

2. **Digestibility/bioavailability of nutrients (e.g. amino acids, fatty acids, vitamins)**

3. **Efficiency of substances which may improve nutrient digestibility/availability (e.g. enzymes)**

EFSA Recommendations

(a) To provide a nutritional assessment of a GM feed ingredient in which a nutrient precursor such as β-carotene has been increased

Treatment structure	Added supplement/comment
T1 Near isogenic parental line	No supplement
T2 Near isogenic parental line	β-Carotene supplement provides β-carotene comparable with T3
T3 GM line, enhanced β-carotene	No β-carotene supplement needed, β-carotene content is comparable with T2
T4 Commercial varieties	Diet composition comparable to T1 and T2; unsupplemented and supplemented

EFSA Recommendations

nutrient such as an amino acid or fatty acids has been increased.

Treatment structure	Added supplement/comment
T1 Near isogenic parental line	No amino acid supplement
T2 Near isogenic parental line	Amino acid supplement provides balanced diet
T3 GM line: enhanced amino acid content	No amino acid supplement needed. Balanced diet comparable with T2
T4 and other commercial varieties	Diet composition comparable with T1 and T2; unsupplemented and supplemented

EFSA Recommendations

(c) To provide a nutritional assessment of a GM feed ingredient when the digestibility of a specific nutrient such as nitrogen or fibre has been increased.

Treatment structure	Level of feeding
T1 Near isogenic parental line	Fixed
T2 GM line: enhanced digestibility	Fixed
T3 Near isogenic parental line	*Ad libitum*
T4 GM line: enhanced digestibility	*Ad libitum*

EFSA Recommendations

(f) To provide a nutritional assessment when the concentration of an anti-nutritional factor such as phytate is decreased in a GM line.

Treatment structure	Added supplement
T1 Near isogenic parental line	No supplement
T2 Near isogenic parental line	Phosphorus supplement added.
T3 GM line: reduced phytate content	No phosphorus supplement added, but dietary phosphorus content comparable with T2
T4 and other commercial varieties	Diet composition with T1 and T2, unsupplemented and supplemented

Food for thought

- **Is livestock feeding study relevant in plants that have been genetically modified through the insertion of one or more genes for Herbicide tolerance and insect resistance?**
- **Is establishing compositional equivalence sufficient?**
- **What is the appropriate groups for enhanced/changed nutritional traits? case-by-case decision is appropriate**

Thank You