Risk of Gene Transfer from GMOs to Livestock and its Consequences for Health and Nutrition.

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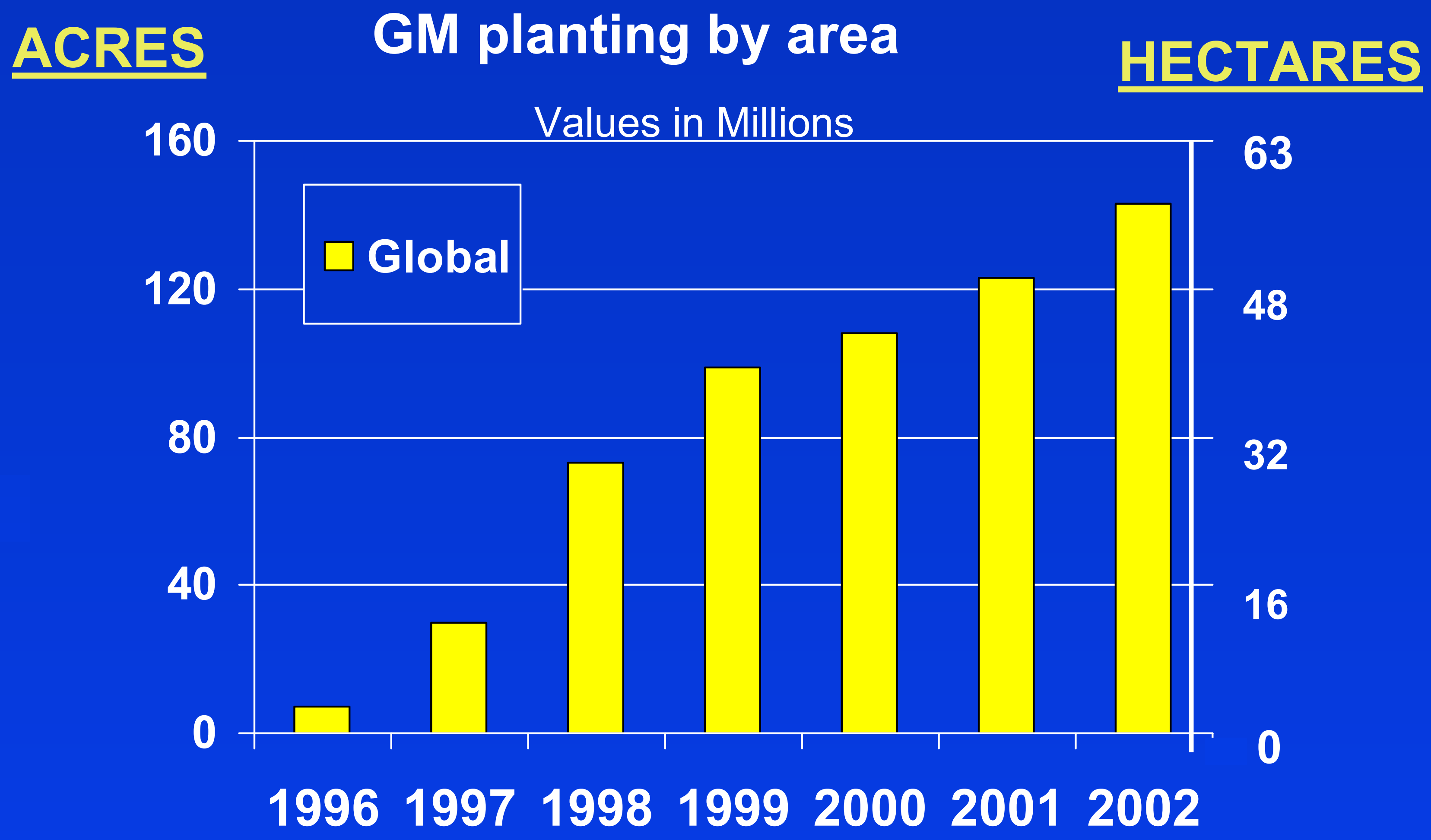
How Much is Grown?



ISAAA 2003

Dominant transgenic crops are:

	Global	GM	% Global
	Millions ha		
Soybean	72	37	51
Maize	140	12	13
Canola	25	3	12
Cotton	34	7	20

Source James

Oilseed Meal Production

	Global	GM	% Global
	Millions t		
Soybean	132	67	51
Canola	18	2	12
Cotton	11	2	20

Source Soy Stats

GM feed ingredients for livestock production

	Soyabeans	
Millions of t imported into EU	2001	2002
USA: Beans	6.1	7.0
Argentina: Meal	7.6	9.5

6 million t of GM soyabeans and 8 million t of GM soyabean meal imported into the EU in 2003

Concerns were expressed about the fate of transgenic DNA

Issue: 1

Would transgenic crops effect animal performance and health?

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Concept of Substantial Equivalence

- This concept is not a safety assessment *per se* but attempts to identify similarities and differences between conventional and GM crops.

- It is based on the idea that existing crops serve as the basis for comparing the properties of a GM crop with an appropriate counterpart considered safe by a long history of safe use.

Substantial Equivalence

- While this concept has been subjected to criticism. It is widely recognized by many national and international organisations as a robust and practical starting point for the safety assessment process

Substantial Equivalence

Compares Conventional and GM crops

- Agronomic and phenotypic characteristics
- Compositional Equivalence
- Nutritional Equivalence

Compositional Equivalence

Roundup Ready Soyabean meal

Composition (% DM)	Control	RR
Dry matter	90.3	91.0
Crude protein	51.5	51.2
Neutral detergent fibre	4.95	4.85
Lysine	3.16	3.09
Meth +Cystine	1.47	1.51

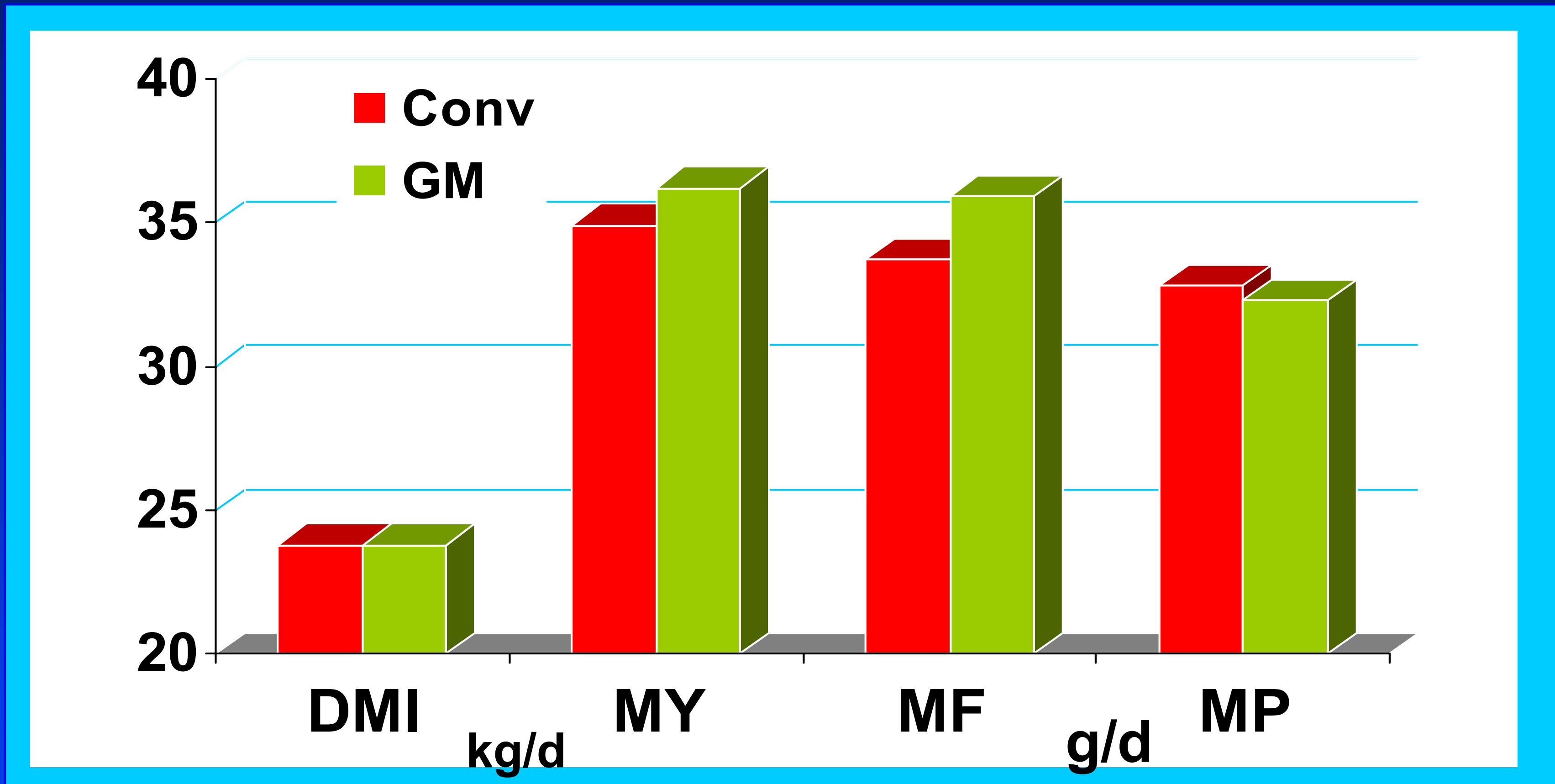
Cromwell et al 2001. Compositionally equivalent in terms of nutritional value.

Compositional Equivalence

The work conducted by Ridley *et al.* (2002), in which over 50 compositional parameters were measured in various plant components from a GM maize crop, a control variety and 15 commercial varieties, grown at different geographical locations over a two year period in both replicated and non-replicated trials and were subjected to statistical analysis to confirm compositional equivalence sets a benchmark for others to follow.

Nutritional Equivalence

Dairy cows: Soybeans



Hammond et al1998

Nutritional Equivalence
Broilers: Bt maize

Broilers: 3 locations	Control	Bt maize	Sig
Final live weight	2621	2693	P<0.01

Piva et al 2001. Improved performance attributed to decrease in Fumonisin B1

Would transgenic crops effect animal performance and health?

- **The answer to the question is:**

There is no evidence that feeding GM crops to livestock will present any more risk to animal health and performance than conventional crops, indeed GM crops may reduce the risk to animal health and improve performance.

Concerns were expressed about the fate of transgenic DNA

Issues: 2

Would tDNA and protein accumulate in meat, milk and eggs derived from animals fed GM feed ingredients?

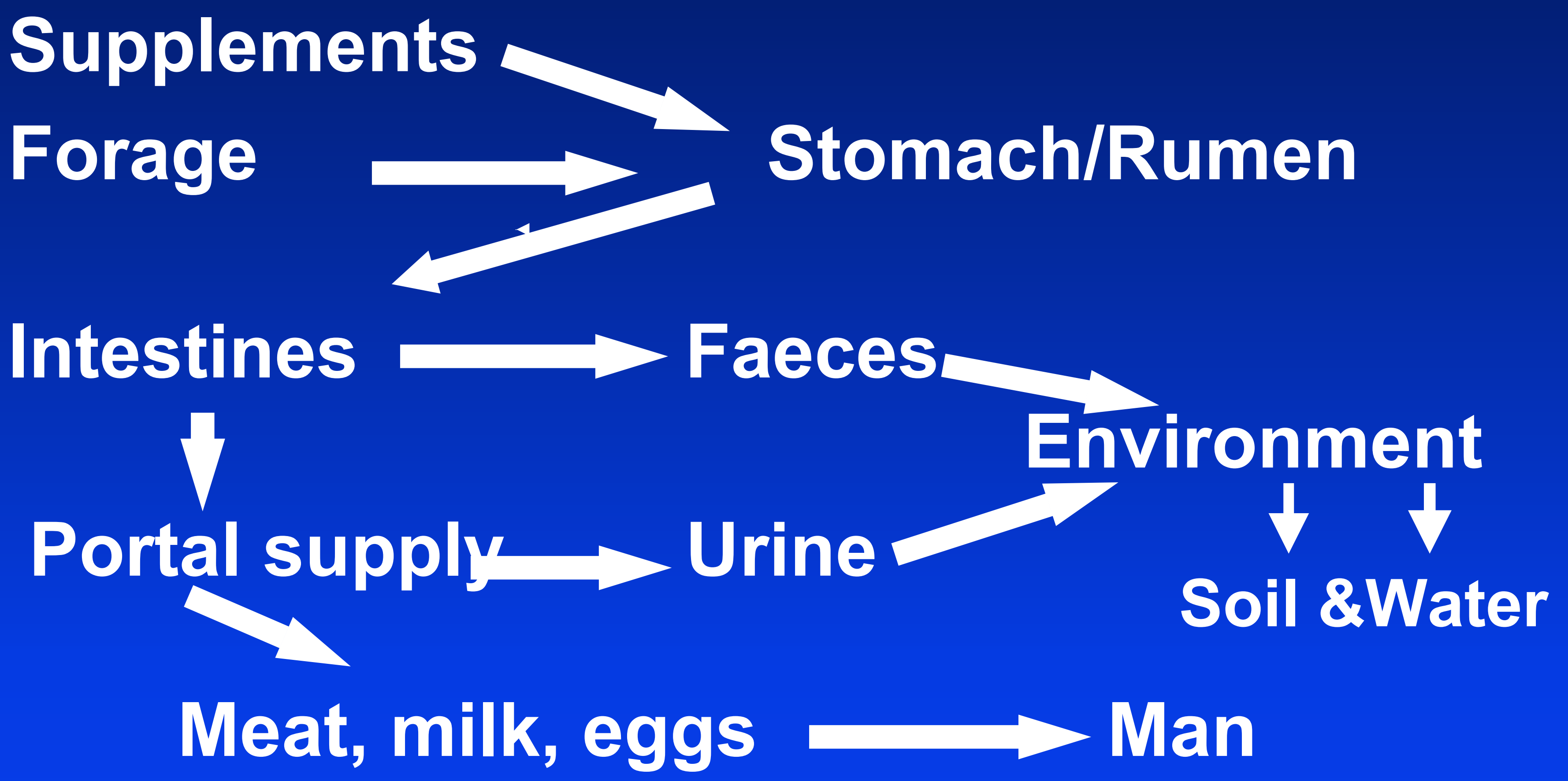
The magnitude of the problem

- Dairy cow consumes 24kg feed DM/d
- Assume 40% as maize silage and 20% maize grain
- Assume all maize to be GM

- Total daily DNA intake; 57.3g
- GM DNA =54µg.
- GM DNA = 0.000094% total DNA

Beever and Kemp

Fate of Ingested DNA

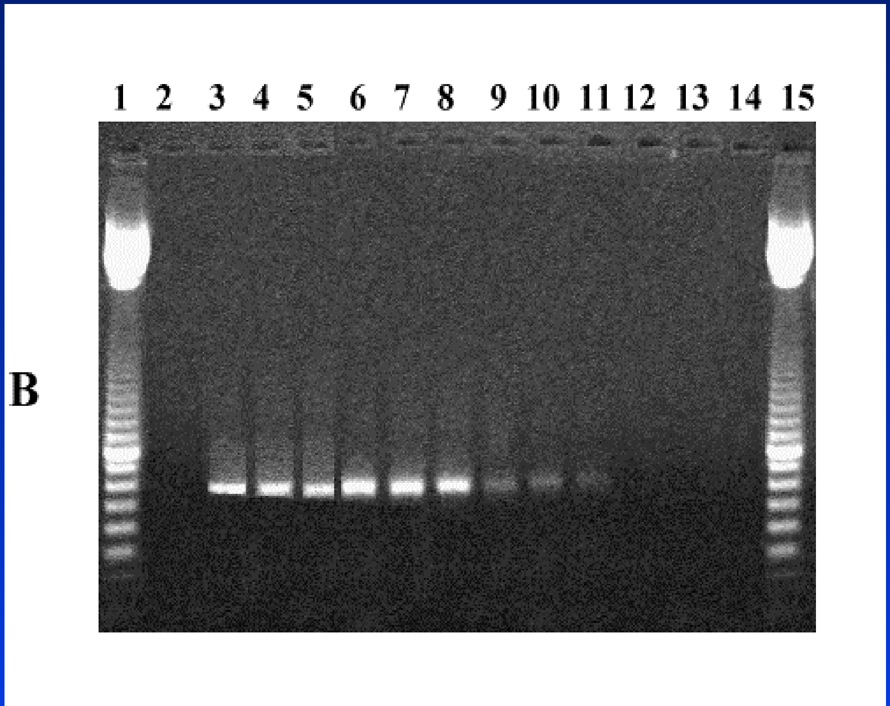


Fate of DNA: Feed processing

- Grinding and milling causes little disruption.
- Mechanical/chemical extraction of oil causes large disruption.
- Dry heat at 90^0C no effect >95^0C and steam caused extensive disruption

Forbes et al Chiter et al

Fate of DNA: Forage Conservation



B. Agarose gel electrophoresis - PCR products from DNA from maize - Rubisco S1 (577 bp fragment)

lane 1 100 bp DNA markers
lane 2 neg. control - no DNA
lane 3 fresh maize leaves
lane 4 dry maize grain
lane 5 maize silage

Chiter, Blair & Forbes

Fate of tDNA: Forage conservation

Ensiling produces a harsh environment that will accelerate DNA degradation.

While silage origin could be confirmed it showed that the ensiling resulted in major fragmentation of tDNA and that the presence of intact, functional genes after an extended time of ensiling was highly unlikely.

Hupfer *et al*.

Fate of DNA

In vivo digestion in ruminants & monogastrics

- **Mouth -** salivary α-amylase, RNAase, DNAase I
- **Rumen -** microbial and physical degradation of feed, microbial nucleases. Microbial nucleic acid synthesis
- **Stomach -** HCl, Pepsins, mucins;
- **Pancreas -** RNAase, DNAase I, DNAase II, lipases, pancreatic proteases, α -amylases
- **Intestines** - Pepsins, DNAase, macrophages, dendritic cells and immune system phagocytes

Rubisco Feed DNA - transfer into cattle and chicken

Einspanier et al. 2001

	cattle	*chicken*
Blood	+	n.d.
Muscle	-	++
Liver	-	+
Spleen	-	+
Kidney	-	+
Milk	(+)	n.d.
Egg	n.d.	-
Faeces	-	-
Embryo	n.d.	-

PCR-detection of chloroplast-DNA (product 199 bp)

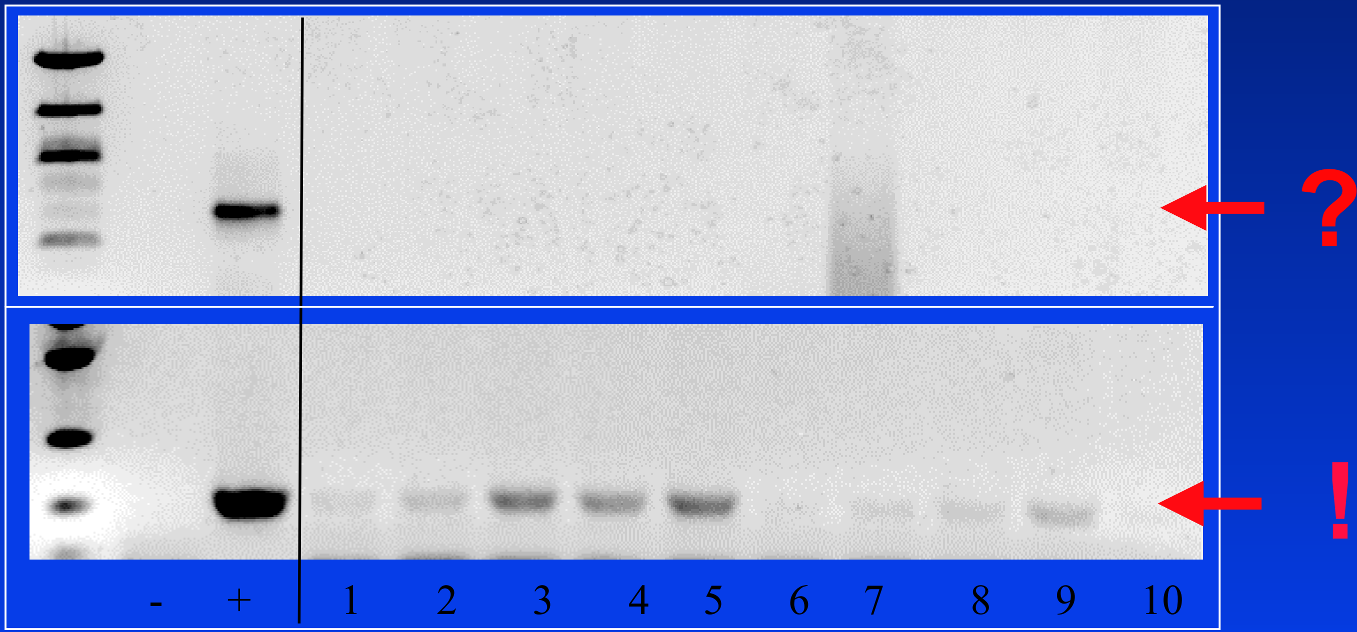
no Bt 176 maize DNA found

Plant DNA in white blood cells of cattle:
Differentiating long vs. short chloroplast DNA fragments

Einspanier et al. 2001

long plant-DNA (532 bp)

short plant-DNA (199 bp)



PCR-detection 40 cycles

Rubisco Feed DNA degradation within the pig

Klotz et al. 2002

Organ	2	4	6	8	12 h time
Stomach	**+**	**+**	**+**	**(+)**	**(+)**
Duodenum	**+**	**+**	**+**	**(+)**	**(+)**
Jejunum	**+**	**(+)**	**(+)**	**(+)**	**(+)**
Ileum	**+**	**(+)**	**(+)**	**(+)**	**(+-)**
Lymph node	**-**	**-**	**-**	**-**	**-**
Blood	**-**	**-**	**-**	**-**	**-**

PCR-detection Rubisco DNA (199 bp)

No Bt 176 maize DNA found in organs

Detection methods

PCR/Southern RUBISCO assay of bovine tissue under either (standard laboratory) conditions or GLP conditions with HEPA laminar flow biohood in lab where no plant DNA had ever been handled.

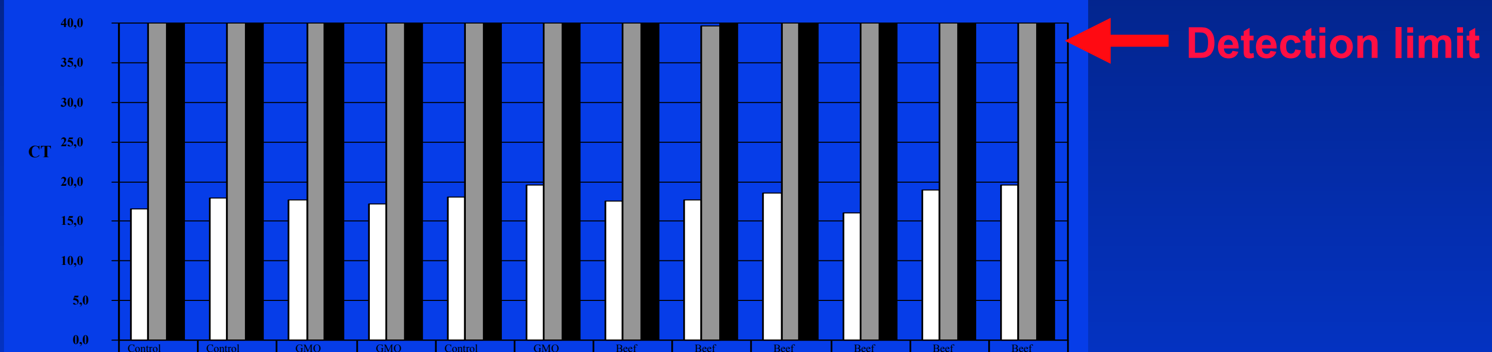
	Kidney	Liver	Spleen
Positive	(0) 0	(6) 1	(10) 0
Inconclusive	(9) 1	(3) 2	(1) 1
Negative	(2) 10	(2) 8	(0) 10
Extract. Contr.	valid	valid	valid
Neg. PCR Contr.	valid	valid	valid
Pos. PCR Contr	valid	valid	valid
Spike	valid	valid	valid

Klaften et al. 2003

Detection methods:

B.) Measurments using qPCR

Measurement of RUBISCO and 18S DNA in beef + chicken spleen



	Control Chicken	Control Chicken	GMO Chicken	GMO Chicken	Control Chicken	GMO Chicken	Beef Spleen	Beef Spleen	Beef Spleen	Beef Spleen	Beef Spleen	Beef Spleen
18S	16,5	17,9	17,6	17,1	18,1	19,6	17,6	17,7	18,6	16,0	18,9	19,5
RbcL 1	40,0	40,0	40,0	40,0	40,0	40,0	40,0	39,6	40,0	40,0	40,0	40,0
RbcL 2	40,0	40,0	40,0	40,0	40,0	40,0	40,0	40,0	40,0	40,0	40,0	40,0

Klaften et al. 2003

Data shown as C_T values, one out of six TaqMan reactions and two independent assays.

Detection of endogenous and transgenic DNA in bovine digestive tract

		Rubisco	GM Soya	GM Maize
Rumen:	Solid	✓	✓	✓
	Liquid	✓	x	x
Duodenum:	Solid	✓	✓	✓
	Liquid	✓	x	x
Faeces		✓	x	x

Phipps et al 2003 In Press

Detection of endogenous and transgenic DNA in bovine blood and milk

	Rubisco	GM Soya	GM Maize
Blood	X/-	x	x
Milk	x	x	x

Phipps et al 2003 In Press

Fate of DNA

Search for Transgenic DNA and Protein in Milk, Meat and Eggs

References: Klotz and Einspanier 1998; Einspanier *et al.*, 2001; Faust, 2000; Phipps and Beever, 2001; Phipps *et al.*, 2002; Ash *et al.*, 2000; Khumnirdpetch *et al.*, 2001; Aeschbacher *et al.*, 2001; Weber and Richert 2001; Klotz *et al.*, 2002; Japan MAFF, 2002; Calsamiglia *et al*,. 2003: Ash *et al.*, 2003; 15, Jennings *et al.*, 2003 a,b, and Phipps *et al.* 2003.

Would transgenic DNA and protein accumulate in meat, milk and eggs derived from animals fed GM feed ingredients?

The answer to the question is:

- To-date no fragments of tDNA have been detected in milk,meat or eggs from animals fed GM feeds.
- However, a number of workers have reported finding fragments of the multi copy rubisco gene in a range of tissue samples including muscle and milk.

Implications for not finding tDNA but finding endogenous DNA in Milk, Meat and Eggs.

- **Is this due to the fact that the Rubisco gene is a multi-copy gene with many copies/cell compared with only one copy/cell of the transgene?**
- **Is the failure to detect tDNA a function of quantity and analytical sensitivity. Even if it is found we must ask the question so what in the light of the WHO statement that there is no inherent danger in consuming DNA including that from GM crops?**

Concerns were expressed about the use of GM feed ingredients

Issues: 3

- Will the consumption of GM crops or animal products derived from GM crops lead to adverse health effects in humans?
- This focuses on the fact that some GM crops contain Antibiotic Resistance Marker genes and concern was expressed about their horizontal gene flow and the possible spread of antibiotics resistance in humans.

The Role of Antibiotic Resistance Marker (ARM) Genes

Why were they used:

Antibiotic Resistant Marker (ARM) genes have been used in the production of some of the first generation GM crops, as an aid in identifying transformed cells, and are present in a number of commercialised GM crops, although having no specific function in that crop.

The Role of ARM Genes

Current position:

"Member States and the Commission shall ensure that GMOs which contain genes expressing resistance to antibiotics in use for medical or veterinary treatment are taken into particular consideration when carrying out an environmental risk assessment, with a view to identifying and phasing out antibiotic resistance markers in GMOs ***which may have adverse effects on human health and the environment.*** *This phasing out shall take place by the 31 December 2004 in the case of GMOs placed on the market according to part C and by 31 December 2008 in the case of GMOs authorised under part B".*

The Role of ARM Genes

The concluding conference of Entransfood, the European Network on Safety Assessment of Genetically Modified Food Crops stated that,

"the risk of gene transfer from foods derived from GM crops that are currently commercially available is deemed negligible."

The Role of ARM Genes

Entransfood, went on to state that

"the use of marker gene expressing resistance to antibiotics in use for medicinal or veterinary purposes has been evaluated and if the antibiotic is widely used or is a tool of last resort, such genes should be avoided.

However, it concluded that, "marker genes coding for neomycin *(npt-11)* or hygromycin *(hpt)* can be used without the risk of compromising human or animal health".

The Role of ARM Genes

The Future?

ARM genes such as the npt-II gene could be removed or replaced by alternative markers. However, should this simple, easy, cheap and efficient technology which has been fully assessed for safety be scrapped and what would be the consequences for ongoing and future work in developing countries?

Horizontal Gene Flow of ARM.

Can it occur?

Has it happened in nature?

What is the probability of it occurring in nature?

While extremely elegant laboratory based experiments have shown that horizontal gene flow between tDNA (ARM genes) and microbes can occur, it has never been shown to have taken place in nature.

Microbiologists consider that the potential for any resistance gene transfer from plants to bacteria is a theoretically possible but the probability that it could occur is extremely low and borders on an evolutionary timescale.

Summary

- Large quantities of GM feed ingredients fed to a wide range of farm livestock with no adverse effects.
- **DNA is not completely degraded in the GI tract.**
- **Fragments of highly abundant plant DNA may be found in organs of some farm animals**
  - ***Chloroplast DNA temporarily present ?***
  - ***Transgenic (GM) DNA never found !***
- **No evidence that milk, meat and eggs from animals fed GM feeds are less safe than that from animals fed conventional feeds, but may be safer.**

Summary

- Final conference of Entransfood, the European Network on Safety Assessment of Genetically Modified Food Crops stated that,

"the risk of gene transfer from foods derived from GM crops that are currently commercially available is deemed negligible."