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FEEDING of GM-CROPS to FOOD PRODUCING ANIMALS and FATE of DEGRADATION of DNA and PROTEIN

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1. Introduction and definitions
2. Types of studies with GM-crops of the 1st generation
   - Experimental design
   - Results
   - Assessment
3. Types of studies with GM crops of the 2nd generation
4. Degradation of tDNA and novel protein
5. Further research need
6. Conclusions

Nutritional view of GM-Crops

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GMP of the 1st generation

- Plants with agrotechnical traits (input traits)
- Without substantial changes in composition/nutritive value
- Substantial equivalent
- Examples:

  Bt-plants (corn, cotton), RR-plants (soybean), PAT-plants (corn, roots) etc.

GMP of the 2nd generation

- Plants with output traits
- With substantial changes in composition/nutritive value
- No substantial equivalent
- Examples:

  Golden rice, low phytate corn, changes in fatty acids or amino acids pattern etc.

Steps for nutritional assessment of feeds from GM-Crops

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Feeds from GMP

	GMP of first generation,	GMP of second generation,
Characterization of GMP	**GMP of first generation,** Plants with input traits, Feeds without substantial changes in composition (substantial equivalent)	**GMP of second generation,** Plants with output traits, Feed with substantial changes in composition
Steps for nutritional assessment	- Determination of major and minor nutrients and undesirable substances, - Principle of Substantial Equivalence (SE) - Case-by-case studies to compare GM-feeds with isogenic counterparts in target animal species/categories	- Determination of major and minor nutrients and undesirable substances, - Determination of bioavailability/bio-potency of changed nutrient/s in target animal species/categories - Case-by-case (long-term) feeding studies to compare GM-feed with variously supplemented isogenic counterparts in target animal species/categories

Studies with GM-crops of the 1st generation

Summary of studies done with feeds from GM-Crops of the 1st generation conducted at the Federal Agricultural Research Centre

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GMP	Analytical measurements	Animal species/categories	Type of study
Bt-maize			
Grain	Crude nutrients, Amino acids Fatty acids, NSP, Minerals,	Growing and fattening pigs	Digestibility
	Mycotoxins		Growing/ fattening
	Crude nutrients	Growing pigs	Digestibility
	Crude nutrients, Starch, NSP, Amino acids, Fatty acids, Minerals	Laying hens	Digestibility, measuring of performance
	Crude nutrients	Broilers	Digestibility
	Crude nutrients	Broilers	Growing
	Crude nutrients, Starch, Amino acids, Fatty acids	Growing and laying quails	10 generations (growing, laying), presently 20th generation
	Crude nutrients, Starch, Amino acids, Fatty acids	Growing and laying hens	4 generations (growing, laying)

Summary of studies done with feeds from GM-Crops of the 1st generation conducted at the Federal Agricultural Research Centre

GMP	Analytical measurements	Animal species/categories	Type of study
Bt-maize			
Silage	Crude nutrients	Growing and fattening bulls	Growing/ fattening
	Crude nutrients	Sheep	Digestibility
Bt-potatoes	Crude nutrients	Broilers	Growing
Pat-maize	Crude nutrients, Starch, Sugar, NSP, Amino acids, Fatty acids,	Pigs	Digestibility
Pat sugar beets			
Roots	Crude nutrients, Sugar	Sheep	Digestibility
	Crude nutrients, Sugar	Pigs	Digestibility
Top silage	Crude nutrients	Sheep	Digestibility
RR-soybeans Roundup Ready soybeans	Crude nutrients, Starch, Amino acids, Fatty acids, Minerals	Pigs	Growing/ fattening

Mycotoxins in isogenic (100 %) and Bt-corn (% of isogenic corn; data from some references)

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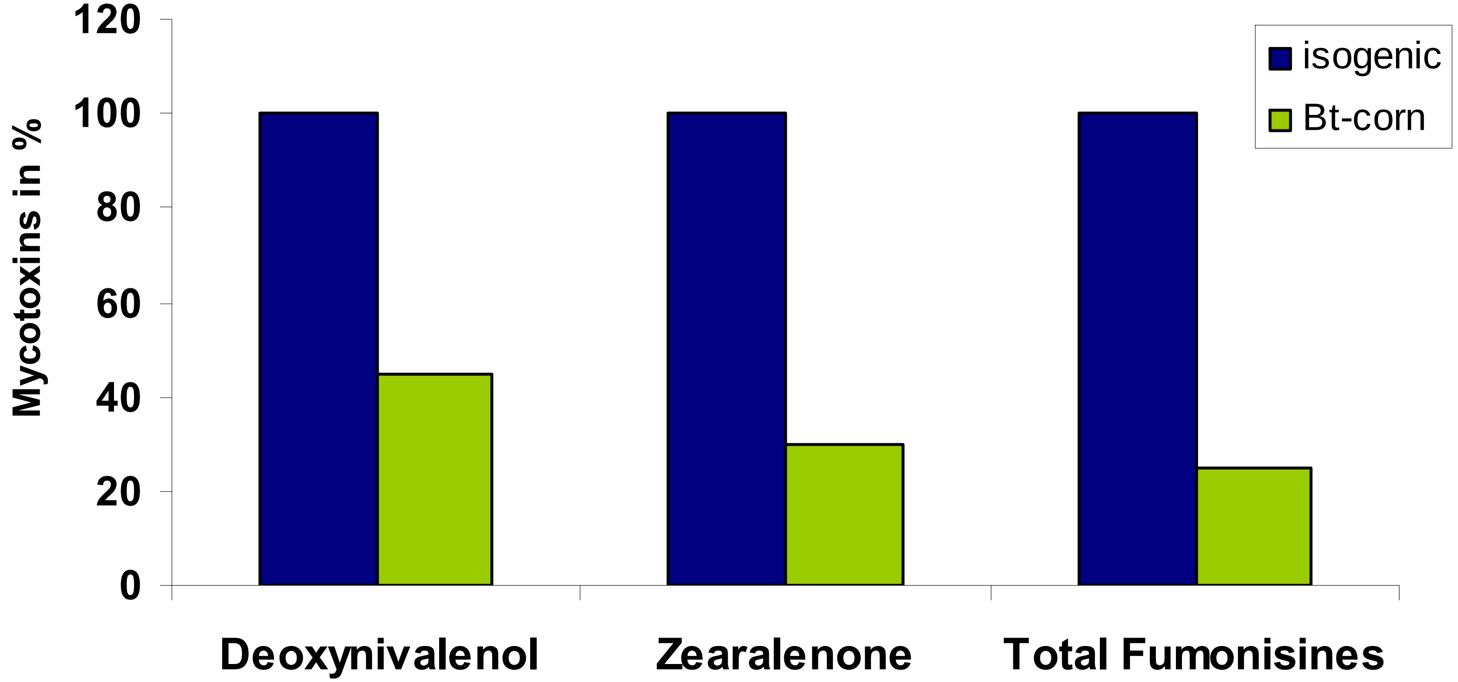

(A) Body weight of female quails (age: 6 weeks), (B) laying intensity and (C) hatchability of quails fed with isogenic (■) and transgenic (Bt, □) corn in a 10 generations experiment

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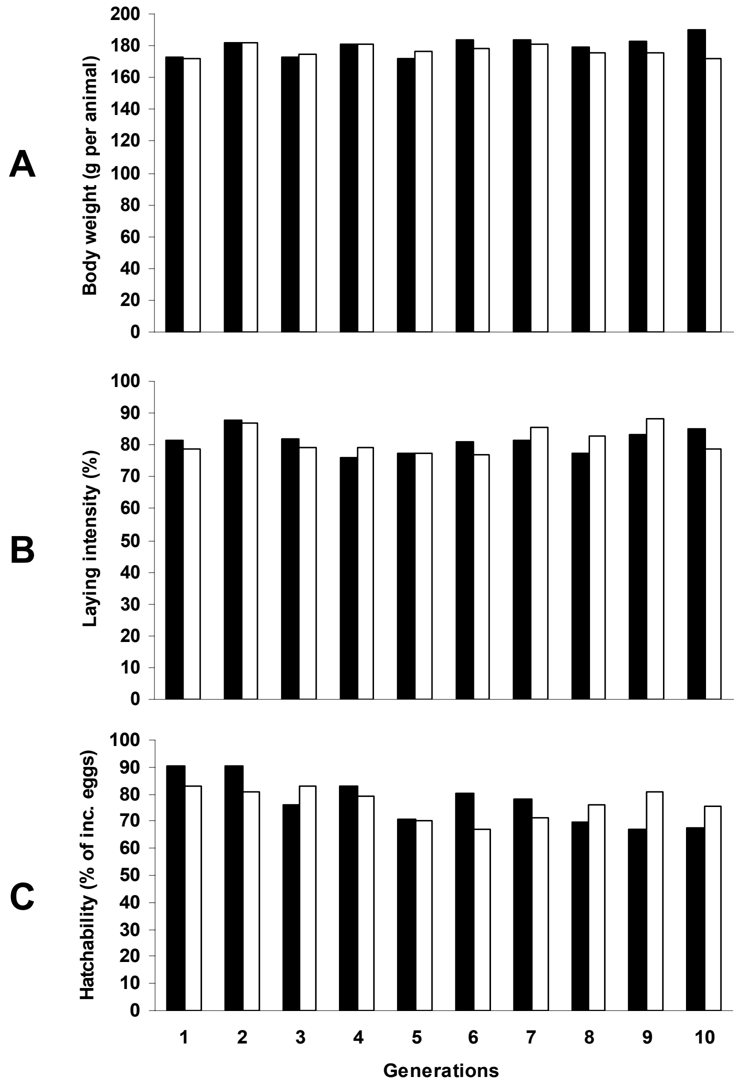

Overall means (Range of generations)

	Isogenic	Transgenic
A	180.1 (172.0 – 190.1)	176.9 (171.9 – 181.7)
B	81.3 (75.9 – 87.8)	81.4 (77.1 – 88.4)
C	77.4 (66.8 – 90.5)	76.7 (67.0 – 83.2)

Summary of studies done with feeds from GM-Crops of the 1st generation conducted by the Federal Agricultural Research Centre

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GMP	Analytical measurements	Animal species/cate-gories	Type of study	Animal number (isogen/ transgen)	Duration (days)	Results (comparison to isogenic plants)				
						Composition of GM-feeds	Digestibility	Zootechnical parameters	Further measurements	References
Bt-maize										
Grain	Crude nutrients, Amino acids Fatty acids, NSP, Minerals,	Growing and fattening pigs	Digestibility	3 times: 6/6	14	NS	NS	-	-	Reuter et al. 2002a
	Mycotoxins		Growing/ fattening	12/36	91	NS	-	NS	Slaughtering data, Fate of DNA	Reuter at al. 2002b
	Crude nutrients	Growing pigs	Digestibility	5/5	14	NS	NS	-	-	Aulrich et al. 2001
	Crude nutrients, Starch, NSP, Amino acids, Fatty acids, Minerals	Laying hens	Digestibility, measuring of performance	6/6	10	NS	NS	NS	Fate of DNA	Aulrich et al. 2001
	Crude nutrients	Broilers	Digestibility	6/6	5	NS	NS	-	-	Aulrich et al. 2001
	Crude nutrients	Broilers	Growing	9/27	35	NS	-	NS	Fate of DNA	Tony et al. 2003
	Crude nutrients, Starch, Amino acids, Fatty acids	Growing and laying quails	10 generations (growing, laying), presently 20th generation	Growers 140/140 Layers 32/32	10 times: 42 42	NS NS	- -	NS NS	Reproduction, Fate of DNA	Halle et al. 2004 Flachowsky et al. 2005b
	Crude nutrients, Starch, Amino acids, Fatty acids	Growing and laying hens	4 generations (growing, laying)	Growers 200/200 Layers 32/32	4 times: 126 91	NS NS	- -	NS NS	Reproduction	Halle et al. 2006

NS = No significant differences

Summary of studies done with feeds from GM-Crops of the 1st generation conducted by the Federal Agricultural Research Centre

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GMP	Analytical measurements	Animal species/categories	Type of study	Animal number (isogen/transgen)	Duration (days)	Results (comparison to isogenic plants)				
						Composition of GM-feeds	Digestibility	Zootechnical parameters	Further measurements	References
Bt-maize										
Silage	Crude nutrients	Growing and fattening bulls	Growing/ fattening	20/20	246	NS	-	NS	Slaughtering data, Fate of DNA	Aulrich et al. 2001
	Crude nutrients	Sheep	Digestibility	4/4	24	NS	NS	-	-	Aulrich et al. 2001
Bt-potatoes	Crude nutrients	Broilers	Growing	9/18	21	NS	-	NS	Fate of DNA	Halle et al. 2005
Pat-maize	Crude nutrients, Starch, Sugar, NSP, Amino acids, Fatty acids,	Pigs	Digestibility	5/5	14	NS	NS	-	-	Böhme et al. 2001
Pat sugar beets										
Roots	Crude nutrients, Sugar	Sheep	Digestibility	4/4	24	NS	NS	-	-	Böhme et al. 2001
	Crude nutrients, Sugar	Pigs	Digestibility	5/5	14	NS	NS	-	-	Böhme et al. 2001
Top silage	Crude nutrients	Sheep	Digestibility	4/4	24	NS	NS	-	-	Böhme et al. 2001
Roundup Ready soybeans	Crude nutrients, Starch, Amino acids, Fatty acids, Minerals	Pigs	Growing/ fattening	12/36	40	NS	-	NS	Slaughtering data, Fate of DNA	Aulrich et al. 2001

NS = No significant differences

Summary of published data to compare feeds from GM plants of the first generation (with input traits) with their isogenic counterparts

Animal (Species/categories)	Number of experiments	Nutritional assessment
Ruminants		No unintended effects in composition (except lower mycotoxins concentration in Bt plants)
Dairy cows	23	
Beef cattle	14	
Others	10	
Pigs	21	No significant differences in digestibility and animal health as well as no unintended effects on performances of animals and composition of food of animal origin
Poultry		
Laying hens	3	
Broilers	28	
Others		
(Fish, rabbits etc.)	8	

Studies with GM-crops of the 2nd generation

GM-crops with output traits (GMP of the second generation)

- **Increased content of desirable/valuable substances**
  - **Nutrient precursors (e. g. β-carotene)**
  - **Nutrients (amino acids, fatty acids, vitamins, minerals etc.)**
  - **Substances which may improve nutrient digestibility (e. g. enzymes)**
  - **Substances with surplus effects (e. g. prebiotics)**
  - **Improvement of sensoric properties/ palatability (e. g. essential oils, aromas)**
- **Decreased content of undesirable substances**
  - **Inhibiting substances (e. g. lignin, phytate)**
  - **Toxic substances (e. g. alkaloids, glucosinolates, mycotoxins)**

Proposal to assess the conversion of nutrient precursors from the second generation of GMP into nutrients (e. g., β-carotene)

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Groups	Diet composition	Measurements
1[1]	Balanced diets including typical levels of isogenic counterpart + β-carotene (level/s adequate to the transgenic crop)	Depends on the claim of genetic modification: - Concentration of converted substances in target organs (e. g., vitamin A in liver)[2]
2[1]	Balanced diets with adequate amounts of transgenic crop	- Metabolic parameters

[1] equal feed for all animals

[2] until a steady state is achieved in the target organs

Proposal to assess the effects of inhibitors of nutrient bioavailability (e. g., phytate)

Groups	Diet composition	Measurements
1	Balanced diet including typical levels of isogenic counterpart, ad lib. feeding	Depends on the claim of genetic modification: - Digestibility of inhibited nutrient - Growing experiment with target animals - Concentration of inhibited nutrient in indicator organs
2	Diets of Group 1 plus inhibited nutrient (e.g., P), ad lib. feeding	
3	Balanced diet including transgenic counterpart in adequate levels to Group 1 (e.g., low phytate crop)	
4	Diets of Group 3 plus inhibited nutrient of Group 2	

Conventional and low-phytate maize (78.5 % of the mixture) in the feed of fattening pigs (from Spencer et al., 2000)

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	Control (0.3 g of available P per kg)		Low-phytate maize (1.7 g of available P per g)	
Inorganic P supplement	-	+	-	+
P content (g/kg)				
29 - 73 kg live weight	3.4	5.4 [1)]	3.4	5.4 [1)]
73 - 112 kg live weight	3.2	4.7 [2)]	3.2	4.7 [2)]
Feed intake (kg/d)	2.23 [a]	2.50 [b]	2.53 [b]	2.51 [b]
Live weight gain (g/d)	730 [a]	870 [b]	900 [b]	880 [b]
Feed per gain (kg/kg)	3.05 [a]	2.87 [b]	2.81 [b]	2.85 [b]
P excreted (g/kg)	4.6 [a]	8.9 [c]	3.8 [b]	8.8 [c]
Strength (4th metacarpal bone, kg)	79.4 [a]	138.5 [bc]	132.2 [b]	153.9 [d]
Ash content (% in 4th metacarpal bone)	53.5 [a]	60.1 [bc]	59.3 [b]	61.2 [c]

[a, b, c, d] Different letters in one line indicate significant differences ($p < 0.05$)

[1)] +2.0 g P/kg [2)] +1.5 g P/kg

Selected crude nutrients, starch, macro-elements and amino acids as well as glycoalkaloids of transgenic inulin synthesising potatoes as compared to those of the parenteral line (Böhme et al. 2005a)

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	Isogenic	Transgenic
Crude nutrients and starch (g/kg DM)		
Crude protein	107	106
Crude starch	55	58
Starch	674	599
Amino acids (g/100 g crude protein)		
Lysine	4.31	4.00
Methionine	1.18	1.39
Threonine	2.49	2.53
Minerals (g/kg DM)		
Ca	0.51	0.56
P	2.10	2.20
Mg	0.90	0.84
Na	0.30	0.23
Glycoalkaloids (mg/kg DM)		
α-Chaconine	524	652
α-Solanine	204	252
Total alkaloids	728	904

Chemical composition of isogenic and transgenic rapeseed (Böhme et al. 2005b)

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	Isogenic	Transgenic
Proximates (g/kg of DM)		
Crude protein	227.9	273.8
Ether extract	440.6	398.6
Amino acids (g/100 g crude protein)		
Lysine	5.61	5.74
Methionine	1.86	1.97
Threonine	4.31	3.99
Minerals (g/kg DM)		
Ca	4.39	4.19
P	7.36	8.41
Mg	3.22	3.92
Fatty acids (% of total fatty acids)		
$C_{14:0}$	0	13
$C_{16:0}$	4	20
$C_{18:1}$	67	39
Glucosinolates (µmol/g DM)		
Alkenyl GSL	9.0	15.4
Progoitrine	7.1	12.1
Total GSL	13.2	20.4

Demonstration of unintended effects by phenotype selection or investigation of defined constituents (by Cellini et al. 2004)

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Host plant	Trait	Unintended effect	References
Potato	Expression of bacterial levansucrase	Adverse tuber tissue perturbations Impaired carbohydrate transport in the phloem	Turk and Smeekens (1999); Dueck et al. (1998)
Wheat	Expression of phosphatidyl serine synthase	Necrotic lesions	Delhaize et al. (1999)
Soybean	Expression of glyphosate (EPSPS) resistance	Splitting stems and yield reduction (up to 40 %) at high soil temperatures (45° C) Higher lignin content (20 %) at normal soil temperatures (20° C)	Gertz et al. (1999)
Wheat	Expression of glucose oxidase	Phytotoxicity	Murray et al. (1999)
Rice	Expression of soybean glycinin	Increased vit. B_6-content (+50 %)	Momma et al. (1999)
Potato	Expression of soybean glycinin	Increased glycoalkaloid content (+16 – 88 %)	Hashimoto et al. (1999a); Hashimoto et al. (1999b)
Potato	Expression of yeast invertase	Reduced glycoalkaloid content (-37 – 48 %)	Engel et al. (1998)
Canola	Overexpression of pytoene-synthase	Multiple metabolic changes (tocopherol, chlorophyll, fatty acids, phytoene)	Shewmaker et al. (1999)
Rice	Expression of carotenoid biosynthetic pathway	Formation of unexpected carotenoid derivates (beta-carotene, lutein, zeaxanthin)	Ye et al. (2000)

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Degradation of DNA and novel protein

Processing of rapeseed (Berger et al., 2003)

Treatment	1	2	3	4
Processing	Crushing	Crushing	Crushing	Crushing
	-	-	Conditioning (96°C, 20 min)	Conditioning (103 - 111°C, 30 min)
	Pressing (69°C)	Pressing (95°C)	Pressing (95°C)	Pressing (95°C)
	-	Extraction	Extraction	Extraction
	-	Desolventizing-Toasting (105°C)	Desolventizing-Toasting (105°C)	Desolventizing-Toasting (105°C)

Determined DNA-fragments in final products of isogenic (i) and transgenic (t) rapeseed

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Treatment		1	2	3	4
Rape-final products		Cake	Toasted meal	Toasted meal	Toasted meal
Determined DNA-fragments (bp) 21000 bp (intact DNA)	i t	+ +	- -	- -	- -
248 bp	i	+	+	+	-
970 bp	i	+	-	-	-
194 bp	t	+	+	-	+
680 bp	t	+	-	-	-
1003 bp	t	+	-	-	-

+ detected, - not detected

Determined DNA-fragments in transgenic Corn Cob Mix (CCM) and Whole Plant Silage (WPS) as influenced by the ensiling period (Aulrich et al., 2004)

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Duration of ensiling (days)	Fragment 1016 bp		Fragment 680 bp		Fragment 194 bp	
	CCM	WPS	CCM	WPS	CCM	WPS
0	+	+	+	+	+	+
2	+	+	+	+	+	+
5	+	+	+	+	+	+
7	-	+	+	+	+	+
14	-	+	+	+	+	+
21	-	+	+	+	+	+
28	-	+	-	+	+	+
35	-	-	-	+	+	+
70	-	-	-	-	+	+
100	-	-	-	-	+	+
200	-	-	-	-	+	+

Studies of the transfer of „foreign“ DNA fragments into experimental animals

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DNA source	Animal species	Results		
		Detection of transgenic DNA	Detection of “foreign” nontransgenic DNA	References
Bt-maize-grain and silage	Broiler Layer Growing bulls Dairy cows	No transgenic DNA in animal tissues	Plant DNA fragments in muscle, liver, spleen, kidneys of broilers and layers, not in blood, muscle, liver, spleen, kidneys of growing bulls, in eggs and feces of broilers and layers and in feces of dairy cows	Einspanier et al. (2001)
Bt-maize-grain	Pig	Transgenic DNA fragments up to 48 hrs up to the rectum, not in blood, organs and tissues	Plant DNA fragments in the gastrointestinal tract, in blood, organs and tissues	Reuter and Alrich (2003)
Bt-maize-grain	Broiler	Transgenic DNA in the gastrointestinal tract, no transgenic DNA in blood, organs and tissues	Plant DNA fragments in the gastrointestinal tract, in blood, organs and tissues	Tony et al. (2003)
Bt-maize-grain	Quails (10 generation)	Transgenic DNA fragments (211 bp) in the stomach and whole gastrointestinal tract, no transgenic DNA fragments in muscle, liver, stomach, spleen, kidney, heart and eggs	Plant DNA fragments in the gastrointestinal tract	Flachowsky et al. (2005)
Bt-potato	Broiler	No transgenic DNA in muscle, liver, kidney and spleen	Plant DNA fragments in muscle, liver, kidney and spleen till 8 h after feeding	El Sanhoty (2004)
Gt-soybeans	Pig	No transgenic DNA in muscle, liver, kidney and spleen	Plant DNA fragments in the gastrointestinal tract	Aulrich et al. (2002)
Inulin-potato-silage	Pig	Transgenic DNA fragment (104 bp) in the stomach, no transgenic DNA fragments in animal tissues	Plant DNA fragments in the gastrointestinal tract, no plant DNA fragments in animal tissues	Broll et al. (2005)

Conclusion: Degradation of DNA

- DNA is a permanent part of food/feed
  (daily intake: men: 0.1 – 1 g; pig: 0.5-4 g; cow: 40-60 g)
- tDNA intake amounted to ~0.005% of total DNA-intake, if 50 % diet come from GM-crops
- DNA is mostly degraded during conservation (silage making) and industrial processing as well as in digestive tract (pH, enzymes)
- Small fragments of DNA may pass through the mucosa and may be detected in some body tissues (esp. leucocytes, liver, spleen)
- There exist no data, that tDNA is characterized by another behaviour as native plant-DNA during feed treatment and in the animals

Conclusion: Degradation of novel Protein

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- In the ruminants feed protein are mostly degraded in the rumen and microbial protein and by-pass protein is degraded by enzymes in the smaller intestine, similar to nonruminants
- The chemical and physiological properties (including microbial and enzymatic degradation) of novel proteins have been intensively tested
- Intact novel proteins were not detected outside of the digestive tract in target animals
- There is no advice, that novel proteins are characterized by other chemical/physical properties as native protein

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Further research need
- Clarification of open questions

Comments to experimental design and statistical methods for bioequivalence testing (Tempelmann 2004)

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Present stage for bioequivalence studies:

Working hypothesis: No mean differences between GM and reference counterparts for various measures

Thesis by Tempelmann (2004)

Statistically nonsignificant effects ($p>0.05$) are not to be presented as evidence of equivalence

His recommendation

Statistical methods for bioequivalence testing have been well developed and applied in pharmaceutical research (FDA 2001). Other experimental designs have to be used (esp. in dairy cows)

Nutritional and physiological responses in Atlantic salmon fed diets with control or Bt corn (MON 810; 82 days of experiment, 3 tanks per treatment with 45 fish each, initial weight: 155 g, Hemre et al. 2006)

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Corn	Control		Bt corn		
Corn portion	Low	High	Low	High	
%	15	30	15	30	p 0.05
Final weight (g)	625 ±117	612 ±116	573 ±126	561 ±138	<
Feed eaten (g per fish)	407 ±13	401 ±30	365 ±27	366 ±12	<
FCR (kg per kg)	0.88 ±0.02	0.88 ±0.01	0.88 ±0.04	0.91 ±0.03	>
Apparent digestibility of DM (%)	93.2	91.6	92.2	91.5	>
Body protein (g kg^{-1} fish)	171	171	170	170	>
Red blood cell count ($10^{12} L^{-1}$)	1.10	1.12	1.13	1.19	<
Haemoglobin (g 100 ml^{-1})	7.87	8.66	8.11	8.57	>

Comments to some studies which certain disturbances after feeding GM-crops

Authors	Study	Results	Comments
Ewen and Pusztai (1999)	Lectin-potatoes to rats	Influence of intestinal-tract, disturbance of reproduction	Scientific study, no practical relevance
Hemre et al. (2005)	RR soybean to salmon	Increase of spleen, influence of spleen functions, more smaller erythrocytes	What is normal? Repetition of study
Kosieradzka et al. (2004)	Transgenic cucumber to rats	Increase of neutrophil granulocytes	What is normal? Repetition of study
Malatesta et al. (2002a,b)	RR soybean to mice; comparison with variety	Increased cell nucleus in liver and pancreas	Methodical weaknesses, comparison with wild variety, What is normal? Relevance of results?
Nordlee et al. (1996)	Transgen of Paranut in soybean and corn	Allergenic reactions in man	Scientific study, no practical relevance
Poulsen et al. (2006)	Feeding of transgenic Lectin-rice to rats	Disturbances in development and fertility	Scientific study, no practical relevance
Scholtz et al. (2006)	Feeding of 50 % Bt-corn in longterm study in quails	Differences in some enzymatic activies between both groups	Physiological relevance, what is normal? Other results after repetition of study

Proposal for the nutritional assessment of GMPs

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	First generation of GMP	Second generation of GMP
- Determination of important constituents		
▪ Crude nutrients	+	++
▪ Genetically modified nutrients (e. g., amino acids, fatty acids, vitamins, enzymes, etc.)	-	++ [2]
▪ Genetically modified undesirable substances (e. g., plant constituents such as lignin, inhibitors, glucosides, etc., or secondary substances, such as mycotoxins, pesticides, etc.)	(+)	++ [2]
- Digestibility, conversion studies, availability of modified nutrients in the target animal species	(+)	++
- In vitro studies of nutritional assessment	(+)	(+)
- Feeding experiments with species/categories of target animal		
▪ Performance of animals and quality of foods of animal origin	(+)	++
▪ Animal health	(+)	(+)
▪ Route taken by modified protein and/or DNA [1]	+	+

- not necessary
(+) may be advantageous
\+ recommended
++ necessary
[1] for scientific purposes
[2] for modified components

Some recommendations from the “Best practices for the conduct of animal studies to evaluate crops genetically modified for input traits (GMP of the first generation)”; adapted from ILSI 2003b

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Animals (species/categories)	Number of animals (coefficient of variation 4 to 5 %)	Duration of experiments	Composition of diets [1]	Measurements
Poultry for meat production	10 to 12 pens per treatment with 9 to 12 birds per pen	5 weeks or more	Balanced diets	Feed intake, gain, feed conversion
Poultry for egg production	12 to 15 replications per treatment with 3 to 5 layers per pen	18 to 40 weeks of age, at least three 28-day phases	Balanced diets	Feed intake, egg production, feed conversion, egg quality
Swine	6 to 9 replications per treatment with 4 or more pigs per replication	Piglets (7 – 12 kg), 4 – 6 weeks Growers (15 – 25 kg) 6 – 8 weeks	Balanced diets	Feed intake, gain, feed conversion, carcass quality
Growing and finishing ruminants	6 to 10 replications per treatment with 6 or more cattle per replication	90 – 120 days	Balanced diets	Feed intake, gain, feed conversion, carcass data
Lactating dairy cows	12 – 16 cows per treatment 28 cows per treatment	Latin square: 28 day periods Randomized block design	Balanced diets	Feed intake, milk performances and composition, body weight, Body Condition Score (BCS), cell counts in milk, animal health

[1] Feed from GMP should be included in high portions in diets and compared with isogenic counterparts

Proposal for a decision tree for nutritional assessment of feeds from GMP of the 1st and / or 2nd generation

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PRINCIPAL QUESTIONS

Are there significant differences in relevant constituents between feedstuffs from isogenic and transgenic plants?

Further questions

- What should be done if no isogenic counterparts exist ?

Further questions

- Which constituents should be studied ?
- What will be used for comparison (isogenic line or natural population) ?

No

No further studies if the principle of substantial equivalence is accepted	Further studies if the principle of substantial equivalence is not accepted
End of evaluation	

Yes

Determination of digestibility, conversion studies
Differences from the isogenic line

- Are side-effects nevertheless to be expected ?
- Can in vitro studies possibly answer further questions ?

- Formulation of rations?
- Comparison (isogenic line or natural population) ?
- What comparison if there is no isogenic counterpart ?

No

No further studies
End of evaluation

Yes

Long-term studies with species/categories of target animal • Animal health • Performance • Product quality • Combine with safety studies (undesirable/unexpected effects)
Physiologically inexplicable differences from the isogenic line

- Are side-effects nevertheless to be expected ?

- Experimental protocol ?
    - o Formulation of rations
    - o Animal species/number
    - o What comparison
- Routes taken by DNA or transgenic protein ?
- Importance of in vitro studies or other less costly studies with representative conclusions?

No

No further studies
End of evaluation

Yes

Further studies with specific objectives (metabolism, etc.)
Physiologically inexplicable differences from the isogenic line

- Are side-effects nevertheless to be expected ?

- What type of further studies ?
- Consideration of F_1 + (F_2)-generation
- Changes in intestinal flora ?

No

No further studies
End of evaluation

Yes

Application for registration of the GMP as animal feed should be deferred

- Extend studies to multidisciplinary studies ?
    - o Histology
    - o Pathology
    - o Toxicology etc.

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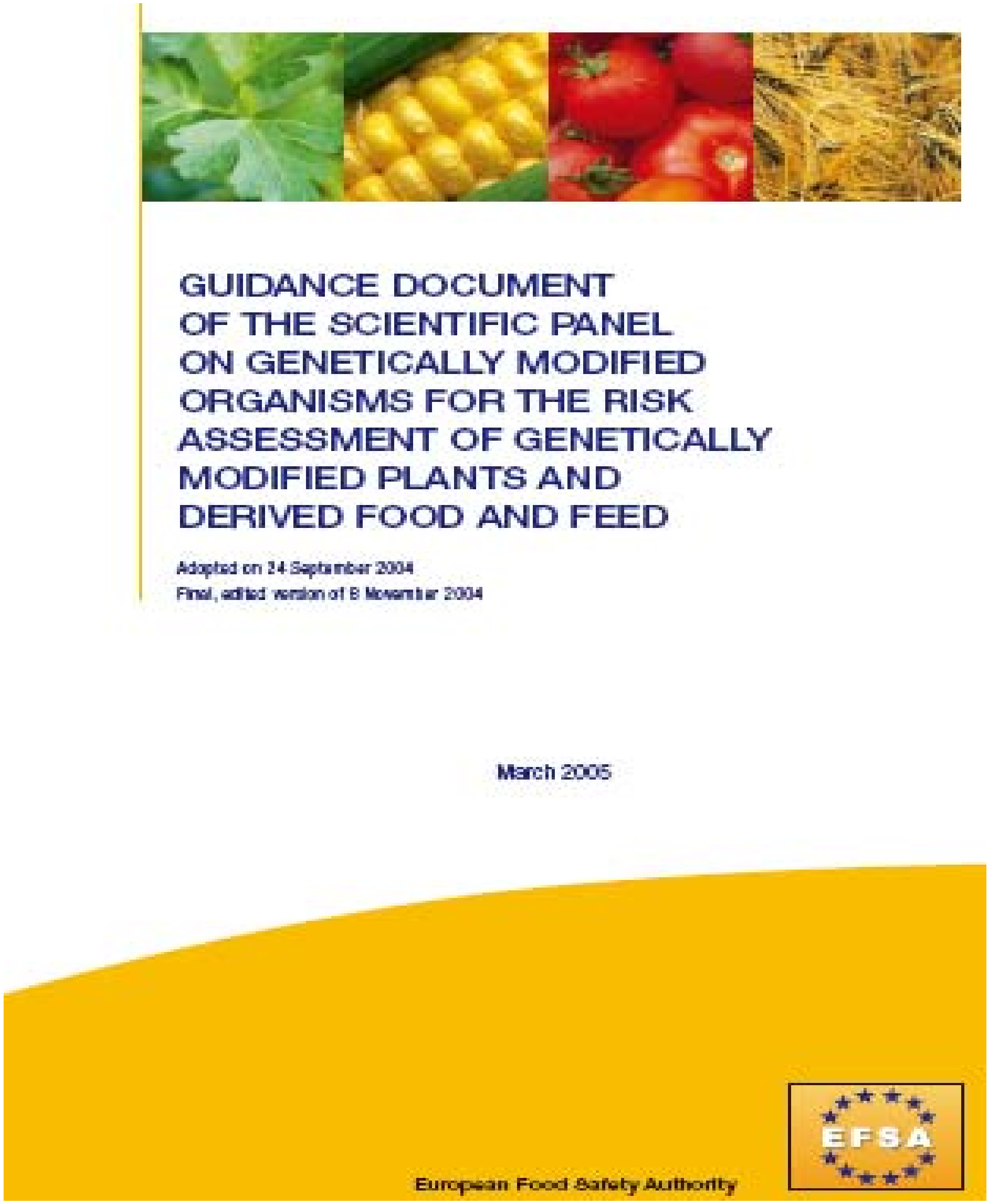

GUIDANCE DOCUMENT OF THE SCIENTIFIC PANEL ON GENETICALLY MODIFIED ORGANISMS FOR THE RISK ASSESSMENT OF GENETICALLY MODIFIED PLANTS AND DERIVED FOOD AND FEED

Adopted on 24 September 2004

Final, edited version of 8 November 2004

March 2005

EFSA

European Food Safety Authority

Conclusions

Conclusions

- Up to now about 500 mio. ha of GM-crops have been cultivated allover the world
- Most animal studies were done with GM-crops of the 1st generation (with input traits)
- No unintended effects in composition (except lower mycotoxins) and nutritional assessment of feeds from GM-crops of the 1st generation were registered in more than 100 studies with food producing animals
- Other experimental designs are recommended for nutritional and safety assessment of feeds from GM-crops of the 2nd generation (with output traits)
- Transgenic DNA and novel protein did not show other properties as plant DNA or nature protein during feed treatment or in the animals
- Furthermore case by case studies seem to be necessary to answer open questions.