Annex 4: Literature Report Health

BEETLE

Biological and Ecological Evaluation
towards Long-Term Effects

Long-term effects of genetically modified (GM) crops on health, biodiversity and the environment: prioritisation of potential risks and delimitation of uncertainties

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Table of Content

1. Introduction

In this literature review the state of knowledge regarding potential long-term effects of GMOs on human and animal health is summarized. The BEETLE team began a prioritization of potential long-term effects on this basis. Furthermore the results were the starting point for an Online Survey Health which had the goal of integrating the expertise of selected stakeholders into the BEETLE project.

More than 81 peer reviewed papers (see References), reports and internet resources were taken into consideration.

2. Methods

The literature research was based on various sources related to genetically modified organisms. Starting points were the existing project partners' expertise. Further sources of information were the internet, various library catalogues (e.g. central catalogue of German libraries, International Centre for Genetic Engineering and Biotechnology (ICGEB with more than 5.600 publications), Washington Library of Congress) and online databases (e.g. ISI Web of Sciences, PubMed (National Library of Medicine and the National Institutes of Health)). Additional unpublished data (e.g. reports from pre-marketing experiments, personal communications from scientific and regulatory experts from science and regulation) were analyzed. Information resources from countries with longer experience of cultivation of genetically modified organisms (Canada, Australia, USA) were explored, as far as they were accessible. It should be taken into consideration that the primary focus of the whole BEETLE project was on potential long-term effects on the environment. Therefore, the BEETLE team worked on the health part less intensively than on the environmental part.

3. Human Health

3.1. Concepts of safety assessment

Strategies for the safety assessment of GM crops have been jointly developed by various international bodies, such as the Organization for Economic Cooperation and Development (OECD), the United Nations World Health Organization (WHO) and the Food and Agriculture Organization (FAO). A cornerstone of the concept of "substantial equivalence" is the comparison of GM crops/foods and existing crops/foods with a "history of safe use". For various GM plants, e.g. maize, soybean, potato, tomato and rice, "substantial equivalence" has been demonstrated (Cellini *et al.* 2004). Using this concept, more than 50 GM crops

have been approved worldwide, and it has been concluded that these foods derived from GM crops are “as safe as those derived from traditional crops”.

The potential occurrence of unintended effects is not a phenomenon specific to genetic engineering. It is well known that traditional plant breeding methods may also result in unexpected changes. In classical breeding programs, backcrossing procedures are applied in order to remove unintended effects (Cellini *et al*. 2004). It has been emphasized that the potential for unexpected and unintended compositional changes will arise with all forms of plant breeding, but that to date, no adverse health effects from the consumption of GM foods have been documented in the human population (NAS 2004).

An extensive study on GMOs in the food supply confirmed that GM foods currently available on the international market have undergone thorough risk assessments and are not likely to present a higher risk for human health than their conventional counterparts (WHO 2005). The lack of any proven adverse effects on humans resulting from the production and consumption of GM crops over the last decade supports these safety conclusions based on the currently applied approaches (Chassy 2004).

In view of the safety assessment of GM crops and conventionally bred crops it has been concluded that the current process of the safety evaluation is not well balanced (Kok *et al.* 2008). GM-derived food is extensively investigated, whereas food derived from conventional farming practice, e.g. cross breeding or mutation breeding, is less thoroughly assessed. In contrast to GM crops, only few traditional foods with a “history of safe use” have been subjected to systematic nutritional and toxicological assessment (Constable *et al.* 2007).

Untargeted analytical methods (transcriptomics, proteomics, metabolomics) are discussed as complementary tools in the safety assessment process of GM crops to detect potential unintended effects (Kuiper *et al*. 2001, Rischer & Oksman-Caldentey 2006). Metabolic profiling techniques have the potential to rapidly identify pathway perturbations (Larkin & Harrigan 2007).

3.2. Nutritional assessment

The biofortification of staple crops through genetic engineering is discussed as a tool to improve the nutritional status of consumers in developing countries (Gilani & Nasim 2007). Various genetic modifications of plant metabolism for nutritional crop improvement, focused on human health benefits, have been described (Davies 2007). For nutritional assessments, integrated food-nutrient databases containing compositional data as well as consumption data are essential (Kok *et al.* 2008). For comparative approaches, crop composition databases have been developed by the ILSI (www.cropcomposition.org) with compositional data gathered by biotechnology companies. It compiles information derived from controlled

field trials that were performed in worldwide locations over a 6-year period using validated analytical methods (Ridley *et al.* 2004). This database can be used when assessing the toxicological and/or nutritional relevance of detected differences between GM and non-GM crops (Kok *et al.* 2008).

3.3. Toxicology

Various strategies to evaluate the safety of food derived from GM crops have been suggested including the safety testing of food composition and toxicology testing (*in vitro* and *in vivo* studies) (Delaney 2007). In addition, the need for an integrated health/food safety and environmental assessment has been discussed (Haslberger 2006).

Various approaches to improve the regimes of toxicity testing of GM foods have been described. Knudsen and Poulsen (2007) discussed the suitability of 90-day rat feeding studies to detect biological/toxicological effects of new gene products in GM food. The use of a new feeding study design allowed the distinction between primary and secondary effects of the new genetic modification event. A 90-day feeding study of rats fed with GM rice expressing snowdrop lectin (*Galanthus nivalis)* showed a number of significant differences between GM and control groups, but they were not considered to be adverse. For concluding on the safety of the GM food, the authors suggested a study including additional group(s) in the experimental design to be able to distinguish whether the observed effects were due to the GNA[1] lectin *per se* or to secondary changes in the GM rice (Poulsen *et al.* 2007a). A spiking procedure was considered as an improvement of testing methodology and suggested as a valuable tool for the future safety testing of GM foods (Poulsen *et al.* 2007b).

Further, a 90-day safety study concerning Bt-rice was conducted (Schroder *et al*. 2007). Analysis of animal behaviour and weight gain revealed no adverse effects. A 30-day feeding study with rats for evaluation of the safety of GM potato (Bt) concludes that GM lines have nearly the same composition and biochemical characteristics as the isogenic line (El-Sanhoty *et al.* 2004).

Rhee *et al*. 2005 reported a multigeneration reproductive and developmental toxicity study in rats of the *bar* gene inserted into GM potato. The specific characteristics of GM food and low-level chronic exposure were examined using a five-generation animal study. No GM potato-related changes in body weight, food consumption, reproductive performance, or organ weight were observed. The authors suggested that genetically modified crops have no adverse effects on multigeneration reproductive and developmental ability. However, these study-specific results on potato cannot be transferred unconditionally to GM crops in general.

[1] GNA = *Galanthus nivalis* agglutinin

So far, no toxic effects have been observed after consumption of Bt crops or derived products. However, due to the absence of a harmonised approach for the statistical analysis and interpretation of data obtained from studies concerning safety assessment of GM crops, the results are subject to controversial discussion (Seralini *et al*. 2007 vs. Hammond *et al*. 2006)[2].

3.4. Allergenicity

Current approaches to test for potential allergenicity are based on the assessment of a series of characteristics of the novel gene product, such as source, structural similarities, digestibility, degradability, and on the results of tests such as antisera binding tests, animal test models, and clinical tests (Kok *et al*. 2008). Overall, the current risk assessment strategy in relation to potential allergenicity has resulted in the absence of transgenic proteins in foods that have been shown so far to cause allergic reactions (Lehrer & Bannon 2005). However, no single factor has been recognized as a primary indicator for allergenic potential, and no validated animal model that is predictive of allergenic potential is available (Delaney 2007).

Adverse long-term effects could arise from the introduction of GMOs which newly express proteins with high allergenic potential. Discovering the unique features responsible for the allergenicity of proteins has led to an allergy assessment strategy that characterizes the potential allergenicity of biotechnology products prior to their commercialization. This testing strategy appears to be effective as shown by the fact that there have been no clinically documented food allergic reactions to any of the biotechnology proteins introduced into food crops (Bannon & Martino-Catt 2007, Burks & Fuchs 1995, Batista *et al*. 2005, Hoff *et al*. 2007). However, the increasing use of GM crops in staple foods will result in an increase in the consumption of novel proteins or proteins from previously not or seldom consumed crops. So far, it is hard to predict if newly introduced proteins will become new allergens that have not yet been confirmed (Bannon & Martino-Catt 2007). For any evaluation of results, allergenicity studies need to consider different sensitivities to allergens e.g. of people from different regions (Goodman *et al.* 2008, Fernandez-Rivas 2006). Furthermore, the future development of GM crops probably will contain more complex traits and/or the increasing use of the stacking of more traits into the same crop, thus increasing the possibility of unintended effects. To face this, in addition to examining features of the introduced proteins themselves, it is essential to examine the overall allergenic potential of the transformed food crop (Bannon & Martino-Catt 2007, Burks & Fuchs 1995, Batista *et al*. 2005).

[2] See also Statement on the analysis of data from a 90-day rat feeding study with MON 863 maize by the Scientific Panel on genetically modified organisms (GMO)
http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178621169104.htm

The guidance document of the EFSA Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants containing stacked transformation events suggests a case-by-case assessment of potentially toxic and/or allergenic effects on humans and animals (EFSA 2007a). Potential effects may arise from additive, synergistic or antagonistic effects of the gene products or of new metabolites and may be particularly relevant where the combined expression of the newly introduced genes has unexpected effects on biochemical pathways.

In addition to pre-market safety assessment, post-market surveillance approaches are discussed as valuable tools to evaluate long-term exposures of GM derived food (Wal *et al.* 2003).

4. Animal Health

Animal health is an extremely broad term. Firstly, there is a need to distinguish between laboratory animals and target animal species (in developed countries mostly food-producing animals) and secondly, between the various animal species (e.g. cattle, sheep, pigs, poultry) and related categories (e.g. dairy cows, beef cattle, heifers, calves as categories of the species cattle). All species/categories have various indicators of health or sickness.

Primarily the aim of this review is focussed to food-producing animals and thus tries to answer the question: “Can animal feeding trials contribute to the assessment of long-term effects?”

4.1. Specifics regarding long-term effects on animal health

The lifespan of food producing animals depends on species and categories of animals as well as the intensity of feeding/keeping (e.g. conventional vs. organic farming). The normal growing/fattening periods (living periods) for meat-producing animals are shown in Table 1. Laying hens and dairy cattle are usually used for longer periods. Laying hens need about 126 – 140 days for growing (pullets) and are kept in the laying period for about 300 – 360 days (one year). Dairy cattle need about 22-36 months for growing (heifers) and are used for up to ten years for lactation (average in Europe two to five years).

Table 1 Examples of lifespan in days for growing/fattening animals in the EU

Livestock	Conventional, more intensive	Organic, more extensive
Chickens for fattening (Broilers)	35 - 42	56 - 84
Turkeys for fattening	56 - 168	70 - 112
Growing/fattening pigs	150 - 300	200 - 400
Fattening calves	80 - 200	-
Growing/fattening bulls	300 - 500	400 - 600

Typically, long-term studies mean in the case of broilers five weeks and in the case of dairy cows five years. In general there is a large difference in long-term effects in various animal species/categories and human beings.

A further aspect of long-term studies deals with the influence of GM-feed on the fertility of animals. More generation experiments are necessary to answer this question.

Based on the difficulties mentioned above the BEETLE report lists in this Annex 4 selected potential long-term effects described in ruminants (cattle, sheep, goats, deer), pigs, poultry (growing and laying chicks, turkeys, ducks, geese) and further food-producing animals (rabbits, fish etc.). But it is known that only a very restricted number of studies with food-producing animals (target animals) comply with the requirements of long-term studies as defined above.

Nutritional values, toxicology, the fate of DNA and of novel proteins are considered for the crops maize, rape seed, soybean, cotton, potato and sugar beet. The relevant traits such as insect resistance and herbicide tolerance (input traits) as well as GM plants with output traits[3] were considered.

During the last few years some reviews on nutritive and safety assessment of feeds from GM-plants were published (e.g. Clark & Ipharraguerre *et al.* 2001, Flachowsky and Aulrich 2001, Aumaitre *et al.* 2002, Flachowsky et al. 2005a, 2007, CAST 2006, Alexander *et al.* 2007). Furthermore the documents by ILSI (Chassy *et al.* 2004, Hartnell *et al*. 2007) and EFSA (2004, 2007b, 2008) summarize also the present state of knowledge regarding feeding GM plants to target animals.

[3] Output traits are intended to enhance the quality of the food and feed, e.g. in the products for consumers. In contrast, input traits are intended for agronomic purposes, e.g. GMHT or Bt plants.

4.2. Nutritional assessment

The nutritional assessment of feeds from GM plants is based on their composition, their digestibility and their nutritive response if fed to food-producing animals. Since 1996 many studies have been carried out to compare feeds from GM plants with their near-isogenic counterparts; mostly GM plants with input traits.

Since 1997, 16 studies have been performed at the Institute of Animal Nutrition in Braunschweig (Germany) to determine the effect of first generation GM plant feeds on the nutrition of dairy cows, growing bulls, growing and finishing pigs, laying hens, chickens for finishing, as well as on growing and laying quails. This research was recently summarized by Flachowsky *et al*. (2007). The majority of feeds tested in the studies (e.g. Bt-maize, HT-maize, HT sugar beet) were grown under similar conditions to their near-isogenic counterparts in the experimental fields of the Friedrich-Loeffler-Institute. The composition of feeds was analysed, and animal studies were used to assess nutritional qualities, including parameters such as digestibility, feed intake, health and performance of target animal species, and effects on the quality of food derived from the animals.

Both chemical analyses and the animal studies reveal no significant differences between GM plant feeds and their near-isogenic counterparts (Flachowsky *et al*. 2007) and hence strongly support their substantial equivalence. The results agree with more than 100 studies published in the literature and reviewed recently by Flachowsky *et al*. (2005a) (Table 2).

In a long term study (10 generations) quails were fed with isogenic or transgenic Bt maize (50% of diet); the study did not show significant effects on body weight of hens, laying intensity or hatchability. Analogous data were registered in a four-generation study with laying hens (Halle *et al*. 2006).

Most experimental designs of previous studies were very simple. The authors compared only one feed with another one and neglected the normal biological range as impressively described in the OECD-consensus documents (OECD 2001a, 2001b, 2002a, 2002b, 2002c, 2003, 2004a, 2004b, 2004c, 2005) or other feed value tables.

Therefore the ILSI[4] documents recommended already in 2003 (Cromwell *et al.* 2003) to compare feeds from GM plants with several commercial counterparts to consider the biological range and to see the effects on the nutritional value. Furthermore the ILSI-document offers recommendations about the number of animals, duration of experiment and further details of experimental design (Table 3). Those recommendations are in accordance with studies for nutritional assessments of feeds, but they are not adequate for long-term studies (except studies with broilers, compare Table 1 and Table 2).

[4] International Life Sciences Institute

Table 2: Summary of published data comparing feeds from GM plants of the first generation (with input traits) with their isogenic counterparts (based on the review by Flachowsky et al. 2005a). The results did not show significant differences in zootechnical parameters between feeds from near isogenic and transgenic plants.

Animal (Species/categories)	Number of experiments	Nutritional assessment
Ruminants		**No unintended effects in composition (except lower mycotoxin concentration in Bt plants)** **No biologically relevant differences in digestibility or animal health, nor any unintended effects on performance of animals or composition of food of animal origin**
Dairy cows	**23**	
Beef cattle	**14**	
Others	**10**	
Pigs	**21**	
Poultry		
Laying hens	**3**	
Broilers	**28**	
Others		
(Fish, rabbits etc.)	**8**	

Table 3: Some 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)"; adapted from Cromwell et al. (2003).

Animals (species/categories)	Number of animals (coefficient of variation 4 to 5 %)	Duration of experiments	Composition of diets	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, feed conversion, carcass data, weight gain**
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 GM plants should be included in high portions in diets and compared with isogenic counterparts

The EFSA-document (EFSA 2008) recommends including an adequate number of commercial varieties to demonstrate the biological range of the parameters which are measured in order to assess any statistically significant differences with respect to the biological relevance between the GM plant and its counterpart.

Table 4: Growth performance and selected slaughtering results of broilers fed a control diet or GM diet (maize event DAS-59122-7 containing Cry 34Ab1 and Cry 35Ab1 genes from Bt-strain and the PAT gene from *Streptomyces viridochromogenes*) as well as the 95 % tolerance interval of all groups (control, GM and 3 commercial hybrids; 60 males and 60 females per treatment, 53, 58 or 70 % maize in starter (0-21 days), grower (22-35 days) or finisher (36-42 days) periods; McNaughton *et al*. 2007).

Criteria	**Control**	**DAS-59122-7**	**S.E.M.**	**Tolerance interval**
Total weight gain (g/animal)	1868	1866	21.0	1625-2092
Mortality (%)	0.83	0.83	0.83	-10.6-14.0
Feed: Gain (g/g)	1.88	1.86	0.02	1.70-2.03
Post-chill carcass (CCW) (g/kg BW)	706	710	3.2	
Males	708	713	4.5	626-792
Females	705	707	4.5	622-791
Breast (g/kg CCW)	269	265	2.2	
Males	270	265	3.0	207-324
Females	267	265	3.0	206-331
Liver weight (g/kg CCW)	35	36	0.6	
Males	35	36	0.8	20-51
Females	34[b]	37[a]	0.8	20-51
Abdominal fat (g/kg CCW)	15	15	0.3	
Males	15	14	0.5	5-24
Females	15	15	0.5	5-24

[a,b] significant differences between control and DAS-59122-7

The study by McNaughton *et al*. (2007) can be considered as an example of how to approach statistical significance and biological relevance (Table 4). No statistically significant differences were observed in any fattening or slaughtering parameters between broilers consuming diets containing DAS-59122-7 maize and those consuming diets containing near-isogenic control grain. One exception was the higher weight of the liver in female broilers fed with DAS 59122 maize (Table 4).

The increase in liver weight of females was not considered significant when adjusting for false discovery rate, and it is unlikely to be biologically relevant because all of the individual liver weights in the female broilers consuming the DAS-59122-7 maize grain diets fell within the tolerance interval calculated from the liver weights of female broilers consuming the reference maize grain (McNaughton *et al*. 2007). Therefore the authors conclude that the performance of broiler chickens fed with maize grain derived from the transgenic maize line DAS-59122-7 that expresses the Cry 34Ab1, Cry 35Ab1, and PAT proteins is nutritionally equivalent to maize grain derived from non-transgenic control maize.

4.3. Toxicology

Altered parameters have only been described in a few toxicity studies with target animals. Scholtz *et al*. (2006, 2008) conducted some metabolic and histological studies in quails fed with either isogenic or Bt maize (50% of diet). Altered enzyme activities and triglyceride concentration were observed in quails fed Bt-maize in the 13th generation of a long-term feeding experiment (Scholtz *et al*. 2006). Later (17th to 20th generation of quails) a few differences in liver weight, hepatocyte nuclear size and AST-activity were observed, but all were within the overall physiological variation. Scholtz *et al*. (2008) concluded that the results do not support the hypothesis that Bt-maize may induce significant effects even when fed over up to 20 generations to quails.

No significant differences were also described by McNaughton *et al*. (2007) in the broiler study referred to above either; although the average liver weight of female chicks increased, all liver weights were in the range seen in chicks fed with commercial maize.

Contradictory conclusions from a rat-feeding study with the transgenic corn MON 863 were presented by Seralini *et al.* (2007) and Hammond *et al.* (2006). Diets of male and female rats contained 11 or 33 % MON 863. Feed intake, weight gain and some physiological functions (58 parameters) were measured after weeks 5 and 14. Altogether 40 of 494 comparisons were statistically significant ($p < 0.05$) in the new analysis by Seralini *et al*. (2007), but the effects were not clear in one direction (sometimes differences in males, sometimes in females; or sometimes after 5 or 14 weeks; or with 11 or 33 % MON 863 in the diet). The authors concluded that the liver and kidneys had been disturbed and that the statistical

methods used by Hammond *et al*. (2006) were not detailed enough to see disruptions in biochemical parameters. Following consideration of the raw data, it is difficult to agree with such conclusions[5]. It appears that clear and general accepted rules to assess statistical results and to see the biological relevance are necessary.

Genetic modifications may be associated with side effects and, the larger the modification, the greater the likely effects (Cellini *et al.* 2004). As the basis for comparative approaches, special animal studies seem to be necessary to examine these questions. Therefore the nutritional and safety assessment of feeds from GM plants of the second generation (GM plants with output traits) is a significant challenge for animal nutritionists. Commercial isogenic counterparts (at least 3) should act as a control to show what is normal in animal studies (EFSA 2008, Flachowsky & Böhme 2005, Hartnell *et al*. 2007).

In contrast, mycotoxin contamination of some GM crops is lower than that of non-GM crops which may be one exception to their substantial equivalence. For example, Bt maize is less severely attacked and weakened by the European Corn Borer and by the Corn Rootworm and might have a greater resistance to field infections, particularly *Fusarium* fungi, which produce mycotoxins. Evidence of reduced mycotoxin contamination in GM crops has been demonstrated in some but not in all cases, as summarized by Flachowsky *et al*. (2005a). In long-term studies, numerous researchers investigated the influence of levels of corn borer infestation of isogenic and Bt maize hybrids on mycotoxin contamination. Most researchers concluded that a lower level of mycotoxin contamination was observed in the Bt maize hybrids, despite the considerable geographical and temporal variation observed.

4.4. The fate of transgenic DNA

The consumption of feeds from GM plants results in the intake of t-DNA and proteins; therefore, studies were conducted on their fate during processing, within the gastrointestinal tract of animals, and the potential extent to which transgenes or their products may be incorporated into animal tissues.

The amount of t-DNA ingested by the animal depends on the concentration of transgenes in feed as well as feed intake. The quantity of DNA in most crops is less than 0.2 g/kg DM (Beever & Kemp 2000), the recombinant gene concentration is much lower (Beever & Phipps 2001, van den Eede *et al*. 2002).

The actual total t-DNA intake may be lower, considering that ensiling GM plants quickly leads to degradation of large plant DNA fragments (Hupfer *et al*. 1999, Aulrich *et al.* 2004). Other

[5] See also Statement on the analysis of data from a 90-day rat feeding study with MON 863 maize by the Scientific Panel on genetically modified organisms (GMO)
http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178621169104.htm

processes such as heat treatment or extraction (Berger *et al*. 2003) will also lower the intake of intact transgenes.

There are many studies available for ruminants, pigs and poultry (more than 30 are published) where the authors analysed the fate of DNA in the digestive tract and in the animal body as summarized by Alexander *et al.* (2007). Some of the studies were done over the whole growing/fattening period and some over the whole life span (see Table 1; e.g. Einspanier *et al.* 2001, Reuter & Aulrich 2003). Most authors did not detect fragments at t-DNA in animal tissues (see Alexander *et al*. 2007, Flachowsky *et al*. 2005a). Also stacked transgenic events (herbicide tolerance [e.g. epsps] and insect resistance [e.g. Cry 1Ab]) in maize silage did not show t-DNA in milk of cows (Calsamiglia *et al*. 2007).

However there are a few studies where t-DNA fragments were detected in tissues. Sharma *et al*. (2006) fed up to 15 % Roundup Ready canola to lambs (n = 11) and pigs (n = 36) to slaughter. Some t-DNA-fragments were detected in the intestinal content samples and in the gastrointestinal tract tissues, but not in visceral tissues (liver, kidney) of lambs or in the spleen from pigs. However, one liver and one kidney sample from the pigs (different animals) were positive for a 278-bp fragment of the transgenic cp 4 epsps.

Mazza *et al.* (2005) fed eight piglets over 35 days with diets containing 50% isogenic or transgenic (MON 810) maize. Blood, spleen, liver, kidney and muscle tissues from all animals were investigated for the presence of plant DNA. Fragments of specific maize genes (Zein, Sh-2) could be detected with different frequencies in all the examined tissues except muscle. A small fragment of the *cry 1Ab* transgene (519 bp) was detected in blood (30%), spleen (10%), liver (14%) and kidney (9% of investigated samples; Mazza *et al.* 2005). The intact *cry 1Ab* gene (3500 bp) or its minimal functional unit (1800 bp) were never detected.

All the authors (e.g. Alexander *et al.* 2007, Mazza *et al*. 2005, Sharma *et al*. 2006) agree that recombinant DNA would be processed in the gut in the same manner as from conventional feed ingested genetic material.

The results indicate that the transfer of DNA-fragments may occur independently of the source and the type of the gene. No data are available showing that t-DNA is characterized by unique behaviour compared to native plant-DNA during feed treatment and in the animals. Intact t-DNA genes or their minimal functional units have not been detected in animal tissues. The authors conclude that it would be unlikely that the occurrence of genetic transfer associated with GM plants is higher than from conventional plants. From the present state of knowledge, the fate of t-DNA of currently registered GM plants does not need to be included in feed safety assessment.

A further aspect is the transfer of DNA (from plants to bacteria) via a non-sexual exchange of genetic material to bacteria in the gastrointestinal tract of humans or animals (Nielsen et al.

1998). In particular the transfer of antibiotic resistance marker genes might lead to potential long-term effects due to loss of therapeutic effects of antibiotics. In this process, several events must occur sequentially, the likelihood of which depends on the availability of intact homologous DNA (see above), the ability of bacteria to undergo transformation with the specific DNA, and the competitiveness of the transformed bacteria. Accordingly, the likelihood of horizontal gene transfer and incorporation of eukaryotic DNA by prokaryotes is extremely low due to genetic incompatibilities and to barriers which prokaryotes evolved to suppress this kind of gene transfer (de Vries & Wackernagel 2005).

Evidence for horizontal gene transfer regarding recombinant plant DNA transferred to bacteria has been obtained up to now only under optimized laboratory conditions (Gebhard & Smalla 1998, de Vries et al. 2004, see 4.4.1 in the Literature Report Environment). Studies of the horizontal gene transfer from plant material to microorganisms in the gastrointestinal tract are rare. However none of studies searching for horizontal gene observed a transfer of functional genes to microorganisms in the gastrointestinal tract of bees (Mohr & Tebbe 2007), cattle (Albrecht 2004) or humans (e.g. Netherwood et al. 2004)

In conclusion, horizontal gene transfer between GM plant material and microorganisms in the gastrointestinal tract is unlikely due to the fate of DNA in the gastrointestinal tract of animal and humans and the evidence that horizontal gene transfer could not be observed under natural conditions. Therefore no potential long-term effects could be expected, in particular regarding the effectiveness of antibiotics. This is supported by the fact that several studies showed that (i) antibiotic resistance is common in microorganisms today (Nwosu 2001; Travis et al. 2006) and (ii) antibiotic resistant pathogenic microorganisms can be isolated from cattle, pigs and hens which have never been fed with feed from GM plants (Guerra et al. 2003).

4.5. The fate of novel proteins

Guidelines have been established by several organizations regarding the assessment of the allergenic risk of each novel protein expressed in a GM plant, prior to market approval (FAO/WHO 2000; Martens 2000; Konig *et al*. 2004). These typically include comparison of amino acid sequence homology of the novel protein to known allergens and digestion of the protein in simulated gastric environments. While allergic reactions are primarily a concern for human consumption of GM foods, certain proteins in soybean have been shown to elicit allergenic reactions in calves and piglets (Van Dijk *et al*. 1988). It is common practice to apply heat to some feedstuffs to inhibit the actions of anti-nutritional proteins. Such is the rationale for toasting soybean, which contains trypsin inhibitors and haemagglutinins.

The fate of novel (transgenic) proteins in feed from GM plants consumed by animals has also generated interest arising from consumers' questions. Recently an extensive review on the food safety of proteins from agricultural biotechnology was edited by Hammond (2008).

Seale and English (2008) analysed the mode of action of bacterial protein toxins and concluded that an interruption of the life cycle at any single step can render the protein nontoxic. For example, insecticidal toxins from *Bacillus thuringiensis* (Bt) have been shown to be rapidly degraded by simulated gastric fluid in *in vitro* studies (Astwood *et al.* 1996). Transgenic proteins in feeds from Bt plants are similarly degraded as bacterial proteins (Federici & Siegel 2008). This is in contrast to the stability of Bt toxins in insect guts where proteolytic activation of the protein occurs, leading to toxicity to insect cells. Betz *et al.* (2000) tested the acute and subchronic oral toxicity of Bt proteins. The "no-observed-adverse-effect-levels" (NOAEL) were in all cases the highest dosages that were tested (e.g. 4000 mg Cry 1Ab-Protein per kg). Hammond and Cockburn (2008) summarized the NOAEL in Acute High-Dose-Studies and in subchronic feeding studies with different proteins introduced into crops developed through agricultural biotechnology. These studies confirm the absence of oral toxicity even though the protein preparations were administered at very high dosage levels.

The amount of transgenic protein ingested by livestock depends on the concentration of the protein in the feed and the amount of feed intake. Transgenic protein concentration varies with the transgenic event and the type of plant tissue in which it is expressed (Stave 2002). The levels of introduced proteins in seeds of biotechnology-derived crops vary between 0.3 (*cry 1Ab* in corn; Yieldgard Corn Borer) and up to 580 mg/kg seed (CP4 EPSPS in cotton; Roundup Ready Flex; Hammond & Cockburn 2008).

Post-harvest feed processing can alter (likely decrease) transgenic protein concentrations (Alexander *et al*. 2007). Anything affecting the concentration of transgenic protein in feedstuffs will also alter the total transgenic protein intake by livestock. Similarly, feed intake, which varies with animal and diet, will influence protein intake. Assuming intake of 10 kg of MON 810 grain per day, total daily Cry 1Ab intake by a dairy cow will be approximately 3.1 mg (10,000 g x 0.31 µg/g fwt = 3100 µg or 3.1 mg, Alexander *et al.* 2007). Feed and protein digestion are also species-dependent. Thus, the presence of transgenic protein throughout animal digestive tracts and potentially in livestock products depends on multiple factors.

Alexander *et al*. (2007) summarized many studies where the authors analysed the fate of novel proteins in ruminants, pigs and poultry. Results from the studies can be summarized as follows:

- In ruminant feed, proteins are mostly degraded in the rumen, and microbial protein and bypass proteins[6] are degraded by enzymes in the smaller intestine, similar to non-ruminants.
- The chemical and physiological properties (including microbial and enzymatic degradation) of novel proteins have been intensively tested.
- Intact novel proteins have not been detected outside of the digestive tract in target animals (also not in food-producing animal tissues and products).
- There is no evidence that novel proteins are characterized by unusual chemical/physical properties distinct from native proteins.

4.6. Improvement of the risk assessment

Feeds from GM plants have been fed to various species/categories of target animals/food producing animals. However there is an ongoing discussion on the quality of the risk assessment and the used methods for food and feed derived from GM plants. Some studies were done over the whole lifespan of the animals (e.g. ≈ 35 days in broilers), but the majority of studies were carried out over a limited period not covering the whole lifespan (especially in long-living animals such as laying hens, dairy cows etc.). A further weakness of most animal feeding studies is the experimental design. Most authors compared only feeds from GM plants with their near isogenic counterpart and did not include commercial varieties to get an impression of the biological range of the investigated parameters. Most feeding studies with food-producing animals were done according to the national rules for such experiments (e.g. to measure the digestibility or the feed value, the feed conversion, the animal yield etc.).

Despite some shortcomings mentioned above the following conclusions can be drawn:

- Is recommended to use an adequate number of commercial crop varieties in feeding trials in order to cover the biological range of the measured parameters. More comparators will help to assess any statistical differences between the GM plant and its counterparts with respect to the biological relevance.
- More studies are necessary for nutritional assessment of feeds from GM plants with output traits.

In the future GM crops with output traits are expected. Therefore more long-term feeding studies with target animals are recommended with feeds from GM plants with these output traits of the so called 2nd GM plant generation.

[6] 'bypass protein' is a commonly used term to refer to dietary protein that is not degraded in the rumen of ruminant animals

4.7. Conclusion

Potential long-term effects of GM plants on animal or human health could not be deduced from recently published literature except for a potential increase in allergenicity due to new or increased exposure to allergens. However, this aspect is already sufficiently covered in the risk assessment. Furthermore food and feed from GM plants do follow a much stronger nutritional and safety assessment than feed from conventionally bred plants. However there is an ongoing discussion regarding the quality of the risk assessment and the methods used for food and feed derived from GM plants. Based on this literature review, questions for an Online Survey Health were prepared to continue the assessment begun by the BEETLE team. Among other things, experts were asked how the risk assessment procedure might be improved.

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