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CONSIDERATIONS FOR THE SAFETY ASSESSMENT OF ANIMAL FEEDSTUFFS DERIVED FROM GENETICALLY MODIFIED PLANTS

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No. 1, Consensus Document on Key Nutrients and Key Toxicants in Low Erucic Acid Rapeseed (Canola) (2001)

No. 2, Consensus Document on Compositional Considerations for New Varieties of Soybean: Key Food and Feed Nutrients and Anti-nutrients (2001)

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No. 4, Consensus Document on Compositional Considerations for New Varieties of Potatoes: Key Food and Feed Nutrients, Anti-Nutrients and Toxicants (2002)

No. 5, Report of the OECD Workshop on the Nutritional Assessment of Novel Foods and Feeds, Ottawa, February 2001 (2002)

No. 6, Consensus Document on Compositional Considerations for New Varieties of Maize (*Zea mays*): Key Food and Feed Nutrients, Anti-Nutrients and Secondary Plant Metabolites (2002)

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OECD Environmental Health and Safety Publications

Series on the Safety of Novel Foods and Feeds

No. 9

Considerations for the Safety Assessment of Animal Feedstuffs Derived from Genetically Modified Plants

Environment Directorate

Organisation for Economic Co-operation and Development

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FOREWORD

The OECD's Task Force for the Safety of Novel Foods and Feeds decided at its first session, in 1999, to focus its work on the development of science-based ***consensus documents,*** which are mutually acceptable among member countries. These consensus documents contain information for use during the regulatory assessment of a particular food/feed product. In the area of food and feed safety, consensus documents provide information on nutrients, anti-nutrients or toxicants, the use for food/feed and other relevant information.

This document addresses considerations in the safety assessment of GM foodstuffs, including the fate of DNA and protein in animal feeding, animal feeding studies, and future GM feedstuffs. As well, there is background material on the various organisms and traits constituting GM plants used as animal feeds.

This document was prepared by Canada and the United Kingdom as the lead countries.

The Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology has recommended that this document be made available to the public. It is published on the authority of the Secretary-General of the OECD.

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PREAMBLE

Food and feed products of modern biotechnology are being commercialised and marketed in OECD member countries. The need has been identified for detailed technical work aimed at establishing appropriate approaches to the safety assessment of these products.

At a Workshop held in Aussois, France (OECD, 1997), it was recognised that a consistent approach to the establishment of substantial equivalence might be improved through consensus on the appropriate components (e.g. key nutrients, key toxicants and anti-nutritional compounds) on a crop-by-crop basis, which should be considered in the comparison. It is recognised that the components may differ from crop to crop. The Task Force therefore decided to develop consensus documents on compositional data. These data are used to identify similarities and differences following a comparative approach as part of a food and feed safety assessment. They should be useful to the development of guidelines, both national and international and to encourage information sharing among OECD member countries.

These documents are a compilation of current information that is important in food and feed safety assessment. They provide a technical tool for regulatory officials as a general guide and reference source, and also for industry and other interested parties and will complement those of the Working Group on Harmonisation of Regulatory Oversight in Biotechnology. They are mutually acceptable to, but not legally binding on, member countries. They are not intended to be a comprehensive description of all issues considered to be necessary for a safety assessment, but a base set for an individual product that supports the comparative approach. In assessing an individual product, additional components may be required depending on the specific case in question.

Although this publication is not a Consensus Document dealing with a specific crop like those which have already been published, it is complementary to those documents.

In order to ensure that scientific and technical developments are taken into account, member countries have agreed that consensus documents will be reviewed periodically and updated as necessary. Users of these documents are invited to provide the OECD with new scientific and technical information, and to make proposals for additional areas to be considered. ***A short, pre-addressed questionnaire is included at the end of this document. The information requested should be sent to the OECD at one of the addresses shown.***

THE ROLE OF COMPARATIVE APPROACH AS PART OF A SAFETY ASSESSMENT

In 1990, a joint consultation of the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) established that the comparison of a final product with one having an acceptable standard of safety provides an important element of safety assessment (WHO, 1991).

In 1993 the Organisation for Economic Co-operation and Development (OECD, 1993) further elaborated this concept and advocated the approach to safety assessment based on substantial equivalence as being the most practical approach to addressing the safety of foods and food components derived through modern biotechnology (as well as other methods of modifying a host genome including tissue culture methods and chemical or radiation induced mutation). In 2000 the Task Force concluded in its report to the G8 that the concept of substantial equivalence will need to be kept under review (OECD, 2000).

The Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology in 2000 (FAO, 2000) concluded that the safety assessment of genetically modified foods requires an integrated and stepwise, case-by-case approach, which can be aided by a structured series of questions. A comparative approach focusing on the determination of similarities and differences between the genetically modified food and its conventional counterpart aids in the identification of potential safety and nutritional issues and is considered the most appropriate strategy for the safety and nutritional assessment of genetically modified foods. The concept of substantial equivalence was developed as a practical approach to the safety assessment of genetically modified foods. It should be seen as a key step in the safety assessment process although it is not a safety assessment in itself; it does not characterise hazard, rather it is used to structure the safety assessment of a genetically modified food relative to a conventional counterpart. The Consultation concluded that the application of the concept of substantial equivalence contributes to a robust safety assessment framework.

A previous Joint FAO/WHO Expert Consultation on Biotechnology and Food Safety (1996) elaborated on compositional comparison as an important element in the determination of substantial equivalence. A comparison of critical components can be carried out at the level of the food source (i.e., species) or the specific food product. Critical components are determined by identifying key nutrients and key toxicants and anti-nutrients for the food source in question. The comparison of critical components should be between the modified variety and non-modified comparators with an appropriate history of safe use. The data for the non-modified comparator can be the natural ranges published in the literature for commercial varieties or those measured levels in parental or other edible varieties of the species (FAO/WHO, 1996). The comparator used to detect unintended effects for all critical components should ideally be the near isogenic parental line grown under identical conditions. While the comparative approach is useful as part of the safety assessment of foods derived from plants developed using recombinant DNA technology, the approach could, in general, be applied to foods derived from new plant varieties that have been bred by other techniques.

EXECUTIVE SUMMARY

1. Animal feed represents an important point of entry of plant products into the food chain. Consequently, it is important that novel feedstuffs be as carefully assessed for safety as those products used directly as human food. This document is intended to provide considerations in the safety assessment of genetically modified (GM) feeds derived from plants, based on the scientific issues involved.

2. The safety assessment of GM food and feed share many common elements, notably the molecular characterisation of the introduced genetic elements, the expression of the novel traits and the impact of these in the newly modified plant. These have been extensively considered elsewhere. This document focuses on those aspects of particular importance to the safety assessment of GM feed, in particular, the wholesomeness of the feed for livestock, and the safety for consumers of products (e.g. meat, milk, eggs) obtained from livestock whose diet includes GM feedstuffs.

3. Establishing the degree of equivalence to other (conventional) varieties is a useful starting point for a safety assessment, and is as relevant to feed issues as to those of foods. Consideration should be given to the differential expression of introduced traits in the plant in the selection of material for comparison, particularly when plant parts not used for food purposes are included in animal feed (e.g. maize stover, cottonseed meal). Studies intended to demonstrate the safety of the isolated product of any introduced gene should take account of the maximum concentration found in any plant part or by-product consumed as feed and the consequent exposure of the animal.

4. The fate of DNA and novel proteins in the digestive tract of both humans and animals has been raised as an issue of concern. Intact DNA and protein can be detected in minimally processed feedstuffs such as hay and silage, but may be degraded by typical feed manufacturing processes. Both DNA and protein are usually extensively digested when consumed by the animal. However, evidence of the degradation of protein during feed preparation should not automatically be assumed to confer safety. Any introduced and expressed protein should be separately examined for its toxic potential regardless of its susceptibility to breakdown.

5. Fragments of non-transgenic plant DNA have been detected in animal tissues including milk. However, there is no basis to suppose that transgenic DNA poses hazards any different to other sources of DNA and the possibility of incorporation of functionally intact DNA (or protein) into animal products is extremely remote. Consequently, unless there is reason for specific concern, the routine testing of animal products for newly introduced DNA or any expressed products is not considered necessary.

6. Many new varieties of plants used as feedstuffs are introduced onto the market based on agronomic and compositional data alone. Feeding trials to confirm safety and/or nutritional value are generally unnecessary. To date, all approved GM plants with modified input traits (e.g. herbicide tolerance) have been shown to be compositionally equivalent to their conventional counterparts. Feeding studies with feeds derived from the approved GM plants have shown equivalent animal performance to that observed with the non-GM feed. Thus the evidence to date is that for GM varieties shown to be

compositionally equivalent to conventional varieties, feeding studies made with target livestock species will add little to a safety assessment and generally are not warranted.

7. For plants engineered with the intention of significantly changing their composition/nutrient bioavailability and thus their nutritional characteristics, suitable comparators may not be available for a nutritional assessment based solely on compositional analysis. In such cases feeding trials with one or more target species may be useful to demonstrate wholesomeness for the animal. Under these circumstances, the duration of feeding studies should be for the production cycle of the animal. Such feeding studies may be usefully supplemented with shorter-term balance studies to confirm that the modification produces the intended nutritional benefit (e.g. higher metabolisable energy value, improved nitrogen retention).

8. In general, the use of a comparative growth study for the screening for any unintended effects of the genetic modification with adverse consequences for the host animal and consumers of animal products not detected by chemical analysis is not warranted for GM varieties any more than for conventionally-derived varieties. However, if concerns remain regarding unintended effects of a particular modification, broiler chicks are useful for comparative growth studies. Because of their rapid weight gain, broiler chicks are particularly sensitive to any change in nutrient supply or the presence of toxic elements in their feed and are particularly useful for this purpose. The young of other livestock tend not to show such rapid growth rates, but may, on occasions, form a more appropriate model. For feedstuffs intended for aquaculture, a fish species such as the catfish may substitute. Milk production is better used in place of growth rate for feed primarily intended for lactating ruminants. It is important to note that standardised and internationally recognised conditions for such tests have not been established.

9. In time, it may be possible to detect unintended effects by non-targeted profiling techniques based on the measurement of the transcriptome or proteome. Alternatively, measures to detect unintended effects may be rendered unnecessary by improved molecular characterisation and understanding of the implications of the molecular events for the metabolism of the plant.

10. Post-market surveillance may be easier to undertake in relation to feed than food. Control of diets, the ability to monitor animal health and the various assurance schemes used in some areas to track animal products to the point of sale would aid the evaluation of long term effects of GM feeds. However, given that there is no basis to indicate any difference in long term effect of GM feeds from those of conventionally-derived feeds, it is not clear under what circumstances such post-marketing surveillance would be warranted. In addition, given the lack of a theoretical basis for the general transfer of functional protein or DNA to animal products, and in the absence of any documented adverse response to products of animals fed GM feedstuffs, post-market surveillance of consumers appears to be of very limited value. Post-market surveillance is likely to be useful only when designed to answer a specific question.

11. Recombinant technology has greatly expanded the opportunities to use plants for the production of a multitude of non-food/feed products. Some of these products raise serious issues of feed security. If a GM plant used for the production of industrial products has a conventional counterpart traditionally used for feed purposes there is a risk of unauthorised material entering the food chain. For this reason a safety assessment for all parts of the industrial GM plant that might enter the feed chain should be conducted. This would inform the risk manager and allow actions proportionate to the risk to be taken. Alternatively, if measures have been implemented to prevent entry of plants producing unauthorized products into the feed supply, a safety assessment of that plant may not be necessary. The processes needed to ensure that the material does not become a component of feed should be proportional to the associated risk.

12. For the present, agronomic (input) traits will continue to dominate new introductions. However, transgenic plants designed specifically to address issues of feed quality, to resist stress and to grow in more marginal areas have reached the stage of field trials.

13. The safety assessment of the "next generation" feeds will involve a case-by-case approach with the probable need to introduce specific elements appropriate to each trait. Sound experimental designs for the assessment of these "value-added" novel products should be required.

14. The approach to safety assessment of novel feeds by national authorities range from use of existing food, feed or environmental legislation to the creation of legislation specific to novel foods and/or feeds. A number of OECD member countries are in the process of developing new legislation on assessment and labelling of GM feeds. Approaches to legislation include establishment of new GM-specific food and feed laws, updating or establishing feed laws, to include GM feeds, or simply considering that the definition of food includes feed.

15. Whatever the regulatory approach taken by national authorities, it is recognised that an assessment of the safety of GM products used as animal feed that is universally accepted and applied and which can be shown to be rigorous in its approach, is fundamental to retaining consumer confidence in animal products.

SECTION 1: SCOPE AND PURPOSE

1.1 Scope and purpose of the document

16. Animal feed represents an important point of entry of plant products into the food chain. Consequently, it is important that novel feedstuffs are as carefully assessed for safety for target animals and for consumers of animal products as those products used directly as human food. Novelty can be introduced into the feed chain in a number of ways: by the novel use of existing resources or by the introduction of novel traits to existing feedstuffs. This consensus document focuses on the latter and considers only those feed ingredients derived from genetically modified (GM) plants. The document is intended to provide considerations in the safety assessment of GM feedstuffs, based on the scientific issues involved.

1.2 Relationship to food safety assessment

17. Many feeds for animals make use of the same plants (or by-products of the same plants) used for human food. Consequently many elements of a safety assessment are common to both. Both require a precise characterisation of the introduced genetic elements, information on substances present as a result of the modification and evidence whether detectable unintended effects occurred because of the insertion(s). These issues have been extensively considered in the context of GM foods. With the exception of genes and gene products unique to feeds and the detection of unintended effects, these common issues will not be further considered here. The approach taken to these common issues is fully described in the reports of the Codex Ad Hoc Intergovernmental Task Force on Foods derived from Biotechnology and the various expert consultations and the references therein (FAO/WHO, 1996, 2000, 2001).

18. Feed use does introduce different concerns and parameters to the safety evaluation than that of human food use. In particular, the assessment of animal feeds must take into account any risk to the animals consuming the feed and any indirect risk to the consumer of animal products (meat, milk and eggs). There is also a potential for a greater exposure of animals to a GM plant or plant by-product than humans as it may comprise a large percentage of the diet fed on a daily basis, often for the complete life span of the animal. A broiler chick for example has a daily consumption of approximately 60g maize kernel/kg body weight and a growing pig about 45g/kg body weight compared to an adult human who consumes about 0.2g/kg body weight each day. In addition, livestock are fed plants and components of plants that humans do not consume and exposure to novel gene products can differ from that of humans. However, feed use does allow the GM plant material to be fed directly to target species, providing tests of wholesomeness and introducing the possibility of using the delivery of nutrients as part of the safety assessment.

SECTION 2: GM PLANTS USED AS ANIMAL FEED

2.1 Established patterns of use of plants for which approved GM varieties exist

19. Currently, maize is the only cereal that has GM varieties in commercial production. It is also the cereal of choice for animal feed in most parts of the world, being replaced by other cereals, particularly wheat or barley, only when local availability and price favour substitution. Use of maize as animal feed accounts for approximately 75% of a total world production of 600 million tonnes annually. Consequently, there is a well established global market for this commodity. Approximately 79 million tonnes were exported in 2001 (FAOSTAT Agriculture Data - http://apps.fao.org/page/collections?subset=agriculture). Maize may be fed as the whole grain or as various by-products of the corn milling industry or as whole crop silage (OECD, 2002). Corn-soybean diets are extensively used for poultry and for growing pigs and maize silage for dairy cattle. In each case the maize provides the major energy source. The protein-enriched maize gluten feed and gluten meal are valuable by-products of starch extraction, the former used for pigs and ruminants and the latter for poultry.

20. Soybean dominates the oilseed market with a global annual production in excess of 150 million tonnes. Feed use accounts for 97% of total production, mostly for domestic animal production, but some 40 million tonnes are traded annually, going to areas, such as the EU, generally not suited to soybean production. It is the preferred source of protein for most pig and poultry production diets (78% of total soybean production) with the remainder being used for ruminants, companion animals and in aquaculture (OECD, 2001). Because of the presence of anti-nutritional factors, there is very limited consumption of the unprocessed bean. Most is fed as the protein-enriched seed meal left after extraction of soybean oil. However, treated whole beans, hulls and the vegetative parts of the plant in fresh or conserved state are also used to a limited extent, primarily for cattle. Such use is domestic and rarely found outside the producer countries.

21. Seed meals left after oil extraction from other oilseed crops can also provide valuable sources of protein and energy for animals, and animal feeding represents the most cost-effective means of disposal of these by-products. Approximately 20 million tonnes of low erucic acid rapeseed meal are used annually in the rations of all classes of livestock, but the high fibre content relative to soybean limits inclusion levels. Rapeseed and rapeseed oil are also occasionally used in small amounts to boost the energy content of some non-ruminant diets. Cottonseed meal (12 million tonnes/year) is fed predominantly to ruminants, which are protected from the toxic effects of gossypol by the presence of a rumen microbial flora capable of its degradation.

22. Other commodities or their by-products have a lesser and often localised role in animal feeding. Of relevance to this document are fodder beet (a sub-species of *Beta vulgaris*) and potato, both of which have approved GM varieties. Fodder beet, roots and tops (leaves), are used exclusively for ruminant feed, traditionally in areas where climatic conditions are less suitable for cereal production. The extent of use of potatoes as a feed ingredient varies considerably depending on locality. When used, either as whole tubers or trimmings, they are generally fed raw to ruminants but are heat-treated before feeding to pigs. Potatoes

used for starch production also generate by-product streams that have found an outlet in animal feeding. These include the fibre-rich pulp remaining after starch extraction and protein-enriched liquid feed used primarily, but not exclusively, by the pig industry.

2.2 Traits introduced into plants used in animal feeding by recombinant DNA technology

23. Virtually all of the GM plants currently grown for commercial production have been modified to improve their agronomic properties. Traits have been introduced to confer resistance to common pests (European corn borer, Colorado beetle), to viral pathogens or to introduce tolerance to selected herbicides for better weed control (Table 1). At present most varieties carry a single introduced trait, but there is growing trend towards "stacked-gene varieties" carrying two or more traits, either introduced simultaneously or obtained by crossing single trait varieties.

Table 1. GM plants used as feedstuffs that have obtained regulatory approval in at least one country grouped by introduced property.

Introduced genetic material	Introduced property	Recipient crops
Insect resistance		
Genes encoding a truncated endotoxin produced by strains of *Bacillus thuringiensis*:	Resistance to attack by:	
cry1A(b) and *cry1A(c)*	Lepidoptera (including European corn borer)	Maize, cotton
cry9C[1]	Lepidoptera (including European corn borer)	Maize
Cry1F	Lepidopteran (including European corn borer, corn earworm, fall army worm and black cutworm)	Maize
cry3A	Coleoptera (including Colorado beetle)	Potato
Virus resistance		
Gene encoding a viral coat protein	Resistance to attack by potato virus Y	Potato
Viral replicase gene	Potato leaf curl virus	Potato
Herbicide tolerance		
epsps (bacterial or engineered plant gene) (more rarely *gox* encoding an oxidoreductase)	Tolerance to glyphosate	Sugar and fodder beet, soybean, rape, cotton, maize
pat encoding PPT acetyl transferase	Tolerance to glufosinate ammonium	Maize, soybean, rice, sugar beet, rape
oxy encoding nitrilase	Tolerance to oxynil herbicides	Cotton, rape
csl-1 encoding an acetolactose synthase	Tolerance to sulphonylurea	Cotton, flax
Modified *als* genes encoding an acetolactose synthase	Tolerance to imidazolines	Maize, rape
Male sterility		
barnase encoding a ribonuclease	Male sterility (pollen)	Maize, rape
barstar encoding a ribonuclease inhibitor	Fertility restorer	Maize, rape
Modified composition		
Sense suppression of *gmFad2-1* encoding a δ-12 desaturase	Increased content of oleic acid	Soybean
Antisense suppression of *gbss* (granule-bound starch synthase)	High amylopectin starch	Potato
Bay *te* encoding 12:0 ACP thioesterase	Increased content of lauric and myristic acids	Rape

Source: Aumaitre *et al*., 2002, Note: some of the plants included in the table have yet to be released on the market.

[1]now removed from the market.

24. To date only a few GM varieties with modified composition have been approved for commercial production. The first to be accepted in the USA in 1995 was an oilseed rape modified to produce high concentrations of lauric acid in the oil for use as food and in the detergent industry. Both this construct and the high oleic acid soybean approved for release in the USA in 1997 and in Canada in 2000 have yet to be grown in commercial quantities.

2.3 The global market – production, use and export of GM plants used in feedstuffs

25. The global area devoted to transgenic plants in 2001 was estimated as 52.6 million hectares (130 million acres), an approximate 19% increase on the previous years plantings. This area increased by a further 11.6% in 2002 reaching 58.7 million hectares (145 million acres). Four countries, USA, Canada, Argentina and China grew 99% of the global crop with a further twelve countries accounting for the remaining 1%. Of these only South Africa and Australia grew more than 100,000 hectares (James, 2002).

26. The USA, which produced 66% of the world total plantings of transgenic crops in 2002, showed a net gain of 3.3 million hectares of crops compared to 2001 as a result of increased plantings of transgenic soybean, maize and cotton. Figures derived from US seed sales in 2002 indicate that transgenic cotton now represents 71% of all cotton seed sales, transgenic maize varieties 32% of the total maize seed sales and transgenic soybean 74% of the total sales (NASS, 2002).

27. The second largest producer of GM varieties, Argentina, showed an overall gain of 1.7 million hectares in 2002 compared to the previous year, which resulted from significant increases in the area of transgenic soybean and cotton and, to a lesser extent, maize. Herbicide resistant soybean now represents greater than 95% of all soybeans produced in Argentina and, for the first time, more than half (51%) of the 72 million hectares of soybeans grown world-wide were GM varieties. In China, the area of transgenic cotton showed a substantial increase rising from 1.5 million hectares in 2001 to 2.1 million hectares in 2002. Because of a high initial uptake of transgenic canola varieties, subsequent use of transgenic crops in Canada has grown relatively slowly compared to other countries. The total area devoted to transgenic varieties in Canada was 3.0 million hectares in 2000, rising to 3.2 million hectares in 2001 and to 3.5 million hectares in 2002.

28. A major factor in determining future demand for non-GM/GM varieties will be the feed market to which the bulk of all maize and soybean is destined. Establishing an assessment of the safety of GM products used as animal feed that is universally accepted and applied and which can be shown to be rigorous in its approach is fundamental to retaining consumer confidence in animal products.

SECTION 3: ASSESSMENT OF GM FEEDSTUFFS

3.1 Characterisation

29. The concept of substantial equivalence forms a useful conceptual basis for a safety assessment, and is as relevant to feed issues as to those of foods. However, the choice of comparators (see 3.2), the key characteristics selected and interpretation of compositional data can differ among different authorities. The OECD consensus documents on common plants that are used for food and feed provide a valuable source of information and can be used to ensure a consistency of approach (http://www.oecd.org/biotrack). These documents delineate key nutrients, antinutrients and toxins contained in common food/feed plants and their products and by-products from common manufacturing processes that are used for food and feed purposes.

30. As indicated in paragraph 17, the characterisation of the host plant, the molecular characterisation of the donor genetic elements, the expression of the novel traits and the impact of these in the newly modified plant are well established elements in the safety assessment of both GM food and GM feedstuffs. While it is not the intention to duplicate what has been extensively covered within the context of GM food safety assessment, animal feedstuffs make use of plants and plant parts not directly consumed by humans. Often these plant parts are not used for human food but are consumed by livestock. In addition, the nature of the genetic modification may be of relevance only to livestock feeding as in the case of forages. As a consequence, although the principles underpinning the safety assessment of food and feed may be similar, they may differ in detail and emphasis.

3.2 By-products and plant parts versus the whole plant

31. Feed manufacturers make considerable use of by-products from other industries using GM plants in which the introduced DNA/novel protein may be virtually absent or, as in the case of seed meals, considerably concentrated (Table 2). This has implications for levels of exposure, choice of comparators and for determining the concentration of novel protein used in acute/sub-chronic toxicity studies made with newly introduced and expressed proteins.

Table 2. Typical protein, oil and cell wall contents (g/kg dry matter) of maize kernel and its by-products of processing fed to animals

Fraction	Protein	Oil	Cell wall (NDF[1])
Whole kernel	102	42	117
Germ meal	108	64	224
Gluten feed	220	51	383
Gluten meal	669	69	84
Fibre	147	42	538

[1]Neutral detergent fibre

32. Consideration also should be given to the differential (spatial and temporal) expression of introduced traits in the plant, particularly when plant parts not used for food purposes are included in animal feed (e.g. maize stover, cottonseed meal). Promoters may be chosen to preferentially express a trait in a given part of the plant prone to pathogen attack or, conversely, to reduce or avoid expression in those parts consumed as human food. For example, a GM plant may be constructed to express the Bt toxin only (or preferentially) in parts of the plant, such as the leaves, which are subject to first generation insect attack (Table 3). While such an approach may serve to reduce human exposure, the exposure of animals that consume most parts of a plant may be substantially increased. As Table 3 shows, dairy cattle consuming maize stover (aerial vegetation) have a substantially greater exposure to the Cry1A(b) protein than animals fed only maize kernel.

Table 3. Concentration of Cry1A(b) protein (µg/g fresh weight tissue) in YieldGard™ (event MON 810) hybrid maize.

Plant tissue	Parameter	1994 USA (6 sites)	1995 USA (5 sites)	1995 EU (4 sites)	1996 EU (3 sites)
Leaf[1]	Mean	9.35	8.95	8.60	12.15
	Std. Dev.	1.03	2.17	0.74	3.86
	Range	7.93-10.34	5.21-10.61	7.59-9.39	7.77-15.06
Forage/whole plant[2]	Mean	4.15	3.34	4.80	4.88
	Std. Dev.	0.71	1.09	0.75	0.52
	Range	3.65-4.65	2.31-4.48	4.11-5.56	4.32-5.34
Kernel	Mean	0.31	0.57	0.53	0.41
	Std. Dev.	0.09	0.21	0.12	0.06
	Range	0.19-0.39	0.39-0.91	0.42-0.69	0.35-0.46

Source: Sanders *et al.*, 1998 with additional data on standard deviation and range kindly provided by the authors.

[1]The mean was calculated from the analyses of plant samples from each field site.

[2]For the 1994 US trials, values represent the analysis of whole plants; for the remaining trials, values represent the analysis of forage tissue. Whole plants were collected two weeks after pollination; forage samples were collected at the soft dough or early dent stage. Means were determined from the analysis of plant samples from one site in the US and all sites in the EU. A plant sample was a pool of two individual plants.

33. Studies intended to demonstrate the safety of the product of any introduced gene should take account of the *maximum* level found in any plant part consumed by animals or in any by-product used as a feed ingredient. A margin of safety then should be established based on this value. This should be done regardless of the frequency of use of the plant part or by-product, or the potential for disruption of protein during any extraction process.

34. Whilst it is desirable to include unprocessed plant material in studies intended to demonstrate tolerance to, or the wholesomeness of, the GM plant, this may not always be possible. Some seeds, such as soybean, are processed before use because of the presence of known anti-nutritional factors (see OECD

soybean consensus document; OECD, 2001). In such cases, the processed seed should be substituted to avoid the possibility of any adverse effects being masked by the effects of the known antinutrients or toxicants. Extrapolation between plant parts is possible, but studies should reflect the botanical nature of the feed and, if necessary, separate studies should be made with seeds and vegetative material. Consideration should also be given to by-product streams in which protein is concentrated or in which lipophilic or hydrophilic metabolites could accumulate. Where a variety of such by-products are produced it may be necessary to include in studies only those at the extreme (e.g. maize gluten meal in preference to gluten feed).

SECTION 4: FATE OF DNA AND PROTEIN IN ANIMAL FEEDING

35. The vast majority of proteins in feeds are not known to present any safety hazards to animals and only when DNA *per se* is consumed in high concentrations are the breakdown products of nucleic acid hazardous to humans (Simmonds, 1990). However, the introduction of GM plants into the food chain has rekindled interest in the fate of DNA in the digestive tract. The use of sensitive molecular biological techniques not previously available has demonstrated that DNA can survive in polymeric form to a far greater extent than was previously recognised and that DNA fragments can be taken up both by host tissues (Schubbert *et al*., 1994, Hohlweg and Doerfler, 2001) and the resident microflora (Mercer *et al*., 2001). The particular issue of the possible transfer of functional DNA to micro-organisms has become associated with recombinant technology, largely because of the use of genes coding for antibiotic resistance as a means of selection.

4.1 Survival of DNA/protein during the harvest and storage of feedstuffs

36. Grain harvested at maturity generally has a relatively low moisture content and can be stored without further treatment until required for use. Little degradation of either protein or DNA occurs under ordinary storage conditions. Vegetative parts of the plant and whole plants harvested before grain maturity have higher moisture content and require further treatment to ensure their stability unless used immediately for grazing animals. Stabilisation may simply rely on air drying (more rarely artificial drying) over a period of days to produce hay or haylage. Ensiling is the preferred method of conservation for plants with a high moisture content and/or high soluble carbohydrate content and the silage so produced is typically used for the feeding of ruminants. Microbial fermentation of soluble sugars and protein rapidly reduces the pH to a point when all further microbial growth is inhibited. Enzymes, micro-organisms, organic acids or added molasses may be added to encourage a rapid development of an acid-producing flora.

37. Autolysis of protein begins immediately after cutting and is retarded by rapid wilting and enhanced by slow wilting. Protein losses continue during ensiling to an extent highly dependent on the ensiled material, the microbial flora that develops and the rate of pH reduction (Fairburn *et al*., 1988). Cry1A(b) protein could not be detected in maize silage prepared from *cry1A(b)* expressing plants (Fearing *et al*., 1997). DNA appears less affected than protein and intact DNA with no evidence of lower molecular weight degradation products has been extracted from wide variety of harvested vegetative materials and from ryegrass and maize silages (Chiter *et al*., 2000, Table 4). Single gene studies reflect the studies made on total DNA stability. The *cry1A(b)* gene was detected in maize seven months after ensiling by amplification of a 211 bp sequence (Hupfer *et al*., 1999). Similarly *rubisco SS*, a plant plastid gene, could be readily amplified from maize silage (Chiter *et al*., 2000).

38. Intact DNA and protein in crops conserved by air drying or ensiling or in pulps obtained by low-temperature aqueous extraction (e.g. sugar beet) can be detected throughout the normal duration of storage (Chiter *et al*., 2000). Unless subject to some other form of processing, which is unlikely in the case of silage or hay, DNA and protein from such sources are consumed by the animal largely in an intact form.

Table 4. Degree of damage to DNA recovered from commercially sourced feed ingredients. Intact >20kb detected, degraded <100 bp.

Commodity	No of samples examined	Degree of damage
Linseed	5	Intact
Linseed - expelled	2	Degraded
Soybean	8	Intact
Soybean - extracted	7	Degraded
Maize kernel	2	Intact
Forage maize	2	Intact
Maize silage	4	Intact
Maize gluten meal	2	Degraded
Rapeseed	3	Intact
Rapeseed - extracted	3	Degraded
Rapeseed-expelled	3	Degraded

Source: Forbes *et al*., 2000.

4.2 Survival of DNA/protein during feed manufacture

39. Manufactured feeds are subject to shear forces and heat treatments of varying severity (pelleting, extrusion, expansion etc.). However, manufacturing processes generally are optimised to protect the nutritional value of the protein in the feed, and to avoid breakdown products and the formation of adducts. Some enzyme additives can partially survive pelleting at 90^{o}C in an active form and are little affected by lower temperatures (Samarasinghe *et al*., 2000). This general protection of proteins against damage during processing is usually ascribed to the other organic fractions of the feed. Consistent with this view is the detection using ELISA of the resistant version of the EPSPS protein, which confers herbicide resistance, in extracted GM soybean meal (Ash *et al*., 2000). Since the detection method was antibody-based, this implies the considerable retention of structural integrity.

40. Grinding and dry milling have little effect on DNA structure unless accompanied by localised heating. More severe commercial treatments, particularly those involving heat and/or chemical extraction, invariably lead to disruption of DNA structure (Smith *et al*., 2003). Only highly fragmented DNA could be recovered from oilseed meals following chemical and mechanical extraction of the oil (Chiter *et al*., 2000). Similarly, no intact DNA could be found in the extensively processed by-products of the maize wet-milling industry (Table 4). This was confirmed in a separate study, where although specific nuclear and plasmid genes could be detected by PCR in the wet gluten and germ fractions, after heat drying the DNA fragments were further degraded and could no longer be detected (Gawienowski *et al.*, 1999).

4.3 Survival of DNA/protein in the digestive tract

41. Most ingested proteins are rapidly degraded by proteases, primarily of host origin in the case of non-ruminants and of microbial and host origin in ruminants. Tests made with simulated gastric and intestinal conditions have confirmed that, with a single exception, protein products of the genes introduced into current commercial crops (Table 1) are as rapidly degraded as other dietary proteins (Noteborn *et al*., 1994; Harrison *et al*., 1996; Wehrmann *et al*., 1996). The exception is the product of *cry9C*, a bacterial lectin, which, in common with a sub-group of other plant and microbial lectins and protease inhibitors, is highly resistant to proteolysis (EPA, 1998).

42. Evidence of the degradation of protein during feed preparation should not automatically be assumed to confer safety. However, the degree of protein degradation occurring during feed processing, if applicable, can add to an established margin of safety. Digestion by livestock of any introduced protein also should be considered in the safety assessment with regard to its impact on the animal and any consequences for consumers of livestock products. While *in vitro* methods to mimic the conditions found in the digestive tract of non-ruminant species are well established, they are less suitable for ruminants. To determine whether a protein will be degraded in ruminants, different methods exist. Rumen fluid may be obtained from fistulated ruminants, however the properties of this fluid are diet dependent (Tilley and Terry, 1963; Goering and Van Soest, 1970). Alternatively to simulate the proteolytic activity of ruminal micro-organisms a protease extract from *Streptomyces griseus* may be used (Mathis *et al*., 2001). Regardless of susceptibility to breakdown during processing, all introduced and expressed protein should be separately examined for its toxic potential. This could involve a search for any structural resemblance to known toxins and/or animal studies.

43. Naked DNA/RNA released from the food matrix is readily degraded in most compartments of the gastro-intestinal tract. The longest periods of survival have been observed in the presence of saliva and in the oral cavity, where DNA has been detected after several hours (Mercer *et al*., 1999, 2001; Duggan *et al*., 2000). Elsewhere, while fragments capable of amplification may be detected for up to 30 minutes, any biological activity is extremely short-lived (Duggan *et al*., 2000). Most experiments have been made under artificial conditions and with naked DNA with the intention of demonstrating a capacity for transfection. While survival on release also may be very short *in vivo*, there is likely to be a constant leaching of DNA into the gut lumen as the feed matrix is disrupted. The *rubisco* gene, or at least an amplifiable fragment of the gene, could be recovered from the intestinal content of mice up to 49 hours after feeding and for a further 70 hours from the caecum (Hohlweg and Doerfler, 2001). Similarly, a 1914 bp DNA fragment containing the entire coding region for cryIA(b) was still amplifiable from the rumen fluid of sheep five hours after consumption of GM maize grain, although not from sheep fed silage prepared from the same maize line (Duggan *et al.*, 2003).

4.4 Uptake of DNA by the microflora of the GI tract

44. Transformation represents the most general and likely mechanism for the acquisition by gut bacteria of DNA released from feed. However, in one of the few attempts to demonstrate this transfer *in vivo*, a β-lactamase introduced into maize and conferring resistance to ampicillin, could only be detected in association with plant material and not with other intestinal contents or in faeces when fed to chicken (Chambers *et al*., 2002). The survival in the gut of the antibiotic marker gene mirrored that of other plant DNA targets and could be detected only in the crop and stomach.

4.5 Detection of transgenic DNA and protein in animal products

45. Following the work of Schubbert and colleagues (Schubbert *et al*., 1994, 1997, 1998; Hohlweg and Doerfler, 2001) the expectation is that DNA fragments of plant origin will be found in the tissues of farm animals, particularly in peripheral lymphocytes and the liver. While fragments of plastid encoded genes are far more likely to be detected than nuclear genes because of their copy number, the same principle(s) determining survival and uptake appear to apply. Whether any particular gene (including a transgene) is detected in tissues thus will be largely a product of the sensitivity of the detection method.

46. As expected, amplifiable fragments of plant DNA have been detected in animal tissues (Klotz and Einspanier 1998; Hohlweg *et al*., 2000; Einspanier *et al*., 2001) and in a variety of animal products including milk, although not in eggs. Fragments of transgenic DNA have yet to be detected in the major

animal products (Table 5). In addition to the data introduced into the public arena, a number of other similar experiments have been completed by plant breeding companies. No transgene (or its expressed product) has been detected in any animal product examined to date.

47. Existing knowledge of the metabolic processes involved in the digestion, absorption and utilisation of amino acid and peptides by livestock species does not wholly preclude the incorporation of intact proteins into animal products although suggests it to be unlikely. Generally, proteins are synthesised *de novo* from an amino acid pool. Studies made of the supply of amino acids to the mammary gland, for example, have shown that the bulk of milk proteins are synthesised *in situ* from single amino acids and some small peptides. However, immunologic (IgG) proteins are taken up from the blood supply (Whitney *et al*., 1976). Uptake is receptor mediated making it unlikely that any ingested protein surviving to be detected in serum would have the physical characteristics necessary for absorption. Egg proteins are generally synthesised in the liver and transported as specifically tagged lipoproteins. Thus it would be exceptionally unlikely for an expressed protein of any plant gene to be found intact in meat, milk or eggs and none have been detected to-date (Table 5).

Table 5. Examination of animal products for the presence of transgenic DNA or protein

Host animal	GM plant	Tissues examined	Outcome
Ruminants			
Dairy cow[1]	Herbicide tolerant soybean	Blood, milk	Transgene not detected
Dairy cow[2]	Bt maize	Blood, milk, digesta, faeces	Transgene detected only in digesta
Beef steer[2]	Bt maize	Blood, muscle, liver, spleen	Transgene not detected
Dairy cow[3]	Bt maize (whole plant)	Milk	Transgene and Cry1A(b) protein not detected
Dairy cow[4]	Herbicide tolerant soybean	Milk	Transgene (*epsps*) not detected
Dairy cow[5]	Bt-maize kernel	Milk	Transgene not detected
Poultry			
Chicken[2]	Bt maize	Muscle, liver, spleen, kidney, eggs	Transgene not detected
Laying hen[6]	Herbicide tolerant soybean	Eggs, liver, faeces	Transgene not detected
Broiler chicken[7]	Herbicide tolerant soybean	Muscle, skin, liver	Transgene not detected
Broiler chicken[3]	Bt maize	Breast meat	Transgene and Cry1A(b) protein not detected
Laying hens[3]	Bt maize	Eggs. liver, white and dark meat	Cry1A(b) protein not detected
Broilers and laying hens[8]	Bt maize	Digesta, meat and eggs	Transgene detected only in feed, maize DNA detected in digesta and liver.
Broilers[9]	Bt maize	Blood, liver and muscle	Transgene (*Cry 9c*) not detected
Pigs			
Grower-finisher pigs[10]	Bt maize	Loin meat	Transgene and Cry1A(b) protein not detected.
Grower-finisher pigs[11]	Bt maize	Blood, muscle, liver, spleen, lymph nodes	Transgene not detected
Grower-finisher pigs[12]	Bt maize	Blood, muscle, liver, spleen, lymph nodes, ovaries	Transgene not detected

References: [1]Klotz and Einspanier 1998; [2]Einspanier *et al.*, 2001; [3]Faust, 2000: [4]Phipps *et al.*, 2002; [5]Phipps *et al.*, 2001; [6]Ash *et al.*, 2000: [7]Khumnirdpetch *et al.*, 2001; [8]Aeschbacher *et al.*, 2001; [9]Anon, 2001; [10]Weber and Richert, 2001. [11]Klotz *et al.*, 2002; [12]Reuter and Aulrich, 2003.

48. Daily exposure by mammals to fragments of food plant and microbial DNA that results in their random incorporation into the nucleus of somatic cell populations has no recognised long-term consequences. There is no basis to suppose that transgenic DNA poses hazards any different to other sources of DNA.

SECTION 5: ANIMAL FEEDING STUDIES AS PART OF A SAFETY ASSESSMENT

49. Unlike foods specifically for human use, GM plants can be fed directly, often in raw or minimally processed form to the target species and the growth, health and welfare of the animal monitored, or the absorption and tissue distribution of particular metabolites measured. Limits to the amount of any one component that can be incorporated into an animal ration usually prevent chronic toxicity studies being made at a relevant dose level with the target species, as is the case with whole-food testing on the human side. The "wholesomeness" of the product based on nutritional efficacy, however, can be directly demonstrated.

50. Most conventional varieties of maize, soybean and other feedstuffs are introduced to the market primarily on the basis of their composition. Experience has shown that nutritional value can be predicted with sufficient accuracy from compositional data to make a feeding trial unnecessary.

51. The traits introduced into most existing commercial GM varieties (Table 1) are agronomic in character and have little or no effect on feed composition or the bioavailability of nutrients. Consequently the gross composition of such GM varieties also falls within the range normally associated with conventional varieties of the same feedstuff and they would be expected to behave as any other variety. Many feeding studies have been made to test this assumption (Table 6). There is no evidence to date from such studies to suggest that the performance of animals fed the GM feed ingredient differed in any respect from those fed the non-GM counterpart or from the performance predicted by the composition of the feed (Faust, 2002). This suggests that for those GM plants with modified input traits, provided that compositional analyses demonstrate no meaningful differences from the comparator(s) nutritional equivalence can be assumed. For such GM varieties, routine feeding studies made with target species will add little to a safety assessment and generally are not warranted (see 5.1).

Table 6. Summary of reported studies made with livestock fed GM feed in comparison to conventional feed.

Animal species	GM plant	Outcome
Ruminants		
Dairy cows[1]	Herbicide-tolerant soybean	No significant differences in milk production or composition but FCM greater (P<0.05) in GM groups.
Dairy cows[2]	Bt maize (chopped plants)	No differences in milk production or composition.
Sheep, beef cattle[3]	Bt maize silage	No significant differences in digestibility, weight gain or DMI.
Dairy cows[4]	Bt maize silage	No significant differences in DMI, milk performance or milk composition.
Dairy cows, sheep[5]	Bt maize and maize silage	No significant difference in performance.
Sheep[6]	Herbicide tolerant sugar beet and leaf silage	No significant differences in the digestibility of nutrients observed.
Dairy cows[7]	Herbicide tolerant maize and maize silage	No significant differences in DMI, milk production or milk composition.
Dairy cows[8]	Bt maize and maize silage	No significant difference in performance.
Dairy cows[9]	Bt maize + Bt maize silage	No significant differences in DMI, milk performance or milk composition.
Beef cattle[10]	Bt maize, maize residues and maize silage	Silage: differences (P<0.05) seen between hybrids not consistently related to Bt. Residue: no significant differences.
Beef cattle[11]	Bt maize silage and maize residues	Silage: no significant difference in ADG and DMI, feed:gain ratio greater in Bt (P<0.05). Residues: no significant differences.
Dairy cows[12]	Herbicide-tolerant cottonseed, Bt cottonseed	No significant differences in body condition, milk yield or milk composition.
Beef cattle[13]	Herbicide-tolerant maize	No significant differences in growth performance, carcass characteristics or meat composition.
Beef cattle[14]	Herbicide-tolerant maize (two events)	No significant differences in growth performance or carcass characteristics.
Dairy cows[15]	Herbicide-tolerant maize/ maize silage	No significant differences in milk production or composition.
Dairy cows[16]	Herbicide-tolerant maize/ maize silage	Intake of GM maize silage and milk production significantly reduced (P<0.05). Ascribed to differences in silage quality.
Sheep[17,18]	Herbicide tolerant sugar/fodder beet	No significant effects on feeding value.
Sheep[19]	Herbicide tolerant canola	No significant effects on digestibility or growth performance
Pigs		
Pigs[20]	Herbicide tolerant maize	No significant effects on nutrient digestibility.
Pigs[21]	Bt maize	No significant differences in DMI or weight gain.
Pigs[22]	Bt maize	No significant difference in nutrient digestibility.
Pigs[23,24]	Bt maize	No significant differences in nutrient digestibility, DMI or weight gain.
Piglets[25]	Bt maize	No significant effects on feed:gain ratio, but ADG significantly increased in Bt-maize fed piglets[*].
Growing pigs[26]	Herbicide tolerant maize,	No significant differences in DE compared to near

	Bt maize	isogenic parent lines.
Growing pigs[27]	Herbicide tolerant soybean	No significant differences in performance parameters or carcass measurements. Sensory scores not significantly influenced by diet.
Pigs[6]	Herbicide tolerant sugar beet	No significant effects on nutrient digestibility.
Pigs[28]	Bt maize	No significant differences in growth compared to near isogenic control.
Growing-finishing pigs[29]	Herbicide-tolerant maize	No significant differences in growth performance or carcass measurements.
Poultry		
Broilers[1]	Herbicide-tolerant soybean	No significant differences in LWG, DMI, feed:gain ratio or survival
Laying hens[30]	Bt maize	No significant effects on nutrient digestibility or AME_n. Egg production not examined.
Broilers[31]	Bt maize	No significant differences in LWG or survival. Feed:gain ratio significantly improved for Bt.
Broilers[32]	Bt maize	No significant difference in LWG, DMI or feed:gain ratio.
Broilers[33]	Bt maize	No significant differences in weight gain or feed:gain ratio and ME values
Broilers[34]	Herbicide tolerant maize	No significant differences in LWG, feed:gain ratio and fat pad weights.
Broilers[35]	Bt-soybean meal	No significant differences in growth, feed:gain ratio or carcass weight of breast meat.
Broilers and laying hens[36]	Bt-maize	No significant effects on production parameters or ME content.
Broilers[37]	Herbicide tolerant maize, Bt maize	No significant differences in performance parameters compared to near isogenic parent lines.
Broilers[23]	Bt-maize	Final liveweight significantly ($P<0.05$) increased in Bt-maize fed birds*.
Other species		
Catfish[1]	Herbicide-tolerant soybean	No significant differences in survival or feed:gain ratio. Weight gain/final weight greater ($P<0.05$) in one of two GM groups.
Rabbits[38]	Rapeseed	No significant differences observed.
Rabbits[39]	Bt-maize	No significant differences observed.

Modified from Flachowsky and Aulrich, 2001.

References: [1]Hammond and Padgette, 1996; [2]Faust and Miller, 1997; [3]Daenicke *et al*., 1999; [4]Rutzmoser *et al*., 1999; [5]Barriere *et al*., 2001; [6]Böhme *et al*., 2001; [7]Donkin *et al*., 2000; [8]Faust, 2000; [9] Folmer *et al*., 2000a;[10]Folmer *et al*., 2000b; [11]Hendrix *et al*., 2000; [12]Castillo *et al*., 2001; [13]Simon *et al*., 2002; [14]Berger *et al*., 2002; [15]Ipharraguerre et al., 2002; [16]Grant *et al*., 2002; [17]Hvelplund and Weisbjerg, 2001; [18]Weisbjerg *et al*., 2001; [19]Stanford *et al*., 2002; [20]Böhme and Aulrich, 1999; [21]Weber *et al*., 2000; [22]Aulrich *et al* 2001; [23]Reuter *et al*., 2001; [24]Reuter *et al*., 2002; [25]Piva *et al*., 2001; [26]Gaines *et al*., 2001a; [27]Cromwell *et al*., 2002; [28]Weber and Richert, 2001; [29]Fischer et al., 2002; [30]Aulrich *et al*., 1998; [31]Brake and Vlachos, 1998; [32]Halle *et al*., 1998; [33]Mireles *et al*., 2000; [34]Sidhu *et al*., 2000; [35]Kan *et al*., 2001; [36]Aeschbacher *et al*., 2001; [37]Gaines *et al*., 2001b; [38]Maertens *et al*., 1996; [39]Chrastinová *et al*., 2002.

*Suggested by authors that improved performance in Bt-maize fed groups due to a lower fumonisin B_1 content.

ADG, average daily gain. AME_n, nitrogen corrected apparent metabolisable energy. DE, digestible energy. DMI, dry matter intake. FCM, fat corrected milk. LWG, liveweight gain. ME, metabolisable energy.

5.1 Value of feeding trials with nutritionally modified feeds

52. Plants modified with the intention of significantly changing their composition and thus their nutritional characteristics may present added issues for a safety assessment. Provided the introduced changes affect only composition and not the bioavailability of individual nutrients (e.g. increased water-soluble carbohydrate in forages), or if the by-products fed are essentially free of the modified component (e.g. seedmeals left after modified oil extraction), valid nutritional comparisons with conventional varieties can still be made. This may involve augmenting the comparator diet to match the composition of the GM variety.

53. If the modification is expected to substantially change bioavailability of a component (e.g. amylopectin-rich starch) then a suitable comparator for feeding studies cannot be designed on the basis of composition alone. In such cases it may be possible only to conduct feeding trials with one or more of the major target species to demonstrate wholesomeness for the animal. Under these circumstances, the duration of feeding studies should be for the production cycle of the animal. Traditionally, other types of studies (e.g. determination of ME (metabolisable energy) value, balance study to demonstrate improved nitrogen retention) have been utilized to evaluate non-GM feedstuffs and could confirm that the intended modification produces the expected outcome.

5.2 Detection of unintended effects of transformation

54. The degree of equivalence to existing varieties is established on the basis of a comparison of compositional and agronomic data. However, even when this is considered “substantial”, there remains a remote possibility of unintended effects of transformation in the plant not detected by a targeted chemical analysis or as a change in growth characteristics of the plant.

55. Unintended effects occurring during the development of new plant varieties are not unique to those produced using recombinant DNA technology but have the potential to occur in all forms of plant breeding. There is at present, no reason to suppose that the incidence of unintended effects is significantly greater when recombinant DNA methods are used. Transgene integration occurs in plants through the same illegitimate recombination mechanisms that allow the chromosomal recombinations that are the basis for conventional plant breeding (Gelvin, 2000). Since no sequence homology is required, no sequences in the genome appear specifically favoured for integration. Consequently, it is not presently possible to predict the site of integration of transgenes into the host genome. However, both chromosomal recombination and transgene integration appear to occur more frequently in gene-rich regions, increasing the likelihood of mutations caused by disruption of gene function (Barakat *et al*., 2000).

56. If other studies (e.g. compositional, agronomic) indicate that unintended effects may have occurred, consideration could be given to the use of comparative growth studies as a means of investigating such effects. Fast growing species such as the broiler chick increase their body weight approximately 45-fold during the approximately 40 days they take to reach market weight. Because of this rapid weight gain, broilers are particularly sensitive to any change in nutrient supply or the presence of toxic elements in their feed. Consequently growth rate studies made with broilers can be used to examine GM products for unintended changes provided that they can be nutritionally matched to a parental line or other suitable control and that they are suitable for inclusion in broiler diets. Broilers have advantages over many other species used in commercial production, as they tend to provide a genetically homogeneous population and can be used in relatively large numbers to increase the statistical power of the experiment.

57. The young of other livestock tend not to show such rapid growth rates, but will, on occasions, form a more appropriate model. For feedstuffs intended for aquaculture, extrapolating results to other fish

species from a comparative growth study made with a fish species such as the catfish, is preferable to an extrapolation from results obtained with broilers. Also the presence of a known toxicant may restrict feeding studies only to those animals known to tolerate the compound. Gossypol limits the use of cottonseed meal in animals other than ruminants. In this case milk production might substitute for growth rate and could be used to screen feedstuffs intended for use with ruminants.

58. Before such studies could become routine, standardized and internationally recognized designs for such tests would need to be established. In particular, the number of animals and the statistical degree of confidence necessary to conclude unintended effects are present need to be specified. Large numbers of animals may be required to achieve appropriate statistical power. It must be recognized that these studies cannot provide definitive evidence of safety, but may contribute to the safety assessment as a whole.

59. In time, it may be possible to detect unintended effects by using non-targeted profiling techniques based on the measurement of the transcriptome or proteome (see 5.3). Alternatively, measures to detect unintended effects may be rendered unnecessary by improved molecular characterisation and understanding of the implications of the molecular events for the metabolism of the plant.

5.3 Non-targeted profiling

60. Non-targeted methods intended to profile gene expression, a significant proportion of the proteome (proteomics) or metabolite production (metabolomics) are being considered as supplements to the targeted methods currently used in establishing the degree of equivalence with parental lines/existing varieties (Fiehn *et al*., 2000). The rapid technical developments in the measurement of the transcriptome or proteome may not immediately allow fully validated methods to be established.

SECTION 6: POST-MARKET SURVEILLANCE/MONITORING

61. Post-market surveillance may be a more practical proposition when undertaken in relation to animal feed than to food since intake is accurately known and recorded and individual animals can be routinely monitored for health. The various "quality assured schemes" also can allow accurate tracing of animal products to their point of sale. However, given the lack of a theoretical basis for the general transfer of functional protein or DNA to animal products (see paragraph 48) and in the absence of any documented adverse response to products of animals fed GM feedstuffs, post-market surveillance of consumers appears to be of very limited value.

62. Surveillance of animals, particularly the longer-lived species, may be useful where the long-term clinical effects of an introduced trait can only be surmised from short-term biological studies. Similarly, it may be advantageous to augment toxicological studies in laboratory animals when livestock are exposed to a novel gene product with multiple biological activities. However, as with human studies, post-market monitoring does not provide a gold standard in safety assurance, nor does it substitute for other components of the assessment. Such large-scale studies may be confounded by many factors such as environment and management (Byers, 1998), although these can be partially offset by the inclusion of a carefully selected control population. Post-market surveillance should be seen as a supplement to the assessment scheme. The specific purpose for post-market surveillance, should be clearly stipulated before the surveillance is initiated.

SECTION 7: BY-PRODUCTS OF INDUSTRIAL CROPS

63. Recombinant technology has greatly expanded the opportunities for the use of plants for the production of high value products, particularly peptide- and protein-based therapeutics (Fischer and Emans, 2000; Walmsley and Arntzen, 2000; Daniell *et al*., 2001). Expression is generally targeted to the seed and to the major seed-producing commodity crops, but there are examples of expression in chloroplasts engineered by plastid transformation (Staub *et al*., 2000). Although many constructs have been produced, generally expression levels are considered too low for commercial exploitation. To date only a few have progressed to field and clinical trials although diagnostic kits based on plant-expressed products are on the market.

64. Intermediate value products such as enzymes also have been introduced into plants used for feed purposes and with some varieties there is the option of extracting the enzyme protein for food use or using the product intact as a feed ingredient (Jensen *et al*., 1996; Denbow *et al.*, 1998). Bulk chemical production intended to provide low cost feedstock has focussed on modifying oil production in oilseeds, particularly rapeseed. One of the first GM plants to obtain release in the USA was an oilseed rape modified to produce high concentrations of lauric acid in the oil for food use, with the meal being used for feed. Subsequently, other modified rape varieties have undergone field trials (Murphy, 1996; Napier and Michaelson, 2001), although none are in commercial production.

65. If the industrial use of crops modified to express high value pharmaceutical agents or bulk feedstock for the chemical industry becomes commonplace, then this raises serious issues of feed security. Usually the cost-effective mechanism for the disposal of residues left after extraction of seeds or vegetative material is into the animal feed chain. While high value, low volume products could absorb the costs of alternative forms of disposal, this is less likely to be the case for lower value bulk products.

66. Although disposal of hazardous by-products can be seen as primarily as a problem of risk management, there is a persuasive argument for completing a safety assessment for all parts of industrial GM plants that could, and if from a conventional plant would, enter the food chain. A full safety assessment made on all plants used for bulk production and their by-products would inform the risk manager and allow actions proportionate to the risk to be taken. This might range from allowing some by-products left after extractions of the modified oil or other primary product to be used as a feed ingredient, to a refusal to allow feed use due to the risk that they would contain residual material of a highly toxic nature. Alternatively, if measures have been implemented to prevent entry of plants producing pharmaceuticals products into the feed supply, a safety assessment of that plant may not be necessary. The processes needed to ensure that the material does not become a component of feed should be proportional to the associated risk.

SECTION 8: AGRONOMIC VERSUS QUALITY TRAITS - FUTURE GM FEEDSTUFFS

67. Transformation events described in the scientific literature address a wide variety of issues including the nutritional, organoleptic and shelf-life of food plants, the expression of plant compounds impacting on public health and the ability of crop plants to resist stress and to grow in more marginal areas. Taking applications for field trials as an indication of varieties likely to be at the forefront of those seeking regulatory approval, it is evident that agronomic (input) traits will continue to dominate new introductions for some time to come.

68. An important input target applied to a wide range of plant species including those used as feeds, has been the development of alternatives to Bt toxins able to offer protection against a broader range of insect pests. Like the Bt endotoxins, most transgenics of this type have introduced genes coding for proteins targeting some aspect of insect gut function. These include a variety of plant-derived lectins and digestive enzyme inhibitors (Schuler *et al*., 1998). Of these the snowdrop lectin (*Galanthus nivalis* agglutinin) has received greatest attention and has been successfully expressed in many different crops including cereals (Rao *et al*., 1998). Feed assessments of plants that have had these compounds incorporated should take into account that protease and amylase inhibitors and some lectins are recognised as anti-nutritional factors and processing of feeds is often required to ensure their removal from the finished feed or feed ingredient. Plant enzyme inhibitors belonging to a general class of defence protein, some of which (e.g. soybean Kunitz-type trypsin inhibitor) are recognised as cross-reacting allergens (Mena *et al*., 1992) and are highly resistant to digestion in the gut.

69. Major cereals, other than maize, have proved recalcitrant to transformation, delaying the introduction of transgenic varieties. However, considerable progress has been made during the last decade and *Agrobacterium*-mediated gene transfer has been added to the biolistic, electroporation and polyethylene glycol-induced methods developed previously (Ingram *et al*., 2001). Wheat and barley have particular implications for feed use. Any new transgenic varieties are first likely to parallel the work done with maize and soybean and focus on herbicide tolerance. Only thereafter are pest resistance and feed quality issues likely to be addressed commercially.

70. Transgenic plants addressing quality issues of importance to animal feeding are assumed likely to be included amongst the next “generation” of transgenic varieties. If so, they will be one of two types, those involving modifications to the composition of plants important in manufactured compound feed (essentially seeds), and those involving forages (essentially vegetation).

71. Seed proteins of both legumes and cereals are considered, from a nutritional standpoint, to have a less than ideal amino acid composition; legumes being considered deficient in sulfur amino acids and cereal grains deficient in lysine and threonine. Introduction of novel seed proteins that have more desirable amino acid profiles (Saalbach *et al*., 1994; Molvig *et al*., 1997) or down-regulating one or more proteins with less desirable characteristics (Kohnomurase *et al*., 1995) have resulted in beneficial changes in amino acid profiles. In an alternative approach, circumventing the normal feedback regulation in the biosynthetic pathway for selected amino acids has been shown to increase the concentration of the free acid with evidence of increased incorporation into storage proteins (Galili *et al*., 1994, 2000; Falco *et al*., 1995).

72. Forage quality is not high on the agenda of most plant breeding companies. Where relevant work has been undertaken, it is in areas such as the modification of lignin biogenesis (Vogel and Jung, 2001) where results have implications for other industries and where forage crops provide valuable, fast-growing models. In comparison, relatively limited work to date has been undertaken addressing other important issues relating to dry matter digestibility (see Herbers and Sonnewald, 1996), protein quality (Bellucci *et al*., 1997) and nitrogen capture.

73. The brief forecast above of traits likely to be encountered, which covers both agronomic traits (disease resistance) and an increased emphasis on quality issues, highlights the need for a case-by-case approach to safety evaluation and the probable need to develop specific assessments appropriate to each event. The OECD Workshop on the Nutritional Assessment of Novel Foods and Feeds (February, 2001) concluded that the concept of substantial equivalence as a starting point in the assessment process, remained a valid tool for assessing novel foods and feeds with nutritionally modified characteristics. It is to be expected that added value plants will require identity preservation to distinguish them from other varieties.

SECTION 9: CURRENT LEGISLATIVE PROCESS APPLIED TO GM FEED

74. The present approach to safety assessment of novel feeds by national authorities ranges from use of existing food legislation (in the case of the United States where feed is considered in legislation to be food), to feed-specific legislation (as in Canada, Czech Republic, Hungary, and others). Other OECD member countries are in the process of developing new legislation specific to GM foods and/or feeds and to the labelling of GM feeds. The European Union has recently proposed legislation to ensure that dual use plants (food and feed applications) are simultaneously assessed for safety for both applications.

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