FOOD DERIVED FROM GLUFOSINATE AMMONIUM TOLERANT SOYBEAN LINES A2704-12 AND A5547-127

A Safety Assessment

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SUMMARY

Glufosinate ammonium-tolerant soybean lines A2704-12 and A5547-127 have been developed primarily for agricultural purposes to provide growers with an additional variety of soybean with tolerance to the broad-spectrum herbicide, glufosinate ammonium. Food derived from this GM soybean line has been evaluated to determine its suitability for human consumption. The evaluation criteria included characterisation of the transferred genes, analysis of changes at the DNA, protein and whole food levels, and assessment of the potential allergenicity and toxicity of any newly expressed proteins. Examination of these criteria has enabled both the intended and unintended changes to be identified, characterised and evaluated for safety.

History of use

Soybeans are grown as a commercial crop in over 35 countries worldwide and have a long history of safe use as human food. The major producers of soybeans are the United States, Argentina, Brazil and China, accounting for 90% of world production. The main food products derived from soybean lines A2704-12 and A5547-127 are seeds, meal and oil. Finished food products containing soybean ingredients include beer, noodles, breads, flours, sausage casings, pastries, crackers, meat substitutes, milk substitutes and confectionery among other things.

Nature of the genetic modification

In soybean lines A2704-12 and A5547-127 the glufosinate ammonium tolerance trait has been introduced by the addition of a bacterial gene encoding the phosphinothricin acetyl transferase (PAT) protein, an enzyme that confers tolerance to glufosinate ammonium. PAT functions by detoxifying phosphinothricin, the active constituent of glufosinate ammonium herbicides.

Soybean line A2704-12 contains two copies of the *pat* gene, inserted at a single locus in a head to tail configuration. They are separated by a DNA fragment containing a non-functional portion from the 5' end of the *bla* gene (an antibiotic resistance marker gene). Soybean line A5547-127 contains a single copy of the *pat* gene. This line also contains two non-functional fragments of the *bla* gene at the same genomic locus. None of the *bla* gene fragments give rise to any detectable transcription products.

Detailed molecular and genetic analyses of soybean lines A2704-12 and A5547-127 indicate that the transferred *pat* gene is stably integrated in the plant genome at a single insertion site and is stably inherited from one generation to the next.

Characterisation of novel protein

Soybean lines A2704-12 and A5547-127 express a single novel protein – PAT. Protein expression analyses indicate that PAT is expressed at low levels or is undetectable in the soybeans and their processed fractions and therefore exposure to the protein through consumption of food derived from soybean lines A2704-12 and A5547-127 would be minimal. In soybean lines A2704-12 and A5547-127, PAT was present at levels ranging from 573-2138 ng/g (up to 0.00056% of total protein) and 10100-20202 ng/g (up to 0.0056% of total protein) respectively. In the soybean hulls, meal, lecithin, refined oil and soy isolate levels of PAT were much lower or undetectable.

The safety of PAT has been assessed on numerous previous occasions by FSANZ. In all instances it has been concluded that PAT is non-toxic to humans and has limited potential as a food allergen.

Comparative analyses

Compositional analyses were done to establish the nutritional adequacy of soybean lines A2704-12 and A5547-127, and to compare them to non-transformed control lines and commercial varieties of soybean. The constituents measured were protein, fat, carbohydrate, ash, moisture, fibre, fatty acids, amino acids, minerals and the anti-nutrients phytic acid, trypsin inhibitor, lectins, isoflavones, raffinose and stachyose.

No differences of biological significance were observed between the transgenic soybean lines and their non-GM counterparts. Several minor differences in key nutrients and other constituents were noted however the levels observed represented very small percentage changes and do not indicate an overall pattern of change that would warrant further investigation. On the whole, it was concluded that food from soybean lines A2704-12 and A5547-127 is equivalent in composition to that from other commercial soybean varieties.

Nutritional adequacy

The detailed compositional studies are considered adequate to establish the nutritional adequacy of the food and indicate that food derived from soybean lines A2704-12 and A5547-127 is equivalent in composition to food from non-GM soybean varieties. The introduction of food produced from soybean lines A2704-12 and A5547-127 into the food supply is therefore expected to have minimal nutritional impact. The nutritional adequacy of food produced from soybean lines A2704-12 and A5547-127 was also confirmed using a feeding study in rapidly growing broiler chicks. This demonstrated that the GM soybeans are equivalent to non-GM soybeans in their ability to support typical growth and wellbeing.

Conclusion

No potential public health and safety concerns have been identified in the assessment of food produced from soybean lines A2704-12 and A5547-127. On the basis of the available data, food produced from these soybean lines can be considered as safe and as wholesome as food produced from other soybean varieties.

FOOD DERIVED FROM GLUFOSINATE AMMONIUM TOLERANT SOYBEAN LINES A2704-12 AND A5547-127:

A SAFETY ASSESSMENT

BACKGROUND

A safety assessment has been conducted on food derived from soybeans that have been genetically modified to be tolerant to the herbicide glufosinate ammonium. The modified soybeans are referred to as glufosinate ammonium-tolerant soybean lines A2704-12 and A5547-127.

Herbicide tolerance is achieved through expression in the plant of a bacterial gene encoding the phosphinothricin acetyl transferase (PAT) protein, an enzyme that confers tolerance to glufosinate ammonium. PAT functions by detoxifying phosphinothricin, the active constituent of glufosinate ammonium herbicides. Glufosinate ammonium (also referred to as phosphinothricin) is a non-selective, contact herbicide that provides effective post-emergence control of many broadleaf and grassy weeds. The mode of action of the herbicide is to inhibit the enzyme glutamine synthetase, an essential enzyme involved with ammonium accumulation and nitrogen metabolism in plants. The inhibition of glutamine synthetase results in an over accumulation of ammonia in the plant, which leads to cell death. PAT is encoded by the *pat* gene from the soil bacteria *Streptomyces viridochromogenes.* The production of PAT by soybean lines A2704-12 and A5547-127 enables the post emergence use of glufosinate ammonium herbicides without risk of damaging the crop. It is thought that the development of GM glufosinate ammonium tolerant soybeans will provide a selective use for glufosinate ammonium, creating a valuable new weed management tool for soybean producers.

There are three major food products derived from soybeans – seeds, oil and meal. Whole soybeans are used to produce soy sprouts, baked soybeans, roasted soybeans, full fat soy flour and the traditional soy foods such as miso, tofu, soy sauce and soymilk. Soybean oil has many food uses including in cooking oils, mayonnaise, margarine, salad dressings, sandwich spreads, and shortenings. Soybeans are also processed into lecithin, an emulsifying agent (food additive) found in a wide variety of foods. Finished food products containing soybean ingredients therefore include beer, noodles, breads, flours, sausage casings, pastries, crackers, meat substitutes, milk substitutes and confectionery among other things.

HISTORY OF USE

Soybean (*Glycine max*), which is grown as a commercial crop in over 35 countries worldwide, has a long history of safe use for both human food and stock feed. Soybean plants are primarily a self-fertilized crop and are generally grown as a seasonal crop in broad acre cropping situations. The major producers of soybeans are the United States, Argentina, Brazil and China, accounting for 90% of world production. The commercially available soybean cultivars A2704 and A5547 have been used as the hosts for the glufosinate ammonium resistance trait.

There is only limited feed use, and no food use, for unprocessed soybeans, as they contain toxicants and anti-nutritional factors, such as lectins and trypsin inhibitors. Appropriate heat processing inactivates these compounds. Before processing, soybeans are graded, cleaned, dried and de-hulled. The soybean hulls are further processed to create fibre additives for breads, cereals and snacks and are also used for stock feed. After de-hulling, soybeans are rolled into full fat flakes that may be either used in stock feed or processed further into full fat flour. Crude soybean oil is then extracted from the flakes by immersing them in a solvent bath. Crude lecithin is then separated from the oil, which is further refined to produce cooking oil, margarine and shortening. After the oil is extracted from the flakes, the solvent is removed and the flakes are dried for use in the production of soy flour, soy concentrates and soy isolates. De-fatted soy flakes are also used in stock feed.

DESCRIPTION OF THE GENETIC MODIFICATION

Methods used in the genetic modification

Soybean lines A2704-12 and A5547-127 were generated by the transformation of soybean shoot apices derived from the soybean lines A2704 and A5547 respectively, using particle acceleration technology. Two purified linear DNA fragments containing the *pat* gene, together with essential regulatory elements, were used in the transformation process. The two linear DNA fragments of 3119 and 957 base pairs were obtained from the plasmid vector pB2/35SAcK by restriction digestion with *Pvu* I (Figure 1).

Following transformation, the cells were induced to produce shoots on plant tissue culture medium containing plant hormones. The shoots that developed from the transformed cells were screened for glufosinate ammonium tolerance by spraying the plantlets in axenic culture with glufosinate ammonium to confirm that transformation had been successful and the *pat* gene was being expressed. Surviving plantlets were transferred to soil, grown in the greenhouse and then screened again for glufosinate ammonium tolerance.

Function and regulation of novel genes

The two *Pvu* I DNA fragments from pB2/35SAcK used in the transformation to produce lines A2704-12 and A5547-127 are illustrated in Figure 1. One of the fragments contains the cassette for expression of the novel protein PAT and the 3' end of the *bla* gene. The other fragment contains the 5' portion of the *bla* gene. Some pUC19 plasmid sequence was also transformed into the soybeans, however this represents only a tiny amount of the total soybean DNA and contains no plant expressible genes. Thus this additional DNA does not have any impact on the safety of food derived from these soybeans. All genetic elements present in the DNA insert are listed in Table 1.

The pat *gene*

The *pat* gene is derived from the soil microorganism *Streptomyces viridochromogenes* strain Tu494. It encodes the enzyme phosphinothricin acetyltransferase (PAT), which modifies and inactivates the herbicide glufosinate ammonium. The bacterial *pat* gene contains a high G:C content that is not typical of plant genes. To optimise expression in plants, a synthetic gene was constructed with a lower G:C content and this has been transferred to soybean. This modified *pat* gene has approximately 70% DNA sequence similarity with the native gene. However, the amino acid sequence of the PAT protein has not been altered.

The *pat* gene can be used as a selectable marker to distinguish GM plant cells from unmodified cells. The *pat* gene has been transferred to the soybean to confer tolerance to glufosinate ammonium herbicides.

The *pat* gene is under the control of the cauliflower mosaic virus (CaMV) 35S promoter and 35S termination signal. The CaMV 35S promoter is widely used as a promoter for high expression of genes in plants and drives constitutive expression of the *pat* gene throughout the plant.

The bla *gene*

The transformation construct also contained an antibiotic resistance marker (the *bla* gene) that confers resistance to some antibiotics, including penicillin and ampicillin. The *bla* gene is under the control of bacterial promoters and was used as a selectable marker to distinguish transformed bacterial cells from non-transformed cells during development of the gene construct. Prior to plant transformation the pB2/35SAcK plasmid was cut with a restriction enzyme to generate two DNA fragments. The restriction enzyme used, *Pvu*I, cuts in the middle of the *bla* gene, thus this gene was disrupted and is no longer

functional. The two plasmid fragments were then used to transform soybean shoot apices. The transformed soybean lines do not contain a functional copy of the *bla* gene.

Table 1: Genetic elements present in the insert DNA

Genetic Element	Size (kb)	Source	Function
pat gene (synthetic)	0.55	*S. viridochromogenes*	Confers tolerance to the herbicide glufosinate ammonium.
bla gene (inactivated)	0.86	*E. coli*	Antibiotic resistance marker used to select for transformed bacteria. Inactivated in the transformed soybean.
P35S	0.54	Cauliflower mosaic virus	Promoter for *pat* gene expression.
T35S	0.20	Cauliflower mosaic virus	Terminator for *pat* gene expression.
ori-pUC	0.55	pUC18 plasmid	Origin of replication (ColE1) of pUC18, has no function in plants.
Right Border	0.054	*A. tumefaciens* Ti plasmid pTiAch5	Right border sequence, has no function in soybean A2704-12 and A5547-127.

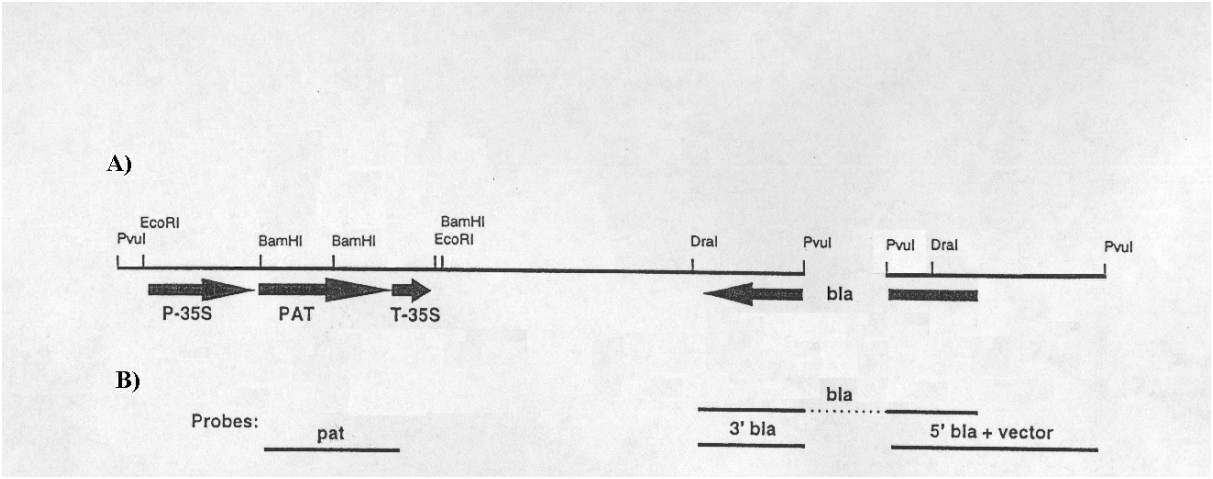

Figure 1: DNA construct used to transform A2704 and A5547 soybeans.
A)The B2/35SAcK plasmid was cut into two pieces with the restriction enzyme *Pvu* I. The 3119 base pair fragment on the left contains the *pat* gene and promoter (P-35S) and terminator (T-35S) sequences as well as the 3' end of the *bla* gene. The 957 base pair fragment on the right contains part of the 5' end of *bla* gene and some plasmid sequence, which has no function in the transformed plants.
B) Shows the locations of the four probes used in Southern hybridisation; pat, bla, 5'bla and 3' bla.

Characterisation of the genes in the plant

Genomic DNA from soybean lines A2704-12 and A5547-127 (generation R_2 homozygous individuals) was isolated and analysed using Southern hybridisation to determine the number of insertion events, the arrangement of the insertion fragments, the copy number of the inserted DNA and the integrity of the inserted cassettes. Polymerase chain reaction (PCR) analysis and DNA sequencing were used to further characterise the insert DNA and insert-to-plant junction regions and to confirm the results of the Southern hybridisation.

Genomic DNA from non-transformed soybean lines A2704 and A5547 was used as the control material. The reference material was plasmid B2/35SAcK, which had been used in the original plant transformation.

The molecular characterisation also included a determination of whether the disrupted *bla* gene, which is present in the transforming DNA, would be expressed in the transgenic plants.

Studies evaluated:

Berghmann, S. and De Beuckeleer, M. (2002). Determination of inserted transgenic sequences in *Glycine max* elite event A2704-12. Aventis CropScience Report No. C021225.

Berghmann, S. and De Beuckeleer, M. (2002). Determination of inserted transgenic sequences in *Glycine max* elite event A5547-127. Aventis CropScience Report No. C021226.

De Beuckeleer, M. and Borrerman, J. (1997). Evaluation of cryptic gene expression of the *bla* gene in LibertyLink soybean event A2704-12. Plant Genetic Systems Report No. A59238.

De Beuckeleer, M. and Borrerman, J. (1997). Evaluation of cryptic gene expression of the *bla* gene in LibertyLink soybean event A5547-127. Plant Genetic Systems Report No. A59239.

De Beuckeleer, M. and Borrerman, J. (1997). Molecular determination of the number of inserted *pat* and *bla* gene copies in LibertyLink Soybean event A5547-127. Plant Genetic Systems N.V. Report No. A59233.

De Beuckeleer, M. (1998). Molecular demonstration of the stability of the integration of *Glycine max* transformation event A2704-12. Plant Genetic Systems N.V. Report No. C001704.

De Beuckeleer, M. and Borrerman, J. (1998).Molecular demonstration of the stability of the integration of *Glycine max* transformation event A5547-127. Plant Genetic Systems N.V. Report No. C001703.

De Beuckeleer, M. and Borrerman, J. (1999). Molecular determination of the number of inserted *pat* and *bla* gene copies in LibertyLink *Glycine max* event A2704-12. Plant Genetic Systems N.V. Report No. B002294.

De Beuckeleer, M. (2002). Analysis of the nature of the flanking sequences from Glycine max elite event A2704-12. Bayer CropScience Report No. C024090.

De Beuckeleer, M. (2002). Analysis of the nature of the flanking sequences from Glycine max elite event A5547-127. Bayer CropScience Report No. C024933.

Van Wert, S. (1999). Transformation system and genetic characterisation of glufosinate resistant soybean event A2704-12. AgrEvo Report No. B002181.

Insert and copy number

To determine the number and nature of DNA insertions that occurred in transformation events A2704-12 and A5547-127, Southern hybridisation was used. Four hybridization probes were made from the transformation plasmid pB2/35SAcK. The *pat* probe covers the entire *pat* gene, the *bla* probe covers the entire *bla* gene, the 5' *bla* probe covers the 5' fragment of the *bla* gene and the 3' *bla* probe covers the 3' fragment of the *bla* gene (Figure 1).

The number and arrangement of the inserts were investigated by digesting genomic DNA from the two transgenic lines A2704-12 and A5547 –127 and genomic DNA from the non-transgenic parental lines with eight different restriction enzyme combinations that cut within the DNA fragment used for the plant transformation. The blots, containing the separated DNA fragments, were then probed with the four different radio-labelled probes. The number and size of each fragment was used to determine the number and arrangement of insert DNA that is present in the transformed soybean genome.

Southern blot analysis of soybean line A2704-12 indicated that two copies of the DNA fragment containing the *pat* gene were integrated at a single locus. These two copies of *pat* are separated by one

copy of the 957 bp *Pvu* I fragment containing the 5' end of the *bla* gene. The 957 bp fragment is inserted in the opposite orientation to its original orientation in the pB2/35SAcK plasmid (see Figure 2).

Soybean line A5547-127 contains a single insert of each of the 3119 bp and 957 bp fragments of pB2/35SAcK. The fragment containing the 5' end of the *bla* gene inserted at the 5' end of the fragment containing the *pat* gene cassette. Thus, line A5547-127 contains one copy of the *pat* gene and single copies of the 5' and 3' portions of the *bla* gene (see Figure 3).

PCR and sequence analysis

PCR was performed on genomic DNA extracted from leaf samples of the two transformed lines and the two non-transformed parental lines. Two separate primer pairs were used, an endogenous control (Soybean actin 1 gene) and primers pairs specific to the inserted DNA. The results of this discriminating PCR confirmed the presence of the insert DNA in soybean lines A2704-12 and A5547-127, and its absence in the non-transgenic parental samples.

Overlapping PCR products spanning the entire length of the insert DNA in A2704-12 and A5547-127 and also including the 5' and 3' junction regions with plant genomic DNA were generated and subsequently sequenced to confirm the results of the Southern blot analyses. The sequence data confirmed the results of the previous characterisations by demonstrating the expected linkage of the elements contained in lines A2704-12 and A5547-127. In both cases, the inserted DNA sequence in the plant is identical to the corresponding transforming plasmid DNA sequences.

Sequencing results for soybean line A2704-12 confirmed the presence of two DNA inserts containing the *pat* gene cassette separated by an inverted 957 bp *Pvu* I fragment from the transforming pB2/SAcK plasmid. Both *pat* gene cassettes are identical to the corresponding transforming plasmid DNA sequence. Part of the 5' fragment of *bla* at the 3' end of the insert has been degraded during the transformation process, however the *bla* gene was already disrupted and expected to be non functional in the plant so this additional degradation of the gene does not raise any concerns.

Sequencing results for soybean line A5547-127 confirmed one copy of the DNA fragment containing the *pat* gene cassette is present and is identical to the corresponding transforming plasmid DNA sequence. The *pat* gene cassette is flanked at the 5' end by a truncated fragment of the 5' end of the *bla* gene and at the 3' end by a truncated fragment of the 3' end of the *bla* gene.

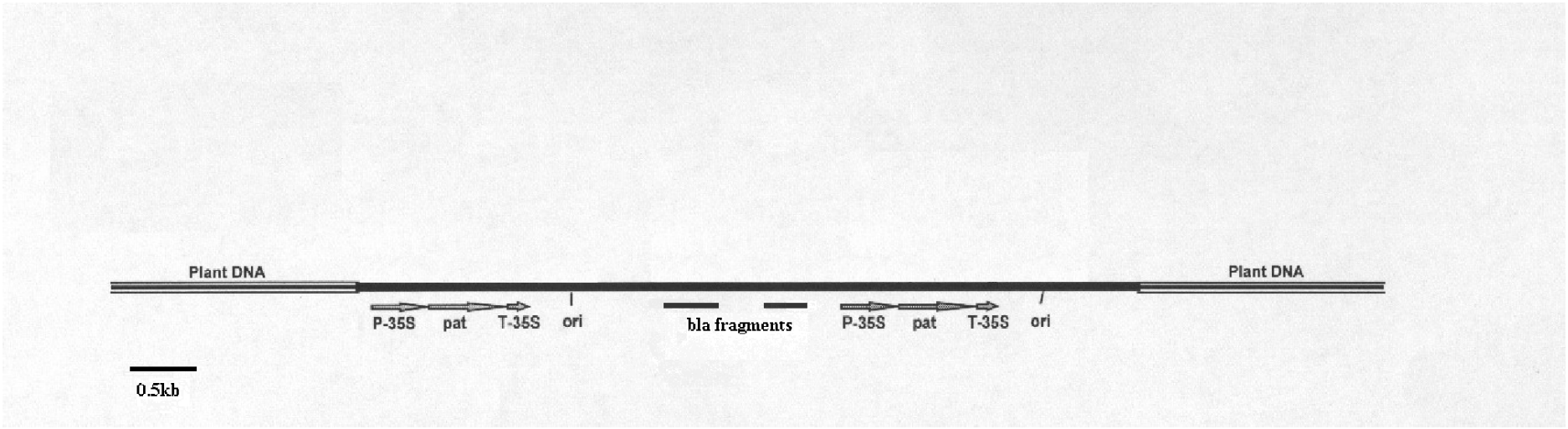

Figure 2: A2704-12 insert in the soybean genome

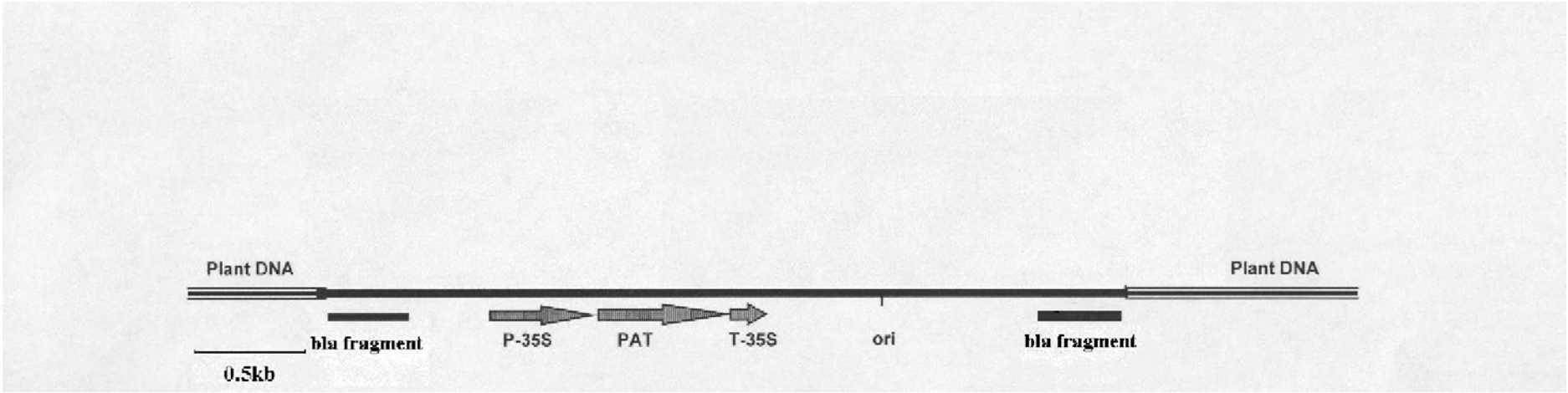

Figure 3: A5547-127 insert in the soybean genome

Detection of bla transcription products

Northern analysis was performed to determine whether the partial *bla* gene sequences present in soybean lines A2704-12 and A5547-127 are expressed in soybean tissues. Total RNA was extracted from leaf, stem and root samples of the transgenic lines A2704-12 and A5547-127 and the non-transgenic parental lines. RNA from each transgenic line and its parental line was run on a gel in parallel to *in vitro* transcribed sense *bla* RNA supplemented with parental line leaf RNA samples. The *in vitro* transcribed sense *bla* RNA was loaded onto the gel at concentrations ranging from 0.1 pg to 4 pg/well. This was done as a positive control for the hybridization reaction as well as to determine the sensitivity of the hybridization probe. The RNA was transferred to a membrane and hybridized with a radio-labelled RNA probe specific to *bla* transcripts.

For soybean line A2704-12 the sensitivity of the reaction was 1 pg. At this level of sensitivity no *bla* transcripts were observed from either the transgenic or non-transgenic leaf, stem or root total RNA samples. For soybean line A5547-127 the sensitivity of the reaction was 2 pg. At this level of sensitivity no *bla* RNA transcripts were observed from either the transgenic or non-transgenic leaf, stem or root total RNA samples. This indicates that *bla* is either not transcribed, or is transcribed at levels that are below the limit of detection. Even if parts of the *bla* gene were transcribed at very low levels it is unlikely that they would be translated into a polypeptide due to the absence of correct translation signals, or that any such polypeptide would be functional.

Analyses of insert flanking sequences

PCR analyses were carried out to determine the nature of the flanking sequences in lines A2704-12 and A5547-127.

Line A2704-12: PCR analysis was performed on a number of templates using primer-pairs targeting the 5' and 3' flanking sequences of soybean line A2704-12. Primers targeting chloroplast tRNA gene sequences were also included in the PCR cocktail. These primers serve as an internal positive control in all PCR reactions. The templates used were genomic DNA from soybean line A2704-12, soybean line A2704, corn (*Zea mays*) variety H99, and canola (*Brassica napus*) variety Ac Excel. The control primer pair produced a PCR product in all four reactions. The primer pairs targeting the 5' and 3' flanking regions produced a PCR product only in reactions containing genomic DNA template from soybean (both A2704 and A2704-12). The reaction with DNA from the transgenic lines resulted in a PCR product the same size as that from non-transgenic lines, which indicates no additional fragments have been incorporated around the primary insertion point. The flanking regions of the A2704-12 insert are of soybean origin.

The flanking soybean genomic DNA regions at the 5' and 3' ends of the insert (198 bp and 299 bp respectively) were sequenced and contained no fragments of plasmid DNA.

Line A5547-127: A similar PCR analysis as that described above was performed on the same control templates and on A5547-127 and A5547 genomic DNA using primer-pairs targeting the 5' and 3' flanking sequences of line A5547-127. The control primer pair produced a PCR product in all four reactions. The primer pairs targeting the 5' and 3' flanking regions produced a PCR product only in reactions containing genomic DNA template from soybeans (both A5547 and A5547-127).

This indicates that the flanking regions of the A5547-127 insert are of soybean origin. The reaction with DNA from the transgenic lines resulted in a PCR product the same size as that from the non-transgenic lines, which indicates no additional fragments have been incorporated around the primary insertion point.

Flanking soybean genomic DNA at the 5' end of the insert (303 bp) and the 3' end of the insert (214 bp) was sequenced and contained no fragments of plasmid DNA.

Conclusion

Detailed molecular analyses have been performed on soybean lines A2704-12 and A5547-127 to characterise the novel genes present in the genome. Results from A2704-12 indicate that two copies of the *pat* gene have been inserted at a single locus in a head to tail configuration. They are separated by a DNA fragment containing a non-functional portion from the 5' end of the *bla* gene. Results from A5547-127 indicate that a single copy of the *pat* gene has been inserted. This line also contains two non-functional fragments of the *bla* gene at the same genomic locus. None of the *bla* gene fragments give rise to any detectable transcription products.

In both lines the *pat* gene is intact and no changes have occurred to its DNA sequence during the transformation process.

Stability of genetic changes

A number of analyses were done to demonstrate the stability of the genetic changes in soybean lines A2704-12 and A5547-127. Southern fingerprint analysis was used to demonstrate the stability of the inserted DNA across three self-pollinated generations and segregation analysis was used to determine the heritability and stability of the *pat* gene in the R_2 and R_3 plants.

Stability of inserted DNA

Southern blot analysis of genomic DNA from three generations (R_3, R_4 and R_5) of soybean lines A2704-12 and A5547-127 indicated that the insert DNA is stable over these generations.

Genomic DNA from the two transgenic lines and the non-transgenic parental lines was digested with *Hind* III and *Nco* I restriction enzymes. Using Southern blot analysis, the digested genomic DNA was hybridized with the 1329 bp *Eco* RI fragment from pB2/35SacK carrying the *pat* gene cassette.

When cut with *Nco* I, genomic DNA from A2704-12 produced the expected fingerprint bands at 4.1 kilobases (kb) and 4.0 kb. The 4.1 kb band represents an internal segment of the insert while the 4.0 kb band represents a border fragment at the 3' end of the insert. The *Hind* III cut A2704-12 genomic DNA also produced the expected fingerprint bands at 4.1 kb and 3.9 kb. The 4.1 kb band represents an internal segment of the insert while the 3.9 kb band represents a border fragment at the 5' end of the insert. No difference in banding pattern between the three different generations (R_3, R_4, and R_5) was observed.

The results for soybean line A5547-127 also showed the expected Southern hybridization fingerprint pattern. When cut with *Nco* I, genomic DNA from A5547-127 produced the expected fingerprint band at 10 kb. The 10 kb band represents the junction between transgenic sequences and plant DNA sequences downstream of the insert. The *Hind* III cut A5547-127 genomic DNA also produced the expected fingerprint band at 2.9 kb. This band represents the junction between transgenic sequences and plant DNA sequences upstream of the insert. No difference in banding pattern between the three different generations (R_3, R_4, and R_5) was observed.

Segregation analysis

Segregation analysis of soybean lines A2704-12 and A5547-127 was carried out on generations R_2 and R_3. To generate these lines, the original transformants were self-pollinated. The glufosinate tolerant progeny were again self-pollinated. This resulted in some individuals producing R_2 progeny (i.e. R_3 plants) that were all tolerant to glufosinate ammonium and some individuals producing R_2 progeny segregating in a 3:1 fashion (glufosinate ammonium tolerant:sensitive). This demonstrates that the *pat* locus is inherited in a Mendelian fashion consistent with a single dominant *pat* locus. Plants that are homozygous with respect to *pat* will produce only glufosinate ammonium tolerant progeny when self-pollinated, whereas heterozygotes will produce a 3:1 mixture of glufosinate ammonium tolerant: sensitive plants. If the *pat* locus were stably inherited, all progeny from the homozygous plants would be glufosinate ammonium tolerant. This was evaluated during subsequent growing seasons with the homozygotes and found to be true. No further testing of heterozygous plants was performed once the expected segregation was confirmed, as none of the progeny from these plants were used to generate commercial lines.

Conclusion

The results of the segregation analysis are consistent with a single site of insertion for the *pat* gene and confirm the results of the molecular characterisation. Molecular analysis of both lines, representing a total of three different generations, indicates that the inserted DNA is stably inherited from one generation to the next.

Antibiotic resistance genes

No complete or functional antibiotic resistant genes were transferred to either soybean line A2704-12 or A5547-127.

CHARACTERISATION OF NOVEL PROTEIN

Biochemical function and phenotypic effects

The herbicide tolerant trait is conferred by the expression of the introduced *pat* gene, which codes for the phosphinothricin acetyltransferase (PAT) protein. PAT functions by detoxifying phosphinothricin (PPT), the active constituent of glufosinate ammonium herbicides. The mode of action of PPT is to inhibit the endogenous enzyme glutamine synthetase, an enzyme involved in amino acid biosynthesis in plant cells. By inhibiting this enzyme, PPT causes rapid accumulation of ammonia in the plant cell, leading to plant death.

In transformed soybean plants, the introduced PAT enzyme chemically inactivates the PPT by acetylation of the free ammonia group, giving rise to herbicide tolerance in the whole plant.

The PAT enzyme expressed in the soybeans is encoded by a synthetic *pat* gene, which shares about 70% homology at the DNA level with the native *pat* gene from S. *viridochromogenes.* The amino acid sequence of the synthetic *pat* gene is however identical to that of the native gene, hence the PAT enzyme expressed in the soybeans is also identical to that derived from the native gene.

The PAT protein consists of 183 amino acids, has a molecular weight of 22 kDa, and exhibits a high degree of enzyme specificity, recognising only one substrate, L-glufosinate, in the acetylation reaction. This high substrate specificity was tested in the presence of each of 21 L-

amino acids at substrate concentrations exceeding 50 times the K_M value for L-glufosinate. None of the tested amino acids substituted as an alternative substrate in the PAT catalysed reaction, but the enzyme reaction with L-glufosinate was not inhibited (Schulz, 1993).

Protein expression analyses

In soybean lines A2704-12 and A5547-127 the only novel protein expected to be expressed is the PAT protein. Expression levels of this protein were determined using an enzyme-linked immunosorbent assay (ELISA) and are reported below. No β-lactamase expression was expected from the *bla* gene as none of the copies of this gene were intact, however both soybean lines were assayed for β-lactamase activity to confirm the absence of expression.

Studies evaluated:

Shillito, R (1997). Phosphinothricin-N-Acetyltranferase protein in processed fractions of group 2 glufosinate resistant soybeans USA, 1996. Performing laboratory: AgrEvo USA Company, United States. Report No. BK-96B-02.

Shillito, R (1998). Phosphinothricin-N-Acetyltransferase protein in processed fractions of group 5 Glufosinate Resistant Soybean, USA, 1997. Performing laboratory: AgrEvo USA Company, United States. Report No. BK97B09.

Shillito, R (1998). Phosphinothricin-N-Acetyltransferase protein in group 2 and group 5 glufosinate resistant soybeans harvested at the forage, hay and seed stages, USA, 1996. Performing laboratory: AgrEvo USA Company, United States. Report No. A59914.

Shillito, R. (2001). Phosphinothricin Acetyltransferase content in grain of Group 2 (Event A2704-12) Glufosinate–tolerant Soybean, USA, 1999. Aventis CropScience Report No. B003247.

Shillito, R. (2001). Phosphinothricin Acetyltransferase content in grain of Group 5 (Event A5547-127) Glufosinate–tolerant Soybean, USA, 1999. Aventis CropScience Report No. B003250.

Shillito, R. (2001). Phosphothricin Acetyltransferase content of Processed Fractions of Group 2 (Event A2704-12) Glufosinate-tolerant Soybean, USA, 1999 (Interim Report). Aventis CropScience Report No. B003248.

Shillito, R. (2001). Phosphinothricin Acetlytransferase content of Processed Fractions of Group 5 (Event A5547-127) Glufosinate-tolerant Soybean, USA, 1999 (Interim Report). Aventis CropScience Report No. B003251.

Schulz, A. (1997). β-lactamase activity in transgenic soybean and equivalency of PAT from transgenic soybean, corn and *E. coli.* AgrEvo Report No. A58682.

PAT protein expression levels

With regard to the safety of food derived from soybean lines A2704-12 and A5547-127, it is important to determine the level of expression of PAT, in order to establish potential dietary exposure to this protein. Soybean lines A2704-12 and A5547-127 were assessed separately for the amount of PAT protein they contain in various food fractions. The results of these are presented below.

Seeds from line A2704-12 and non-transformed A2704 were planted at a single trial site in Iowa and grown to maturity (Shillito 1997). None of these plants were sprayed with glufosinate ammonium herbicide. Seeds from line A5547-127 and non-transformed A5547 were planted at a single trial site in Texas and grown to maturity (Shillito 1998). Tolerant plants were sprayed with glufosinate ammonium (40 oz/acre) six weeks after planting (two months prior to harvesting).

Approximately 750 pounds of soybeans from each A2704-12 and A2704, and 98 pounds of soybeans from each A5547-127 and A5547 were harvested and shipped to the Food Protein Research laboratory, Bryan, Texas where they were processed into the following fractions; whole soybeans, soybean hulls, non-toasted meal, toasted meal, crude lecithin, refined oil, refined-bleached-deodorized oil, and soy isolate. A sample of whole soybeans was also removed for analysis. All samples were analysed for PAT content and the results are described below.

In another study, transgenic soybean line A2704-12 and the non-transgenic counterpart were planted at a four trial sites during the 1999 growing season – Illinois, Nebraska, Wisconsin and Ontario, Canada. Transgenic soybean line A5547-127 and its non-transgenic counterpart were grown at four trial sites also – Florida, Mississippi, Arkansas and North Carolina. The plants in this study were grown under conditions typical of production practises. There were six transgenic plots of each line, three sprayed twice with glufosinate ammonium and three unsprayed. There were also three (unsprayed) non-transgenic plots of each parental line. Soybean samples were collected from the plots of each regime and analysed for PAT content. Soybean samples were collected from Illinois (A2704-12 and A2704) and Florida (A5547-127 and A5547) field trials and transported to Texas A&M University for processing into hulls, defatted meal (non-toasted and toasted), crude lecithin, refined bleached deodorized oil, and soy isolate. These samples were also analysed for PAT protein content as described below.

A double-antibody ELISA was used to quantify the PAT protein in the whole soybeans and their fractions from transgenic and non-transgenic plants. The ELISA detects both intact and degraded PAT, therefore the results are likely to be overestimates of the level of active protein. In addition to this, the immunoreactive PAT detected by the ELISA was not assayed to determine whether or not it retained enzyme activity. It is unlikely that the enzyme is active, as temperatures reach up to 100°C during the cooking processes, sufficiently high to inactivate the enzyme. The limit of quantitation of the immunoassay was 0.4 ng/mL.

1996-1997 Study:
No PAT was detected in the control plants. Levels of PAT found in the transgenic seeds and their processed fractions are shown in Tables 2 and 3.

1999 Study:
A trace amount (approximately 6 ng/g) of PAT protein was detected in the A2704 control soybeans and hull samples. This result could be due to contamination of the control soybean sample by the transgenic sample to the order of 1 part in 300. No PAT protein was found in the other control fractions. There was no significant difference in PAT content between sprayed and unsprayed A2704-12 soybeans ($p = 0.25$) or sprayed and unsprayed A5547-127 soybeans ($p = 0.36$). The processed fractions were not analysed statistically. PAT content of transgenic soybean grain and processed fractions are shown in Tables 4 to 6.

Table 2: PAT protein in A2704-12 seeds and processed fractions from 1997 field trial

Fraction	PAT/Sample (ng/g)	Crude protein (%)	PAT protein as a % of crude protein
Whole seed	573	37-45%	0.00016%
Hulls	380	12.5%	0.00030%
Defatted Meal	ND		-
Toasted Defatted Meal	ND		-
Crude Lethicin	ND		-
Refined Oil	ND		-
Refined Bleached Deoderised Oil	ND		-
Soy Isolate	ND		-

ND= not detected

Table 3: PAT protein in A5547-127 seeds and processed fractions from 1998 field trial

Fraction	PAT/Sample (ng/g)	Crude protein (%)	PAT protein as a % of crude protein
Whole seed	10800	37-45%	0.00292%
Hulls	2260	10.8-12.5%	0.00209%
Defatted Meal	44.6	43-48%	0.0000104%
Toasted Defatted Meal	4.96	43-48%	0.00000115%
Crude Lethicin	ND		-
Refined Oil	ND		-
Refined Bleached Deoderised Oil	ND		-
Soy Isolate	35.1	90%	0.0000039%

ND = not detected

Table 4: PAT in A2704-12 and A5547-127 soybean grains from 1999 field trials

Line	Average PAT (ng/g sample) Mean (SD)		Average PAT as % of crude protein	
	Unsprayed	Sprayed	Unsprayed	Sprayed
A2704-12	862 (272)	879 (264)	0.000227%	0.000227%
A5547-127	9971 (940)	10100 (816)	0.00283%	0.00285%

Table 5: PAT in processed fractions of A2704-12 grain

Commodity	PAT (ng/g fresh weight)		% crude protein present in matrix		PAT protein expressed as % crude protein	
	Sprayed	Unsprayed	Sprayed	Unsprayed	Sprayed	Unsprayed
Whole Soybean	2138	1948	38.9%	38.5%	0.00050%	0.00056%
Hulls	1596	1653	20.5%	17.4%	0.00081%	0.00092%
Meal	11.03	5.18	54.5%	55.4%	0.00000095%	0.0000020%
Toasted Meal	ND	ND	-	-	-	-
Crude lecithin	ND	ND	-	-	-	-
Refined Oil	ND	ND	-	-	-	-
Refined Bleached Deodorised Oil	ND	ND	-	-	-	-
Soybean isolate	ND	9.0	89.3%	89.3%	-	0.000001%

ND = not detected

Table 6: PAT in processed fractions of A5547-127 grain

Commodity	PAT (ng/g fresh weight)		% crude protein present in matrix		PAT protein expressed as % crude protein	
	Sprayed	Unsprayed	Sprayed	Unsprayed	Sprayed	Unsprayed
Whole Soybean	17471	20202	36.5%	35.8%	0.0048%	0.0056%
Hulls	9521	11416	25.6%	23.6%	0.0037%	0.0048%
Meal	69.5	105.5	51.9%	54.9%	0.000013%	0.000019%
Toasted Meal	13.4	35.9	51.9%	54.9%	0.000013%	0.000019%
Crude lecithin	ND	ND	-	-	-	-
Refined Oil	ND	ND	-	-	-	-
Refined Bleached Deodorised Oil	ND	ND	-	-	-	-
Soybean isolate	80.9	41.4	88.9%	87.9%	0.000009%	0.0000047%

ND = not detected

Western blot analysis

Western blot analysis was performed to determine that the PAT protein expressed in lines A2704-12 and A5547-127 conforms to the expected size and identity. This analysis can also be used to determine if the plant-expressed PAT protein has been subject to any post-translational modifications that would be detected as altered molecular weight, e.g. glycosylation. Crude protein extracts from leaves of soybean lines A2704-12 and A5547-127 and purified PAT protein expressed from *E. coli* (using the same *pat* gene used to transform the soybeans) were compared by SDS-PAGE/Western blotting using polyclonal rabbit anti PAT antiserum. This demonstrated that the PAT proteins from these sources all have the same apparent molecular weight, as determined by visual analysis of the Western blot. This indicates that the soybean-expressed PAT protein conforms to expected size and identity and also that it does not appear to have undergone any significant post-translational modifications of a type that would alter the molecular weight/electrophoretic mobility of the protein.

Assay for β-lactamase activity

When the *bla* gene is functional it expresses the enzyme β-lactamase, which is able to break down certain β-lactam antibiotics such as ampicillin and penicillin, thus conferring resistance. It was previously shown by Northern analysis that the *bla* gene fragments present in lines A2704-12 and A5547-127 are either not transcribed or transcribed at levels that are below the limit of detection. Moreover, none of the *bla* gene fragments are intact, therefore even were they to be transcribed, it is most unlikely they would be translated to produce functional β-lactamase. To confirm the absence of protein expression, an assay for β-lactamase activity was done on crude protein extracts from the transgenic soybean lines.

In this study, crude protein samples extracted from leaves of A2704-12, A2704, A5547-127 and A5547 were incubated with radio-labelled penicillin at 37ºC for 5 minutes and 75 minutes to determine if any β-lactamase protein was present. Samples were analysed for the breakdown of penicillin by β-lactamase using High Performance Liquid Chromatography (HPLC). HPLC is used to separate different compounds based on the speed at which they travel through a column. In this case, degraded penicillin can be distinguished by size from intact penicillin. Controls used in this experiment were 1) a negative control reaction of labelled penicillin only and 2) a positive control reaction of the penicillin resistant bacteria *E. coli*/pUC18, which is known to produce β-lactamase, incubated with labelled penicillin for 5 and 75 minutes. Radio-labelled penicillin alone eluted from the column after 21.5 minutes.

The 5 and 75 minute β-lactamase treated penicillin eluted at 17 minutes indicating that it has been broken down into several smaller degradation products. When crude protein extracts from soybean lines A2704-12, A2704, A5547-127 and A5547 were analysed by HPLC after incubation with labelled penicillin, the penicillin eluted from the column at 21.5 minutes, the same time as the intact penicillin. This was the case for samples incubated for 5 and 75 minutes, indicating that no degradation occurs even after 75 minutes incubation. This showed that β-lactamase is not expressed in either of the two transgenic lines studied or their parental controls and confirms that the bla gene fragments are non-functional.

Potential toxicity of novel protein

Potential toxicity of PAT

There is no evidence available indicating that the PAT protein is toxic to either humans or other animals (OECD, 1999). In addition, data demonstrating the absence of acute oral toxicity of the PAT protein in mice have been evaluated by FSANZ on a number of previous occasions where it was concluded that the protein is non-toxic to humans (FSANZ 2000, 2001a,b, 2003). This conclusion also applies to the PAT protein present in soybean lines A2704-12 and A5547-127, as it is identical in amino acid sequence to the PAT protein assessed for toxicity on previous occasions. In addition to consideration of acute toxicity, the amino acid sequence of the PAT protein has also been compared to that of known protein toxins.

Studies evaluated:
Herouet (2002). Phosphinothricin Acetyltransferase (PAT) *pat* gene product: Overall amino acid sequence homology search with known toxins and allergens. Bayer CropScience Report No C024489.

Similarities with known protein toxins

Bioinformatic analyses were done to assess the PAT protein for any similarity with known protein toxins. The amino acid sequence homology search was carried out by comparing the complete amino acid sequence of the PAT protein with all protein sequences present in the following reference databases: SwissProt, trEMBL, GeneSeq-Prot, PIR, PDB, DAD and GenPept. The BLASTP program was used to compare the PAT amino acid sequence to all the sequences available in the different databases. Comparisons were made in a pair wise fashion. The similarity was shown by local alignments of the two sequences that included only the most similar local region(s).

No relevant similarity between the PAT protein and known toxins was found, based on a 35% identity over an 80 amino acid segment. As expected, the main similarities observed were to other PAT proteins from various origins.

Potential allergenicity of novel protein

There are concerns that new proteins introduced into food will cause allergic reactions in some individuals. The potential allergenicity of a novel protein is evaluated using an integrated, step-wise, case-by-case approach relying on various criteria used in combination, since no single criterion is sufficiently predictive of either allergenicity or non-allergenicity. The assessment focuses on the source of the novel protein, any significant amino acid similarity between the novel protein and that of known allergens, and the structural properties of the novel protein, including susceptibility to degradation in simulated digestion models. Applying such criteria systematically provides reasonable evidence about the potential of the newly introduced proteins to act as an allergen (Lehrer and Reese 1998; Jones and Maryanski 1991).

The only novel protein expressed in soybean lines A2704-12 and A5547-127 is PAT. This protein was assessed using these criteria for its potential allergenicity.

Studies submitted:
Herouet (2002). Phosphinothricin Acetyltransferase (PAT) *pat* gene product: Epitope homology and glycosylation searches. Bayer CropScience Report C024490.

Schneider, R. (1993). Fate of introduced DNA in gut: Degradation of phosphinothricin acetyltransferase gene from transgenic rape HCN 92 (Brassica napus) in stomach fluids from pig, chicken and cow. Hoechst Report No. A51613.

Schulz, A. (1993). L-Phosphinothricin N-Acetyl transferase – Inactivation by pig and cattle gastric juice. Hoechst Report No. A51230.

Schulz, A. Lutge, K. and Taggeselle, P. (1997). Stability of the Phosphinothricin acetyl transferase enzyme: Heat Stability and Digestion in Simulated Gastric Fluid and Simulated Intestinal Fluid. AgrEvo Report No A58686.

Similarity to known allergens

The amino acid sequence of PAT was compared with epitopes found on known allergens. The purpose was to identify any short sequence of amino acids that might represent an isolated allergenic epitope. The amino acid sequence of the PAT gene was compared with epitopes of all known allergens present in the publicly available protein databases SwissProt, trEMBL , GeneSeq-Prot, PIR, PDB, DAD and GenPept.

The criterion indicating allergenicity was a 100 per cent identity on a window of eight amino acids with an allergenic protein. The results of this search showed no identity with known allergens.

Glycosylation and Heat Stability

Common plant food allergens are usually glycosylated proteins and most are tolerant to heat denaturation, remaining stable during the high temperatures involved in cooking or processing. However, the PAT protein lacks glycosylation sites, and according to Western blot analysis does not appear to be glycosylated when expressed in soybean lines. Studies have determined that the enzyme is heat labile and is completely inactivated by temperatures above 75°C. In addition to this, experiments conducted by Shulz *et al*. (1997) found that the purified protein is denatured at temperatures above 40°C.

In vitro digestibility

Typically, most food allergens tend to be stable to the peptic and acidic conditions of the digestive system if they are to reach and pass through the intestinal mucosa to elicit an allergic response (Kimber et al 1999; Astwood et al 1996; Metcalfe et al 1996). The PAT protein was therefore investigated for its digestibility in simulated digestion models.

Two studies were done – one looking at the digestibility of the PAT protein in simulated gastric fluid (SGF) and one looking at digestibility in simulated intestinal fluid (SIF). SGF contains pepsin and SIF contains pancreatin, a mixture of enzymes including amylase, trypsin, lipase, ribonuclease and protease. PAT from a crude protein extract from glufosinate ammonium tolerant corn leaves was treated with SGF and was found to be digested rapidly (in under 5 seconds). No degradation occurred when SGF lacked pepsin. Purified PAT in SIF is completely digested within 15 minutes.

Conclusion

Soybean lines A2704-12 and A5547-127 express a single novel protein – PAT. PAT is expressed in soybean seeds at low levels with levels ranging from 573-2138 ng/g (up to 0.00056% of total protein) in A2704-12 and 10100-20202 ng/g (up to 0.0056% of total protein) in A5547-127. In the soybean hulls, meal, lecithin, refined oil and soy isolate levels of PAT were much lower or undetectable.

A large number of studies have been done on the PAT protein to determine its potential toxicity and allergenicity. These studies demonstrate that the protein is non-toxic to mammals, including humans, and has limited potential to be allergenic.

COMPARATIVE ANALYSES

A comparative approach focussing on the determination of similarities and differences between the GM 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 GM foods (WHO 2000). The critical components to be measured are determined by identifying key nutrients, key toxicants and anti-nutrients for the food source in question (FAO 1996). The key nutrients and toxicants/anti-nutrients are those components in a particular food that may have a substantial impact in the overall diet.

These may be major constituents (e.g., fats, proteins, carbohydrates) or minor components (e.g., minerals, vitamins). Key toxicants are those toxicologically significant compounds known to be inherently present in the plant, such as those compounds whose toxic potency and level may be significant to health (e.g., solanine in potatoes if the level is increased). The key components of soybean that should be considered in the comparison include proximates, amino acids, fatty acids, phytic acid, trypsin inhibitors, lectins, isoflavones and phosphatides (OECD 2001). Table 7 shows the analyses done on each soybean line. As part of the analysis, the allergenic potential of the transgenic soybean lines was compared to the parental controls.

Table 7: Analyses performed on Soybean Fractions of lines A2704-12 and A5547-127 and their non-transgenic counterparts

Matrix	Analyses Performed
Soybean Grain	Proximates, amino acids, fatty acids, phytic acid, trypsin inhibitor, calcium, phosphorous, potassium, stachyose, and raffinose, lectins, isoflavones
Soybean Hulls	Proximates
Refined Bleached Deodorised Oil	Fatty acids
Soy Isolate	Crude protein, amino acids
Defatted Meal (non-toasted)	Proximate, amino acids, trypsin inhibitor, phytic acid, isoflavones, lectins
Defatted Meal (toasted)	Proximates, amino acids, phytic acid, trypsin inhibitor, isoflavones, lectins

Studies evaluated:
Amann, M. and Eickhoff, J. (1998). Variability of Nutrients in Soybeans: Ranges of Reported Nutrient Values. AgrEvo Report No. C000924.

Barraj, (1998). Statistical Analysis of compositional and nutritional data from glufosinate resistant soybean event A2704-12 and its Nontransgenic counterpart A2704. AgrEvo USA Report No. A59917.

Oberdorfer, R. (2001). Nutritional Impact Assessment Report on Glufosinate Ammonium Tolerant Soybean Transformant A2704-12. Aventis CropScience Report No Co13150.

Shillito, R.D. (2001). Composition of Processed Fractions of Group Two (Event A2704-12) Glufosinate-tolerant Soybean, USA, 1999. Aventis Report No B003146.

Shillito, R.D. (2001). Composition of Processed Fractions of Group Five (Event A5547-127) Glufosinate-tolerant Soybean, USA, 1999. Aventis Report No B003148.

Shillito, R. (2001). Composition of Processed Fractions of Group 2 (Event A2704-12) Glufosinate-tolerant Soybean, USA, 1999. Aventis CropScience Report B003146.

Shillito, R. (2001). Composition of Raw Agricultural Commodities of Event A2704-12 Group 2 Glufosinate Tolerant Soybean and the Non-transgenic Counterpart A2704, USA, 1999. Aventis CropScience Report B003145.

Nutrient analysis

To determine whether unexpected changes had occurred in the nutrient composition of soybean lines A2704-12 and A5547-127 as a result of the modification, and to assess the nutritional adequacy of these lines, compositional analyses were done on whole soybean seed and processed fractions including soybean hulls, toasted and non-toasted soy meal, soy isolate, and bleached deodorised refined oil.

A total of 42 components were analysed in soybean seed from A2704-12 and A5547-127 transgenic plants and their non-transgenic counterparts. The components measured were proximate content (protein, fat, carbohydrate, ash, moisture), acid detergent fibre (ADF), neutral detergent fibre (NDF), amino acids, fatty acids, minerals (calcium, phosphorous and potassium), stachyose, raffinose, phytic acid, and trypsin inhibitor.

Transgenic soybean line A2704-12 and its non-transgenic counterpart A2704 were grown in three different locations (Illinois, Iowa and Nebraska) in 1996 and in four different locations (Illinois, Nebraska, Wisconsin and Ontario, Canada) in 1999. Transgenic soybean line A5547-127 and its non-transgenic counterpart A5547 were grown in three different locations in the United States (Arkansas, Florida and North Carolina) and in Puerto Rico in 1996 and in 4 different locations (North Carolina, Florida, Mississippi and Arkansas) in 1999. In every trial a plot for the non-transgenic control was planted as the closest relative and grown under the same conditions. The plants grown in the 1996 field trials were not treated with glufosinate ammonium. The plants grown in the 1999 field trials consisted at each location of three non-transgenic plots, and six transgenic plots, three of which were treated with glufosinate ammonium herbicide.

Seed grown from each of the 1996 sites was analysed, and the results were compared statistically within location for any location effect, within type (transgenic versus non-transgenic) for any type effect and a two way analysis was also carried out to test for any interaction between type and location in regard to nutrient content. Seeds and processed fractions, hay and forage of all plants were analysed in regard to nutrient content and values compared to the literature values for each component. No statistical analysis was conducted on the data from the processed fractions as they contained only one replicate.

Soybean grain, soybean hulls, soy isolate, defatted meal (toasted and non toasted) and refined, bleached, deodorised oil from the 1999 plants were analysed for proximates, amino acids, fatty acids, phytic acid, trypsin inhibitors, lectins, and isoflavones. Samples from the A2704-12 and A2704 soybeans were compared to the literature range only. From examination of this data it was concluded that treating the transgenic A2704-12 soybeans with glufosinate-ammonium had no effect on the composition of the plant, compared to the non-transgenic control.

The nutrient values of both sprayed and unsprayed soybean line A2704-12 plants and the A2704 control plants were within the literature range in almost every instance. Where differences were seen, both the transgenic and the non-transgenic samples tended in the same direction.

Soybean samples from soybean lines A5547-127 and A5547 grown in 1999 were statistically analysed for differences between the non-transgenic plants and the transgenic plants (sprayed with glufosinate ammonium and unsprayed). These results are discussed below.

Proximate analysis of whole soybeans

A2704-12
The results of the proximate analysis on whole soybeans are shown in Table 8. Results were compared within location for any location effect, within type (transgenic versus non-transgenic) for any type effect and a two way analysis was also carried out to test for any interaction between type and location in regard to nutrient content. A significant difference was observed for protein with the A2704-12 transgenic plants having 2.3% more protein than the A2704 control non-transgenic plants. However, the values reported for both A2704-12 and control soybeans protein levels were within the literature reported range, therefore this difference is not considered to be biologically significant.

A5547-127
Proximate analysis was done on whole soybeans from the trials in Arkansas, Florida and Puerto Rico (1996) (See Table 9). There were no significant differences between the glufosinate ammonium-tolerant soybeans and the control soybeans in regard to fat, protein, and carbohydrate content. Ash content was significantly different between types (transgenic higher than control) at two of the three sites, however when analysed across all three locations no significant differences were observed indicating that variance within the sites alone may have contributed to the significant differences. In any case, the ash levels fall within the literature range. The neutral detergent fibre (NDF) content of the transgenic plants was significantly higher than the control at one of the locations. This is likely to be a location effect as when analysed at the other two locations and across all locations (for type and interaction effects), no significant differences were observed. This is also the case for acid detergent fibre (ADF) content.

Proximate analysis on soybeans grown in the 1999 trials showed no significant differences between the transgenic grain (whether sprayed with glufosinate ammonium or not) and the control grain for any of the variables except carbohydrate. Carbohydrate levels were reported as significantly different however as the carbohydrate value is calculated from the other proximate values the reported differences ($p=0.0123$) are likely to be due to the slight differences seen in the other proximate measurements. Thus there are no nutritionally

significant differences between the transgenic soybeans and the non-transgenic controls in regard to proximate content.

Amino acid analysis of whole soybeans

A2704-12
Of the 18 amino acids analysed in whole soybeans, there were a number of significant differences between the glufosinate ammonium–tolerant soybeans and the control soybeans (table 10). In all cases, the transgenic samples showed higher levels of amino acids than the controls. This was consistent with the slightly higher total amino acid content of the transgenic seeds compared with the control seeds.

The amino acids cysteine, glutamic acid, glycine, histidine, methionine, tryptophan, and valine showed no significant differences between the transgenic and non-transgenic samples. All the amino acids that had a significant difference between the transgenic and non-transgenic soybeans were within the literature reported ranges.

The largest mean difference was only 8.5% for tyrosine and this and the other reported differences are considered to be biologically insignificant.

A5547-127
Amino acid content of whole soybeans grown at Florida and Puerto Rico test sites was analysed. Of the 18 amino acids analysed there were no significant differences between the transgenic A5547-127 plants and the non-transgenic control plants (see Table 11). The mean alanine content in transgenic seeds as a percentage of the control seeds was 28%. This is explained by the result that control seeds varied somewhat in their alanine content as can be seen by the standard deviation of 0.78. No significant difference was reported for alanine.

For the seeds grown in the 1999 test sites the concentrations of amino acids in all three treatments were in the published range. The are no statistically significant differences between the non-transgenic grain, the transgenic sprayed grain and the transgenic non-sprayed grain for alanine, aspartic acid, cysteine, glutamic acid, glycine, methionine, proline, threonine, and tyrosine. Some significant differences were found for the other amino acids, however, these differences appear to be due to natural variation rather than directly attributed to the genetic modification, and are not considered to be biologically significant as they were not observed in the 1996 trials.

Mineral content of whole soybeans (A2704-12 and A5547-127)

The minerals calcium, phosphorus and potassium were analysed in whole soybeans from lines A2704-12, A2704, A5547-127 and A5547 (Tables 13 and 14). There was no significant difference between the transgenic and non-transgenic samples for either A2704-12 or A5547-127 in regard to calcium and potassium.

In regard to phosphorus content of A2704-12 soybeans and their control there was no significant difference between transgenic and non-transgenic samples at two of the locations (Illinois and Iowa), however at Nebraska the transgenic plants had higher levels than the control plants. When all samples were analysed, there was no significant type difference, however a significant difference was observed following the two-way analysis. In A5547-127 soybeans there was a significant difference between transgenic and non-transgenic samples in

regard to phosphorus levels at one of the locations (Florida) where the transgenic plants were found to have higher levels than the control plants. However, when all samples were analysed, there was no significant type difference. Nor was there a significant difference observed following the two-way analysis. The level of phosphorus in both A2704-12 and A5547-127 soybeans was within the literature range.

Fatty acid analysis of refined soybean oil (A2704-12 and A5547-127)

The results of compositional analysis of refined soybean oil from transgenic soybean lines A2704-12 and A5547-127 and their parental control lines are shown in tables 14 and 15. The fatty acids C8:0, C10:0, C11:0, C12:0, C13:0, C14:0, C14:1, C15:0, C15:1, C16:1, C16:2, C16:3, C17:0, C17:1, C18:4, C20:2, C20:3, C20:4, C20:5, C21:5, C22:1-6, C24:0 and C24:1 were below the limit of quantitation (<0.10%). No statistical analysis was done on the refined soybean oil fatty acid values.

For transgenic soybean line A2704-12, the percentage difference between the control and transgenic seeds ranged from –4.3% to 10%. The fatty acid content of the samples was similar to the USFDA's reference values for soybean oil. For transgenic line A5547-127, the difference in means of the transgenic oil compared to the control oil ranged from –2.5% to 2.1%. The fatty acid profile of the refined transgenic soybean oil is the same as the profile of the control soybean oil. The amino acid content of the samples was similar to the US FDA's reference value range for soybean oil. Thus there is no biologically significant difference between the oils from transgenic and control lines.

Toasted and Non-toasted Defatted Meal (A2704-12 and A5547-127)

Proximates, amino acids, and anti-nutrients were measured in the toasted and non-toasted defatted soybean meal from lines A2704-12, A2704, A5547-127, and A5547. Values were compared with each other and standard literature ranges. No differences of nutritional impact were observed.

Amino acid analysis of soy isolate (A2704-12 and A5547-127)

Amino acid analysis was carried out on soy isolate of lines A2704-12, A2704, A5547-127 and A5547, and compared with the standard literature values. For A2704-12 soy isolate, both transgenic and non-transgenic soy isolate amino acid values were slightly below the standard range for all amino acids other than methionine, threonine and tyrosine. However, the transgenic soy isolate was very similar to the control. The values for each amino acid in the transgenic soy isolate differed from the control values by only –1.1% to 1.6%.

For A5547-127 soy isolate, the percent difference between the mean levels of each amino acid in the transgenic soy isolate compared to the non-transgenic soy isolate did not exceed 20%. Five (asparagine, cysteine, methionine, threonine, and tyrosine) of the 18 amino acids measured were within the literature range for both lines and in all but three of the other cases (glutamine, glycine and serine) the transgenic plants were closer to the literature than the non-transgenic plants. The differences reported in both soybean lines A2704-12 and A5547-127 are not nutritionally significant.

Phospholipid Profile of Crude Lecithin (A2704-12 and A5547-127)

The results of the phosphatide analysis are shown in tables 16 and 17. For line A2704-12 and its parental control, values were within the literature range in all cases other than phosphitid acid. Values for the control and transgenic samples are similar and are unlikely to be nutritionally significant. For line A5547-127 and its parental control, no correspondence was found between the literature range and the levels determined for both the transgenic and non-transgenic samples. However, the transgenic and non-transgenic samples showed good correspondence with each other with the levels of phospholipids in the transgenic plants ranging from –16% to –9.7% of the control values.

Table 8: Comparison of fibre and proximate content in A2704-12 whole soybeans and control (1996)

Constituent (% of Dry Matter)	All Locations Mean (standard deviation)		Mean difference (% of control)	P value (one way)	P value (two way)	Literature Range[1]
	A2704-12	Control				
Fat	21.53 (0.90)	21.69 (1.09)	-0.7	NS	NS*	14.9-31.4
Protein	42.28 (0.52)	41.32 (1.29)	2.3	NS	**0.041**	28.9- 47.9
Ash	5.23 (0.44)	5.14 (0.39)	1.7	NS	NS*	4.5-6.4
Fibre (NDF)	10.39 (0.89)	10.14 (0.60)	2.4	NS	NS	1.1-15
Fibre (ADF)	7.50 (0.74)	7.16 (0.98)	4.7	NS	NS	9.0-11.3
Carbohydrates	31.08 (0.78)	32.41 (2.48)	4.1	NS	NS	30.2-44.9

[1] Literature range comes from the literature review by Amann and Eickhoff, 1998, including data from the USDA, 1997, and OECD consensus document on soybeans, 2001.
NS = not significant
* Significant location effect reported

Table 9: Comparison of fibre and proximate content in A5547-127 whole soybean and control (1996)

Constituent (% of Dry Matter)	All Locations Mean (standard deviation)		Mean Difference (%)	P value (one way)	P value (two way)	Literature Range
	A5547-127	Control				
Fat	21.23 (1.24)	21.68 (0.66)	-2.07	NS	NS *	14.9-31.4
Protein	41.35 (1.14)	41.23 (1.12)	0.29	NS	NS *	28.9- 47.9
Ash	5.54 (0.31)	5.32 (0.19)	4.1	NS	NS *	4.5-6.4
Fiber (NDF)	10.22 (2.67)	9.97 (1.56)	2.5	NS	NS *	1.1-15
Fiber (ADF)	7.10 (0.46)	7.02 (0.58)	1.1	NS	NS *	9.0-11.3
Carbohydrates	31.88 (1.61)	31.76 (1.33)	0.38	NS	NS *	30.2-44.9

NS= not significant
* Significant location effect only

Table 10: Comparison of amino acid content in A2704-12 whole soybeans and control (1996)

Constituent (% of Dry Matter)	All Locations Mean (standard deviation)		Mean difference (% of control)	P value (one way)	P value (two way)	Literature Range
	A2704-12	Control				
Alanine	1.58 (0.03)	1.53 (0.05)	3.2	**0.038**	**0.022***	1.40-2.04
Arginine	2.64 (0.10)	2.45 (0.15)	7.7	**0.003**	**0.002**	2.45-4.05
Aspartic acid	4.47 (0.09)	4.35 (0.10)	2.8	**0.016**	**0.022**	3.05-5.46
Cysteine	0.48 (0.03)	0.48 (0.02)	0	NS	NS*	0.45-1.08
Glutamic acid	6.12 (0.76)	6.03 (0.64)	1.4	NS	NS*	6.47-9.13
Glycine	1.57 (0.04)	1.54 (0.05)	1.9	NS	NS*	1.05-2.50
Histidine	1.11 (0.04)	1.07 (0.07)	3.7	NS	NS*	1.0-1.64
Isoleucine	1.61 (0.04)	1.57 (0.05)	2.5	**0.035**	**0.020**	1.23-2.44
Leucine	2.79 (0.05)	2.70 (0.07)	3.3	**0.005**	**0.005**	2.2-4.0
Total Lysine	2.27 (0.05)	2.21 (0.08)	2.7	NS	**0.042***	2.20-2.95
Methionine	0.55 (0.02)	0.54 (0.04)	1.8	NS	NS	0.42-0.87
Phenylalanine	1.81 (0.04)	1.74 (0.06)	4.0	**0.006**	**0.002**	1.6-2.62
Proline	1.89 (0.09)	1.81 (0.16)	4.4	NS	**0.038***	1.73-2.90
Serine	1.98 (0.04)	1.92 (0.04)	3.1	**0.015**	**0.003***	1.76-2.91
Threonine	1.52 (0.05)	1.49 (0.03)	2	NS	**0.028***	1.38-1.96
Tryptophan	0.43 (0.04)	0.41 (0.03)	4.8	NS	NS	0.51-0.67
Tyrosine	1.14 (0.02)	1.05 (0.11)	8.5	**0.015**	**<0.001***	1.11-2.15
Valine	1.68 (0.05)	1.64 (0.05)	2.4	NS	NS	1.27-2.44

NS= not significant

* Significant location effect only

Table 11: Comparison of amino acid content in A5547-127 whole soybean and control (1996)

Constituent (% of Dry Matter)	All Locations Mean (standard deviation)		Mean difference (% of control)	P value (one way)	P value (two way)	Literature Range
	A5547-127	Control				
Alanine	1.73 (0.04)	1.35 (0.78)	28	NS	NS	1.40-2.04
Arginine	2.91 (0.09)	2.92 (0.09)	-0.3	NS	NS	2.45-4.05
Aspartic acid	5.00 (0.10)	4.99 (0.11)	0.2	NS	NS	3.05-5.46
Cysteine	0.60 (0.05)	0.57 (0.03)	5.3	NS	NS *	0.45-1.08
Glutamic acid	6.39 (0.14)	6.63 (0.38)	-3.6	NS	NS *	6.47-9.13
Glycine	1.72 (0.04)	1.73 (0.04)	-0.6	NS	NS	1.05-2.50
Histidine	1.25 (0.06)	1.28 (0.07)	2.3	NS	NS *	1.0-1.64
Isoleucine	1.70 (0.11)	1.71 (0.04)	-1.7	NS	NS *	1.23-2.44
Leucine	3.06 (0.08)	3.06 (0.07)	0	NS	NS	2.2-4.0
Total Lysine	2.61 (0.14)	2.56 (0.09)	1.9	NS	NS *	2.20-2.95
Methionine	0.54 (0.03)	0.55 (0.02)	-1.8	NS	NS	0.42-0.87
Phenylalanine	2.01 (0.05)	2.02 (0.06)	-0.5	NS	NS	1.6-2.62
Proline	2.09 (0.04)	2.09 (0.04)	0	NS	NS	1.73-2.90
Serine	2.22 (0.02)	2.20 (0.04)	0.9	NS	NS	1.76-2.91
Threonine	1.67 (0.02)	1.68 (0.04)	-0.6	NS	NS	1.38-1.96
Tryptophan	0.49 (0.02)	0.49 (0.04)	0	NS	NS	0.51-0.67
Tyrosine	1.26 (0.04)	1.27 (0.02)	-0.8	NS	NS	1.11-2.15
Valine	1.79 (0.10)	1.82 (0.03)	-1.6	NS	NS *	1.27-2.44

NS= not significant
* Significant location effect only

Table 12: Mineral content of whole A2704-12 soybean and control (1996)

Constituent (% of Dry Matter)	All Locations Mean (standard deviation)		Mean difference (% of control)	P value (one way)	P value (two way)	Literature Range
	A2704-12	Control				
Calcium	0.26 (0.05)	0.27 (0.06)	-3.7	NS	NS*	0.19-0.36
Phosphorus	0.61 (0.08)	0.57 (0.07)	7	NS	**0.001***	0.47-1.02
Potassium	1.86 (0.12)	1.87 (0.13)	0.5	NS	NS*	0.47-2.47

NS= not significant
* Significant location effect

Table 13: Mineral content of whole A5547-127 soybean and control (1996)

Constituent (% of Dry Matter)	All Locations Mean (std dev)		Mean difference (% of control)	P value (one way)	P value (two way)	Literature Range
	A5547-127	Control				
Calcium	0.31 (0.02)	0.31 (0.02)	0	NS	NS	0.19-0.36
Phosphorus	0.67 (0.01)	0.66 (0.03)	1.5	NS	NS	0.47-1.02
Potassium	1.83 (0.06)	1.85 (0.06)	-1.1	NS	NS *	0.47-2.47

NS = Not significant
* Significant location effect reported.

Table 14: Fatty acid content of refined soybean oil (A2704-12)

Fatty Acid	Per cent of Oil			
	A2704-12	Control	Mean difference %	Literature Range
Saturated				
tetradecanoic (C14:0)	<0.10	<0.10	0	0.10
palmitic (C16:0)	9.13	9.48	-3.4	6.7-14.5
stearic (C18:0)	4.73	4.59	3.1	0.5-8.9
arachidic (C20:0)	0.36	0.35	2.9	0.1-0.9
behenic (C22:0)	0.35	0.35	0	0
Total	14.57	14.77	-1.4	13.6-14.4
Mono-unsaturated				
palmitoleic (C16:1)	0.11	<0.10	10	0.2-0.5
oleic (C18:1)	23.55	23.04	2.2	14.3-28.7
eicosenoic C20:1	0.22	0.23	-4.3	0.2-0.5
Total	23.77	23.27	2.1	14.3-28.7
Poly-unsaturated				
linoleic (C18:2)	53.05	53.35	-0.6	36.5-60.0
linolenic (C18:3)	7.96	8.00	-0.5	1.9-14.7
Total	61.01	61.35	-0.5	38.4-72.5
Grand Total	99.35	99.35	0	-

Table 15: Fatty Acid content of refined soybean oil (A5547-127)

Fatty Acid	Percent of oil			
	A5547-127	Control	Difference in means (% of control)	Literature Range
Saturated				
tetradecanoic (C14:0)	<0.1	<0.1	0	0.10
palmitic (C16:0)	11.70	11.58	1	6.7-14.5
stearic (C18:0)	4.05	4.07	-0.5	0.5-8.9
arachidic (C20:0)	0.46	0.46	0	0.1-0.9
behenic (C22:0)	0.56	0.57	-1.8	0
lignoceric (C24:0)	0.22	0.22	0	-
Total	16.99	16.90	0.5	13.6-14.4
Mono-unsaturated				
palmitoleic (C16:1)	<0.1	<0.1	0	0.2-0.5
oleic (C18:1)	22.40	22.21	0.8	14.3-34
Eicosenoic (C20:1)	0.28	0.27	3.7	0.2-0.5
Total	22.68	22.28	1.7	14.3-28.7
Poly-unsaturated				
linoleic (C18:2)	52.88	52.98	-0.2	36.5-60.0
linolenic (C18:3)	7.00	7.18	-2.5	1.9-14.7
Total	59.88	60.16	-0.5	38.4-72.5
Grand Total	99.55	99.34	2.1	-

Table 16: Phospholipid profile of A2704-12 crude lecithin

Phospholipid	% Fresh Weight		Difference (% of control)	Literature Range
	A2704-12	Control		
Phosphatidyl Choline	18.03	17.93	0.5	13-23.5
Phosphatidyl Ethanolamine	14.81	14.55	1.7	14-20
Phosphatidyl Inositol	10.45	10.48	-0.3	9-14
Phosphitid Acid	<1.0	<1.0	0	3-8

Table 17: Phospholipid profile of A5547-127 crude lecithin

Phospholipid	% Fresh Weight		Difference (% of control)	Literature Range
	A5547-127	Control		
Phosphatidyl Choline	4.55	5.04	-9.7	13-23.5
Phosphatidyl Ethanolamine	4.65	5.31	-12	14-20
Phosphatidyl Inositol	4.11	4.91	-16	9-14
Phosphitid Acid	<1.0	<1.0	0	3-8

Key toxicants

The only naturally occurring toxins in soybeans are lectins. Lectins are proteins that bind to carbohydrate-containing molecules and which inhibit growth and sometimes cause death in animals. It is reasonable to assume that similar effects would occur in humans. Lectins, however, are rapidly degraded upon heating, and therefore only become an issue when raw soybeans are consumed. There are no human food uses for raw soybeans.

Lectin content has been analysed and compared between the transgenic soybeans and the non-transgenic controls (see tables 18 and 19). In line A2704-12 the lectin content was significantly lower than the control and was also well below the reported literature range, which itself is highly variable. Given the large amount of variation in lectin content among soybeans, the difference between the transgenic and control soybeans is unlikely to be biologically meaningful. Also, as lectins are a natural toxicant, any significant decrease in their level is not considered to pose a safety concern. In line A5547-127 lectin levels were 15 – 22 % higher than the control, but were still well below the literature range therefore this difference does not represent a food safety concern, nor is it biologically meaningful given the large amount of variation among soybeans for lectin content.

Levels of anti-nutrients

Anti-nutrients in soybeans include trypsin inhibitor, phytic acid, stachyose, raffinose and isoflavones (daidzein and genistein and glycitein). These components were analysed in soybean seeds of both transgenic lines and their parental controls. Trypsin inhibitor and phytate were analysed by two different laboratories, Woodson-Tenent Laboratories and Ralston Analytical Laboratories. The results of these analyses are shown in tables 18 and 19, and described below.

Trypsin inhibitor and phytate levels in the A2704-12 transgenic plants are between 0 and 10.8% higher than the control seeds and are within the literature ranges. Trypsin inhibitor is present in A5547-127 and control soybeans at levels within or slightly lower than the literature range. There were no significant differences in trypsin inhibitor between sprayed and unsprayed transgenic seeds and the control seeds. The phytate levels for A5547-127 soybeans were within the range reported in literature.

Stachyose and raffinose are low molecular weight carbohydrates and are considered to be anti-nutrients due to the gas production and resulting flatulence caused by their consumption. The levels of raffinose and stachyose in soybean lines A2704-12 and A5547-127 are shown in tables 19 and 20. Overall they were within or below the literature range. The minor variation between the transgenic seeds and their non-transgenic counterparts are not consistently observed and are consistent with the high level of variation found among soybeans of all types regardless of whether they are GM or conventional varieties.

The levels of the isoflavone glycitein in soybean line A2704-12 is 6.3% higher than the level in the control with both levels being higher than the literature range. In the soybean line A5547-127 transgenic soybeans and their controls, glycitein levels are higher than the literature range but the transgenic level is only 2.8% higher than the control level. Amounts of the other isoflavones (daidzein and genistein) are well within or slightly below the literature ranges and total isoflavone levels are also within the literature range. Overall the A2704-12 transgenic seeds contain 4.2% less isoflavones than their controls and the A5547-127 transgenic seeds contain 4.4% more isoflavones than their controls, but these small differences are not nutritionally significant.

Allergenic potential

Saline extracts of commercially available soybeans have been reported to contain several antigenic properties, which can stimulate the rabbit immune system after injection and/or orally sensitise guinea pigs, calves, pigs, and humans.

The presence of these allergenic proteins in the diet of hypersensitive individuals can cause severe adverse reactions in the gastrointestinal tract (OECD 2001).

To determine whether soybean lines A2704-12 and A5547-127 are similar to the parental strains in terms of allergenic potential, the parental soybean varieties and lines A2704-12 and A5547-127 were tested using sera obtained from 16 soy-reactive human volunteers. There was no significant difference observed in the endogenous soybean allergen content of the extract obtained from the transgenic soybeans compared to the extract obtained from the parental line. Thus there was no significant increased risk of allergenic potential in the soybean line A2704-12 compared with A2704, or A5547-127 compared with A5547.

Conclusion

The comparative analyses do not indicate that there are any compositional differences of biological significance in the grain and processed fractions derived from transgenic soybean lines A2704-12 or A5547-127, compared to the non-GM controls (A2704 and A5547 respectively) or any differences in allergenic potential. Several minor differences in key nutrients and other constituents were noted however the levels observed were within the range of natural variation for commercial soybean lines and do not indicate an overall pattern of change that would warrant further investigation. On the whole, it can be concluded that A2704-12 and A5547-127 soybeans are equivalent in composition to non-GM soybeans.

Table 18: Anti-nutrients and other compounds in A2704-12 soybean seed

Anti-nutrients	A2704-12 Not sprayed	A2704-12 Sprayed	Control	Difference (% of control mean)		Literature Range
				Not sprayed	Sprayed	
Trypsin Inhibitor (WT) TIU/g dm	58202	60659	54940	5.9	10	40000-73600
Trypsin Inhibitor (Ral) TIU/g dm	33437	34487	31137	7.4	10.8	40000-73600
Lectin HU/mg	4.72	4.54	6.53	-28	-30	14.8-129
Phytate (WT) %dm	1.46	1.42	1.37	6.6	3.6	1.0-2.74
Phytate (Ral) %dm	1.27	1.25	1.25	1.6	0	1.0-2.74
Total Isoflavones ppm	1446	-	1510	-4.2	-	470-63600
Daidzein ppm	684	-	678	0.9	-	206-2060
Genistein ppm	628	-	689	-8.9	-	430-2040
Glycitein ppm	134	-	143	-6.3	-	82-109
Raffinose (%dm)	0.57	0.56	0.51	11.8	9.8	1.10-1.28
Stachyose (%dm)	3.79	3.76	3.66	3.6	2.7	3.70-6.30

dm = dry matter
ppm = parts per million
WT = Woodson-Tenent Laboratories
Ral = Ralston Analytical Laboratories

Table 19: Anti-nutrients and other compounds in A5547-127 soybean seed

Anti-nutrients	A5547-127 Not sprayed	A5547-127 Sprayed	Control	Difference (% of control value)		Literature Range
				Not sprayed	Sprayed	
Trypsin Inhibitor (WT) TIU/g dm	67931	66606	63696	6.6	4.5	40000-73600
Trypsin Inhibitor (Ral) TIU/g dm	38596	37894	37559	2.7	0.9	40000-73600
Lectin HU/mg	10.46	9.85	8.56	22	15	14.8-129
Phytate (WT) %dm	1.71	1.67	1.66	3.0	0.6	1.0-2.74
Phytate (Ral) %dm	1.57	1.60	1.52	3.3	5.3	1.0-2.74
Total Isoflavones ppm	946	-	906	4.4	-	470-63600
Daidzein ppm	358	-	348	2.9	-	206-2060
Genistein ppm	407	-	383	6.2	-	430-2040
Glycitein ppm	180	-	175	2.8	-	82-109
Raffinose (%dm)	0.83	0.78	0.86	-3.5	-9.3	1.10-1.28
Stachyose (%dm)	3.27	3.32	3.51	-6.8	-5.4	3.70-6.30

dm = dry matter
ppm = parts per million
WT = Woodson-Tenent Laboratories
Ral = Ralston Analytical Laboratories

NUTRITIONAL IMPACT

In assessing the safety and suitability of a GM food, a key factor is the need to establish that the food is nutritionally adequate and will support typical growth and wellbeing. In most cases, this can be achieved through an understanding of the genetic modification and its consequences, together with an extensive compositional analysis of the food.

To date, all approved GM plants with modified agronomic production 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 with target livestock species will add little to a safety assessment and generally are not warranted.

For plants engineered with the intention of significantly changing their composition/nutrient bioavailability and thus their nutritional characteristics, however, 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.

In the case of soybean lines A2704-12 and A5547-127, the extent of the compositional and other available data is considered to be adequate to establish the nutritional adequacy of the food. However, a feeding study has been conducted on these two transgenic soybean lines and is evaluated below as additional supporting information.

Animal feeding studies

Studies evaluated:
Leeson, S. (1997). The effect of the soybean hybrid 2704-12 on the growth of male and female broiler chickens. Department of Animal and Poultry Science. Report No. C011985

Leeson, S. (1998). The effect of Glufosinate resistant soybeans (A5547-127) on the growth of female broiler chickens. Department of Animal and Poultry Science Report No. A57547.

The study was done to compare the wholesomeness of transgenic soybean lines A2704-12 and A5547-127 compared to the non-transformed parental soybean lines when fed to rapidly growing broiler chicks. The rapidly growing broiler is considered to be sensitive to changes in nutrient quality in diets, and therefore is often used as a model to assess the wholesomeness of feed.

Seventy two male and seventy two female commercial strain female broiler chickens were obtained at one day of age to compare the soybeans from line A2704-12 with their control and two hundred and forty commercial strain female broiler chickens were obtained at one day of age to compare the soybeans from line A5547-127 with their control. For the A2704-12 feeding study, birds were weighed and allocated (by sex) at random to one of two treatment groups, replicated six times with six birds per replicate. The birds were reared on one of two diet treatments as prepared by the Arkell Research Station Feed Mill. Each diet treatment was prepared for the starter period and are conventional corn-soybean type diets commonly used in Southern Ontario. The treatments vary only in the source of soybeans used in each diet. The source of soybean meal for the first diet was conventional while the alternate treatment used soybean line A2704-12.

Birds were fed starter diets to 15 days at which time feed intake was measured and all birds weighed individually. All occurrences of mortality were submitted to the Ontario Veterinary College, Department of Pathology for post mortem examination.

For the soybean line A5547-127 feeding study, birds were weighed and allocated at random to one of two treatment groups, replicated six times with twenty birds per replicate. The birds were reared on one of two diet treatments as prepared by the Arkell Research Station Feed Mill. Each diet treatment was prepared for starter, grower and finished periods and are conventional corn-soybean type diets commonly used in Southern Ontario. The treatments vary only in the source of soybeans used in each diet. The source of soybeans for the first diet was conventional while the alternate treatment used soybean line A5547-127.

Birds were fed starter diets to 17 days of age at which time feed intake was measured and all birds weighed individually. Grower diets were fed between days 17 and 31 at which time feed intake was measured and all birds weighed individually. Finisher diets were fed between days 31 and 42 and again feed intake was measured and all birds weighed individually. On day 42, 8 birds were randomly selected from each pen. These birds were eviscerated and the abdominal fat pad was removed and weighed. Carcasses and right and left breast muscles were weighed.

There were no significant differences (calculated by a T-test) between the diets containing either line A2704-12 or A5547-127 soybeans and the diets containing conventional soybeans in terms of initial and final weight, weight gain, feed intake and feed intake to body weight

gain ratio. Carcass characteristics were unaffected by the source of soybeans in the experimental diets. These data indicate that soybean lines A2704-12 and A5547-127 are equivalent to conventional soybean lines in terms of their ability to support the rapid growth of broiler chicks and confirm the results of the compositional analyses.

Conclusions

On the basis of the compositional data evaluated in regard to soybean lines A2704-12 and A5547-127, it was concluded that these herbicide tolerant soybean lines are equivalent to other commercially available soybeans in terms of their composition and nutritional adequacy.

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