Determination of the Safety of Bayer's
Soybean A5547-127(Herbicide Tolerant)
For Direct Use as Food, Feed or for Processing

Food and Feed Safety

The product dossier Soybean A5547-127was reviewed for safety and nutritional differences in comparison with the conventional soybean. The review was focused on any new or altered expression trait and changes in composition and nutritional content or value relative to the conventional soybean. After thorough safety assessment, the following conclusions were made: Soybean A5547-127 is as safe as its conventional counterpart based on compositional and nutritional analyses. Soybean A5547-127 is safe to humans, animals and as nutritious as conventional soybean.

A biosafety permit for Soybean A5547-127 and all progenies from crosses with this was issued to Bayer CropScience, Inc. on June 23, 2011. The validity of the permit is only five years.

This approval is for use as food and feed or for processing of soybean A5547-127 in the Philippines. Food and Feed use of soybean A5547-127 and its by-products is therefore authorized as of June 23, 2011. The biosafety permit (No. 11-054) for direct use stated that "soybean A5547-127 "and all progenies from crosses of this product except when stacked with other biotech traits has undergone satisfactory assessment and found to be as safe as conventional soybean and can be a substitute for its traditional counterpart as food and feed or for processing"

I. Brief Identification of the Genetically Modified Organism (Living Modified Organism)

Designation:	Soybean A5547-127
Applicant:	Bayer CropScience, Inc 3/F Bayer House Canlubang, Calamba City, Laguna 4028 Philippines
Plant Species:	
Crop:	Soybean (*Glycine max.*)
Parent Material:	Cultivar A5547
Center of Origin:	China
Antinutrients:	Trypsin inhibitors, lectins, isoflavones, stachyose, raffinose and phytic acid.
Trait Description:	Tolerance to glufosinate-ammonium herbicide
Trait Introduction Method:	particle acceleration transformation (microparticle bombardment)
Donor Organism:	*Streptomyces viridochromogenes*
Pathogenicity:	*Streptomyces viridochromogenes*, the donor organism of *pat* gene, is not known to be a human pathogen nor has it been associated with other properties (e.g production of toxins) known to affect human health.

Proposed Use: For direct use as food, feed or for processing

II. Background Information

Bayer CropScience Inc. has developed a new genetically modified soybean that is tolerant to glufosinate-ammonium herbicides. Soybean has been modified to express phosphinothricin acetyltransferase (PAT) in the cell tissue to produce the glufosinate-tolerant soybean designated as A5547-127. This soybean event will allow the use of glufosinate-ammonium as a post-emergence herbicide, thus providing an alternative weed control option in soybean production and reduces reliance on soil incorporated herbicides.

On May 17, 2010, Bayer Crop Science, Inc. submitted an application to the Bureau of Plant Industry requesting a biosafety permit under DA A.O. #8 for soybean which has been genetically modified (GM) for herbicide tolerance.

BayerCropScience,Inc. has provided information on:

- the safe history of use of the crop,
- the source of the donor gene,
- the molecular characterization of Soybean A5547-127,
- description of the modification method,
- data and information on the stability of the gene insertions,
- characterization and levels of PAT protein expression in the Soybean A5547-127,
- establishment of the very low potential for allergenicity of the PAT protein through amino acid sequence comparisons with known protein allergens and digestibility studies using simulated gastric and intestinal fluids,
- analysis for the potential of allergenicity associated with PAT protein and Soybean A5547-127 as well as the nutrient composition of the soybean grain,
- forage and grain processed fractions, and
- overall food and feed safety of Soybean A5547-127 plants.

Relevant scientific publications were also supplied.

SoybeanA5547-127has been evaluated according to BPI's safety assessment procedures by concerned agencies including: Bureau of Animal Industry (BAI), Bureau of Plant Industry (BPI), Bureau of Agriculture Fisheries and Product Standards (BAFPS) and a Scientific Technical Review Panel (STRP).

The process involved an intensive analysis of the nature of the genetic modification with a consideration of general safety issues, toxicological, allergenicity and nutritional issues associated with the modified soybeans.

The petitioner/applicant published the said application in two (2) widely circulated newspapers (Malaya Business Insight and The Daily Tribune) on July 27, 2010 for public comment/review. BPI received positive comments on the petition supporting the direct use for food, feed or for processing of SoybeanA5547-127 during the 30-day comment period.

Review of results of evaluation by the BPI Biotech Core Team completed the approval process.

III. Description of Novel Protein (Introduced Traits)

Soybean A5547-127 has been genetically modified to express a single copy of the synthetic *pat* gene isolated from the common aerobic soil actinomycete, *Streptomyces viridochromogenes* that resulted in the expression of the enzyme phosphinothricin N-acetyl transferase (PAT). *Pat* gene

confers tolerance to glufosinate-ammonium-herbicides, the active ingredient in phosphinothricin herbicides, as a weed control option in soybean crops.

In addition, the glufosinate-ammonium-tolerant Soybean A5547-127 also contains fragments of the bacterial selection marker gene *bla*, which are not expressed. The soybean transformation event A5547-127 has been obtained by transformation with the vector pB2/35SAcK (has the same backbone structure derived from pUC plasmid) that inserted only one functional gene, the phosphinothricin acetyl transferase, *pat.* Prior to transformation, the vector was digested with a restriction enzyme to disrupt the coding sequence of the *bla* gene removing any remote possibility of its expression. The partial *bla* gene sequences coding for a truncated *β*-lactamase are not sufficient to express a functional *β*-lactamase. The *pat* gene is under the control of the 35S cauliflower mosaic virus promoter and terminator.

Safety of the Expressed Proteins

The inserted *pat* gene is present in Soybean A5547-127 and expresses phosphinothricin acetyltransferase. *Streptomyces viridochromogenes*, source of the *pat* gene, is not known to be pathogenic, toxic or allergenic for human or animals. *Pat* gene is composed of the same basic natural nucleic acids as found in any DNA from known food constituents consumed as part of human or animal diets. It undergoes the same breakdown process in the digestive system as typical food genes and poses no direct toxicity on human health.

The PAT protein was shown to degrade rapidly in gastric or intestinal fluid and has no amino acid sequence similarity with any known toxins or known allergens. Additionally, repeated dose oral toxicity and acute toxicity by intravenous injection studies with mice demonstrated lack of any toxic effects related to the PAT protein.

The PAT protein lacks allergenic and toxic potential, it has no amino acid sequence homology to other known allergens or toxins as demonstrated by overall amino acid and epitope homology searches and it is rapidly and completely degraded in human Simulated Gastric Fluid (SGF) and Simulated Intestinal Fluid (SIF). This minimizes the likelihood that this protein could survive in the human digestive tract and be absorbed as intact protein.

In homology searches using the amino acid sequence of the PAT protein encoded by the *pat* gene, no potential N-glycosylation sites were identified, which supports that it is unlikely that the PAT protein possesses allergenic properties.

In conclusion, it is considered that the *Streptomyces viridochromogenes* (source of gene) is non pathogenic and the *pat* gene as well as its encoded PAT protein is not toxic to mammals. PAT also does not possess characteristics associated with food allergens. There is a reasonable certainty that no harm will result from the inclusion of the PAT protein (encoded by the *pat* gene) in human food and in animal feed.

IV. Nutritional Composition (Compositional Analysis)

Based on the detailed characterization provided (nutritional composition and agronomic data of the modified plants compared to unmodified comparators) it is unlikely that any unintended effects are present in Soybean A5547-127.

The evidence provided by Bayer CropScience, Inc. supports that the nutritional composition of Soybean A5547-127 is substantially equivalent to conventional soybean varieties. Analysis of the nutritional components of Soybean A5547-127 and products derived from the crop indicated no significant differences and thus are found to be nutritionally equivalent to their traditional counterpart. There is no impact on the nutritional value of Soybean A5547-127 as a result of the genetic modification.

The components which were used for compositional and nutritional analyses were proximates (moisture, ash, protein, crude fat, detergent fiber and carbohydrate), amino acids, fatty acids, minerals (sodium, potassium, magnesium, calcium, iron and phosphorus), isoflavones and vitamins (incl. tocopherols).

The statistical evaluation of the composition data of Soybean A5547-127 seeds compared to their non-GM counterpart, A5547 showed that for most components, no significant differences for the majority of the by site comparisons were determined. For most of the analyzed components, no difference between the GM and non-GM soybean were observed. A slight statistical significant difference has no nutritional impact, since the absolute difference is very small and as all results obtained are comparable to reference ranges for commercial products.

Based on the statistical evaluation of the analytical data and an assessment of the nutritional impact of the different observations, Soybean A5547-127 is found to be nutritionally equivalent to non-GM soybean.

The nutritional equivalence of SoybeanA5547-127 to conventional soybean was further confirmed in the feeding study with broiler chickens. Results of the study confirmed the safety of the SoybeanA5547-127which showed that the performance of broilers fed with Soybean A5547-127 is comparable to those fed with the non GM soybean.

Thus SoybeanA5547-127 can be used in food and feed formulations and can substitute 100% for conventional soybean.

V. Anti-Nutritional Factors

Like all oilseed protein crops, soybeans contain natural toxins. After conventional processing, products contain antinutrients in very low amounts and below the thresholds considered to raise a food safety concern. These include trypsin inhibitors, lectins, isoflavones, stachyose, raffinose and phytic acid, quantities of which are in the same range as found in conventional soybean.

Statistically significant differences were only detected for raffinose and oleic acid in comparisons between the non-GM soybean and the GM soybean seed from glufosinate-treated soybean plants. These findings were not confirmed by the overall comparison between the non-GM control and neither by comparing the GM seeds obtained from non glufosinate-ammonium treated soybean plants with the control.

Studies showed that the statistical findings have no nutritional relevance since the absolute treatment differences in the raffinose contents are very small. The amounts of oleic acid are slightly higher in the GM samples, as most of the mean values are within the reference ranges reported from the literature. The mean values determined from composition analyses of the non-GM and GM soybean seeds were compared to reference ranges taken from chemistry reference guidelines and most mean values obtained are within the reported ranges.

VI. Regulatory Decision

After reviewing the scientific data and information relevant to the application of Bayer CropScience, Inc. it is concluded that SoybeanA5547-127 and all progenies derived from crosses of the product with any conventionally-bred soybean, and soybean containing approved biotech events for direct use is as safe and substantially equivalent to its unmodified counterpart and is therefore approved for direct use as food, feed and for processing.

Bayer CropScience Inc. shall also duly inform the public of the approval for direct use by way of publishing in any one (1) of the top three (3) leading newspapers in the country that imports of this

product are covered by conditions for approval as provided in Department of Agriculture Memorandum Circular No. 8, Series of 2002. In the meantime Bayer CropScience, Inc. had duly complied with this by publishing the approval in the July 8, 2011 issue of the nationally circulated newspaper, The Manila Bulletin.