

Safety Assessment of Roundup Ready[®] Soybean Event 40-3-2

Executive Summary

Using modern biotechnology, Monsanto Company has developed Roundup Ready[®] soybean varieties that confer tolerance to glyphosate, the active ingredient in Roundup[®] agricultural herbicides, by the production of the naturally occurring glyphosate-tolerant CP4 enolpyruvylshikimate-3-phosphate synthase (EPSPS) protein. The EPSPS enzyme is present in the shikimic acid pathway for the biosynthesis of aromatic amino acids in plants and microorganisms. Inhibition of this enzyme by glyphosate leads to a deficiency in the production of aromatic amino acids and lack of growth in plants. The aromatic amino acid biosynthetic pathway is not present in mammalian, avian or aquatic animals. This explains the selective activity in plants and contributes to the low risk to human health and the environment from the use of glyphosate according to label directions.

Roundup Ready soybean event 40-3-2 was produced by introduction of the glyphosate-tolerant *cp4 epsps* coding sequence derived from the common soil bacterium *Agrobacterium sp.* strain *CP4* into the soybean genome using particle-acceleration transformation. The CP4 EPSPS protein is a member of the class of EPSPS proteins found ubiquitously in plants and microorganisms.

The tolerance of Roundup Ready soybeans to glyphosate has been demonstrated since 1991 in field trials conducted throughout the United States and since 1996 with commercial production in the United States, Canada and Argentina. Roundup Ready soybeans were planted in 1996 on less than 5% of the U.S. soybean acres. In the 2001 growing season, 71% of the soybeans -- approximately 54 million acres of the 75.4 million acres of the soybeans grown in the U.S. -- were Roundup Ready soybeans. In Argentina, where the adoption rate is estimated to be greater than 95%, Roundup Ready soybeans were grown on over 25 million acres in 2001. Globally, Roundup Ready soybeans made up 63% of all transgenic crops grown in 2001. One of the reasons growers have rapidly adopted the Roundup Ready soybean is the simplicity it offers in weed control. Since Roundup agricultural herbicides are highly effective against the vast majority of annual and perennial grasses and broadleaf weeds, growers planting Roundup Ready soybeans are able to reduce the number of herbicides used to control the economically destructive weeds that grow in their fields and thereby realize a savings in weed control costs. This reduction in herbicide use has benefited the environment by reducing the number of herbicide applications and also allows growers to implement integrated weed management practices in their fields -- practices that are generally not possible when pre-plant or pre-emergent herbicides are used.

The food, feed and environmental safety of Roundup Ready soybean was established based upon: the evaluation of the functional and structural similarity of the CP4 EPSPS protein to a

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diverse family of EPSPS proteins typically present in food and feed derived from traditional plant and microbial sources; the low dietary exposure to the CP4 EPSPS protein; the lack of toxicity or allergenicity of EPSPS proteins in general; and by direct safety studies of the CP4 EPSPS protein. Furthermore, the nutritional equivalence and wholesomeness of Roundup Ready soybeans compared to conventional soybeans was demonstrated by the analysis of key nutrients, including proximates, amino acid and fatty acid composition, as well as anti-nutrients. The nutritional equivalence of Roundup Ready soybeans to conventional soybeans was confirmed in numerous feeding studies with rats, cows, pigs, broiler chickens, fish and quail. The environmental impact of Roundup Ready soybeans is also comparable to conventional soybeans. The results of these studies demonstrate that Roundup Ready soybeans are as safe as conventional soybeans with respect to food, feed and environmental safety.

Introduction

Monsanto Company has developed Roundup Ready soybean plants that confer tolerance to glyphosate, the active ingredient in Roundup agricultural herbicides. The primary mode of action of glyphosate is the competitive inhibition of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). This enzyme is part of the shikimate pathway that is involved in the production of aromatic amino acids and other aromatic compounds in plants (Steinrucken and Amrhein, 1980). When conventional plants are treated with glyphosate, the plants cannot produce the aromatic amino acids needed to survive. Roundup Ready soybean plants, developed using modern biotechnology, produce the CP4 5-enolpyruvylshikimate-3-phosphate synthase protein (CP4 EPSPS), which is derived from a common soil bacterium. The CP4 EPSPS protein is naturally less sensitive to inhibition by glyphosate and thus plants expressing this protein are tolerant to glyphosate.

Roundup agricultural herbicides are used as a foliar-applied, non-selective herbicides and are effective against the majority of annual and perennial grasses and broadleaf weeds. Glyphosate has no pre-emergence or residual soil activity (Franz *et al.*, 1997). Furthermore, glyphosate is not prone to leaching, degrades in soil over time, and will not cause unreasonable adverse effects to mammals, birds or fish under normal use conditions (U.S. EPA, 1993; WHO, 1994; Giesy *et al.*, 2000; Williams *et al.*, 2000).

Roundup Ready soybeans offer growers an additional tool for improved weed control. Control of weeds in the soybean crop is essential, as weeds compete with the crop for sunlight, water and nutrients. Failure to control weeds within the crop results in decreased yields and reduced crop quality. In addition, weeds reduce the efficiency of the mechanical harvest of the crop.

The tolerance of Roundup Ready soybeans to glyphosate has been demonstrated since 1991 in field trials conducted throughout the United States and since 1996 with commercial production in the United States, Canada and Argentina. Roundup Ready soybeans were introduced in 1996 on less than 5% of the U.S. soybean acres. In the 2001 growing season, 71% of the soybeans -- approximately 54 million acres of the 75.4 million acres of the soybeans grown in the U.S. -- were Roundup Ready soybeans (James, 2001). In Argentina, where the adoption rate is estimated to be greater than 95%, Roundup Ready soybeans were grown on over 25 million acres in 2001 (James, 2001). Globally, Roundup Ready soybeans made up 63% of all transgenic crops grown in 2001. One of the reasons growers have rapidly adopted Roundup Ready soybean is the simplicity it offers in weed control. Since Roundup agricultural herbicides are highly effective against the majority of annual and perennial grasses and broadleaf weeds, growers planting Roundup Ready soybeans are able to reduce the number of herbicides used to control the economically destructive weeds that grow in their fields and thereby realize a savings in weed control costs (Carpenter, 2001). This reduction in herbicide use has benefited the environment by reducing the number of herbicide applications (Carpenter, 2001) and also allows growers to implement integrated weed management practices in their fields – practices that are generally not possible when pre-plant or pre-emergent herbicides are used.

Roundup Ready soybeans provide the following environmental and economic benefits:

- Improved efficacy in weed control compared to herbicide programs used in conventional soybeans, as specific pre-emergent herbicides that are used for prevention are replaced by a broad-spectrum post-emergent herbicide that can be used on an ‘as needed’ basis (Culpepper and York, 1998; Roberts *et al.*, 1999). The introduction of Roundup Ready soybeans in the U.S. has eliminated 19 million herbicide applications per year – a decrease of 12%, even though the total soybean acres increased by 18% from 1996-1999 (Carpenter, 2001). This decrease in herbicide applications means that growers make fewer trips over their fields to apply herbicides, which translates into ease of management and reduced fuel use.
- A reduction in herbicide costs for the farmer. It’s been estimated that U.S. soybean growers saved \$216 million in 1999 compared to 1995, the year before Roundup Ready soybeans were introduced, including the technology fee (Carpenter, 2001).
- High compatibility with Integrated Pest Management and soil conservation techniques (Keeling *et al.*, 1998; American Soybean Association, 2001), resulting in a number of important environmental benefits including reduced soil erosion and improved water quality (Baker and Laflen, 1979; Hebblethwaite, 1995; CTIC, 1998), improved soil structure with higher organic matter (Kay, 1995; CTIC, 2000), improved carbon sequestration (Reicosky, 1995; Reicosky and Lindstrom, 1995) and reduced CO₂ emissions (Kern and Johnson, 1993; CTIC, 2000).

In summary, weeds are a severe constraint in the production of soybeans worldwide. Soybeans cannot compete effectively in early growth stages and must be protected from the invasion of aggressive weeds. Present management systems interweave cultural and mechanical practices with herbicides to overcome the competitive effect. The introduction of Roundup Ready soybeans has reduced the number and cost of herbicide applications, resulting in equal or better control of the weeds. The use of Roundup Ready soybeans also offers environmental benefits associated with the use of conservation-tillage and integrated weed management practices.

Molecular Characterization of Roundup Ready Soybeans

Roundup Ready soybean event 40-3-2 was developed by introducing the *cp4 epsps* coding sequence into soybean variety A5403, a commercial soybean variety of Asgrow Seed Company. A5403 is a maturity group V cultivar that combines consistently high yield potential with resistance to races 3 and 4 of the soybean cyst nematode (SCN). It also possesses good standability, excellent emergence and tolerance to many leaf and stem diseases. The Roundup Ready trait has since been transferred into more than one thousand commercial soybean varieties by traditional breeding techniques.

Roundup Ready soybean event 40-3-2 was produced by particle-acceleration transformation (McCabe *et al.*, 1988; Christou *et al.*, 1988) of the non-transgenic parental soybean line

A5403 utilizing DNA derived from plasmid PV-GMGT04 (Figure 1). The primary insertion of DNA derived from the transformation plasmid PV-GMGT04 includes a single *cp4 epsps* gene cassette which consists of the cauliflower mosaic virus (CaMV) E35S promoter, a chloroplast transit peptide and *cp4 epsps* coding sequence, and the *nos* 3' polyadenylation signal. The *cp4 epsps* coding sequence is fused to a chloroplast transit peptide (CTP4) sequence from petunia. The CTP targets the CP4 EPSPS protein to the chloroplast, the location of EPSPS in plants and the site of aromatic amino acid biosynthesis (Kishore and Shah, 1988). CTPs are typically cleaved from the "mature" protein following delivery to the plastid (della-Cioppa *et al.*, 1986). The *cp4 epsps* coding sequence encodes 455 amino acids, is terminated by tandem stop codons, and results in the synthesis of the full length and functional ~46 kDa CP4 EPSPS protein in Roundup Ready soybean event 40-3-2 as confirmed by western blotting, enzyme-linked immunosorbent assay (ELISA) and EPSPS enzyme activity assays (Padgett *et al.*, 1995; Re *et al.*, 1993; Padgett *et al.*, 1994; USDA, 1994; Harrison *et al.*, 1996; Padgett *et al.*, 1996a).

In addition to the primary functional insert, Roundup Ready soybean event 40-3-2 contains two small segments of inserted DNA: a 250 base pair segment of *cp4 epsps* DNA located adjacent to the *nos* 3' polyadenylation signal of the primary, functional insert and a 72 base pair segment of *cp4 epsps* DNA that co-segregates with the primary functional insert. Although these two *cp4 epsps* segments are present, they are not functional genes. This has been demonstrated by western blot analysis, in which only the predicted, full-length CP4 EPSPS protein encoded by the functional *cp4 epsps* gene cassette was detected (Rogan *et al.*, 1999).

The stretch of DNA adjacent to the 3' end of the primary insert has also been characterized by Monsanto and others (Windels *et al.*, 2001). This DNA has been shown to be soybean genomic DNA. Several low abundance mRNA transcripts were detected that contain the region flanking the 3' end of the primary insert. This is not unexpected since plants often utilize and recognize multiple transcript polyadenylation signals, which results in multiple transcripts for a given gene (Rothnie, 1996; Hunt, 1994; Gallie, 1993). Given the structure of the *cp4 epsps* gene and surrounding genetic elements, and the nature of the plant's protein-producing machinery, the secondary transcripts, if translated at all, would be expected to produce only the CP4 EPSPS protein. The only CP4 EPSPS-containing protein detected in event 40-3-2 using sensitive western blot analysis is the expected ~46 kDa CP4 EPSPS protein (Rogan *et al.*, 1999).

The inserts described are inherited in the expected Mendelian pattern and the stability of the inserts has been demonstrated by molecular analysis of the R3 through the R6 generations of event 40-3-2. These inserts were shown to be present in the plant materials that were used to confirm the safety of Roundup Ready soybeans and hence are constituents in the safety studies that are described in the sections below. In addition, Roundup Ready soybean event 40-3-2 has been in commercial production on over 240 millions acres (100 million hectares) globally since 1996 with consistent product performance and with no confirmed adverse food, feed or environmental effects.

CP4 EPSPS Protein Levels in Roundup Ready Soybean Plants

An enzyme-linked immunosorbent assay (ELISA) (Harlow and Lane, 1988) method was developed and optimized to quantitate CP4 EPSPS protein levels in soybean leaf and seed matrices (Padgett *et al.*, 1995; Rogan *et al.*, 1999). Protein levels are presented in Table 1 for soybean seed and leaf tissues collected from Roundup Ready soybean plants that were either unsprayed or sprayed with the original Roundup herbicide in 1992 and 1993 U.S. field trials (Padgett *et al.*, 1995; Taylor *et al.*, 1999).

The mean level of CP4 EPSPS protein in soybean seed from the 1992 trials was 0.301 µg/mg fresh weight for plants treated with the original Roundup herbicide and 0.288 µg/mg fresh weight for unsprayed plants. The mean protein levels in seed from the 1993 trials were 0.218 µg/mg fresh weight for plants treated with the original Roundup herbicide and 0.201 µg/mg fresh weight for unsprayed plants. This level of CP4 EPSPS protein in the seed represents approximately 0.08% of the total protein in the seed. Mean CP4 EPSPS protein levels in soybean leaf tissue from the 1993 trials were 0.489 µg/mg fresh weight for plants treated with the original Roundup herbicide and 0.415 µg/mg fresh weight for unsprayed plants.

As expected, the CP4 EPSPS protein was not detected in soybean leaf or seed tissue from the non-transgenic A5403 parental variety in either year above the limit of detection.

Safety Assessment of the CP4 EPSPS Protein in Roundup Ready Soybeans

The safety assessment of the CP4 EPSPS protein produced in Roundup Ready soybean event 40-3-2 includes protein characterization, functional and structural comparisons of the CP4 EPSPS protein to ubiquitous plant and microbial EPSPS proteins with a history of safe consumption, *in vitro* digestibility in simulated gastric and intestinal fluids, acute oral toxicity in mice, and amino acid comparison to known toxins and allergens.

CP4 EPSPS Protein Characterization

The CP4 EPSPS protein produced in Roundup Ready soybeans is functionally similar to a diverse family of EPSPS proteins typically present in food and feed derived from plant and microbial sources (Levin and Sprinson, 1964; Harrison *et al.*, 1996). The EPSPS proteins are required for the production of aromatic amino acids in plants and microbes. Genes for numerous EPSPS proteins have been cloned (Padgett *et al.*, 1988, 1991, and references therein), and active site domains are conserved among the known EPSPS proteins (Padgett *et al.*, 1988; 1991). Bacterial EPSPS proteins have been well characterized with respect to the three-dimensional X-ray crystal structure (Stallings *et al.*, 1991) and the detailed kinetic and chemical reaction mechanisms (Anderson and Johnson, 1990). The enzymology and known function of EPSPS proteins generally, and the CP4 EPSPS protein specifically, establish that this class of enzymes performs a well-described and understood biochemical role in plants.

From the perspective of safety, this characterization demonstrates that the metabolic effect of the expression of the CP4 EPSPS protein is limited to conferring tolerance to glyphosate. Part of this evaluation includes the known structural relationship of the CP4 EPSPS protein with other EPSPS proteins found in food, the comparison of the amino acid sequences with conserved identity of the active site residues, the expected conserved three-dimensional structure based on similarity of the amino acid sequence, and the fact that EPSPS proteins catalyze a non-rate-limiting step in the aromatic amino acid pathway. Hence increases in the level of EPSPS proteins would not be expected to affect the flux through the aromatic amino acid pathway. With respect to amino acid sequence, there is considerable divergence among known EPSPS proteins. For instance, the amino acid sequence of the CP4 EPSPS protein is 41% identical at the amino acid level to *Bacillus subtilis* EPSPS protein, whereas the soybean EPSPS protein is 30% identical to *Bacillus subtilis* EPSPS protein. Thus, the divergence of the CP4 EPSPS protein amino acid sequence from typical food EPSPS protein sequences is on the same order as the divergence among food EPSPS proteins themselves (Harrison *et al.*, 1996).

The detailed enzymology (Harrison *et al.*, 1996) and biochemical composition evaluations (Padgett *et al.*, 1996a) confirm that the CP4 EPSPS protein, as expressed in Roundup Ready soybeans, has the predicted metabolic effect: the production of aromatic amino acids via the shikimic acid biosynthetic pathway.

In vitro Digestibility of the CP4 EPSPS Protein

Simulated mammalian gastric and intestinal digestive fluids were used in *in vitro* assays to assess the susceptibility of the CP4 EPSPS protein to proteolytic digestion. Rapid degradation of the protein correlates with limited exposure to the gastrointestinal tract and little likelihood that the protein can produce pharmacological, toxic or allergenic effects. The method of preparation of the simulated digestion solutions used is described in the United States Pharmacopeia (1989).

The CP4 EPSPS protein was rapidly digested in the *in vitro* digestive system (Harrison *et al.*, 1996). Enzyme activity and immunoblot analyses were used to monitor the degradation of the CP4 EPSPS protein and demonstrated that the half-life of the protein in simulated gastric fluid was less than 15 seconds and less than 10 minutes in simulated intestinal fluid. To put the rapid degradation of the CP4 EPSPS protein in the simulated gastric system into perspective, solid food has been estimated to empty from the human stomach by about 50% in two hours, while 50% of liquid intake has left the stomach within approximately 25 minutes (Sleisenger and Fordtran, 1989). If the CP4 EPSPS protein were not degraded in the gastric system, it would be rapidly degraded in the intestine. Proteins that are rapidly degraded in the gastrointestinal tract are unlikely to confer toxicity or allergy (Astwood *et al.*, 1996; Astwood and Fuchs, 2000).

Assessment of Acute Oral Toxicity of the CP4 EPSPS Protein in Mice

Few proteins are toxic when ingested and those that are toxic typically act in an acute manner (Sjoblad *et al.*, 1992). To confirm the lack of acute toxicity, an oral toxicity study with CP4 EPSPS protein as the test material was performed on mice to directly assess any potential toxicity associated with the protein (Harrison *et al.*, 1996). Acute administration was considered sufficient to assess the safety of the CP4 EPSPS protein, since proteins that are toxic act via acute mechanisms. There were no treatment-related adverse effects in mice administered CP4 EPSPS protein by oral gavage at dosages up to 572 mg/kg of body weight. This dose represents a significant – greater than 1000-fold – safety margin relative to the highest potential human consumption of CP4 EPSPS protein and assumes that the protein is expressed in multiple crops (Harrison *et al.*, 1996). Results from this study demonstrated that the CP4 EPSPS protein is not acutely toxic to mammals. This result was expected since the CP4 EPSPS protein is readily digested in gastric and intestinal fluids *in vitro* and the protein is from a ubiquitous family of proteins with a history of safe consumption and no biologically plausible mechanism of toxicity to animals.

Assessment of Structural Homology of the CP4 EPSPS Protein to Known Protein Toxins

Another aspect used for the assessment of potential toxic effects of proteins introduced into plants is to compare the amino acid sequence of the protein to the sequences of known toxic proteins. Homologous proteins derived from a common ancestor have similar amino acid sequences, are structurally similar and often share common function. Therefore, it is undesirable to introduce DNA that encodes for a protein that is homologous to a protein that is toxic to animals and people. Homology is determined by comparing the degree of amino acid similarity between proteins using published criteria (Doolittle *et al.*, 1990). The CP4 EPSPS protein does not show meaningful amino acid sequence similarity when compared to known protein toxins.

Assessment of Allergenic potential of CP4 EPSPS Protein

Although there are no single predictive bioassays available to assess the allergenic potential of proteins in humans (FDA, 1992), the physicochemical and human exposure profile of the protein provides a basis for assessing potential allergenicity by comparing it to known protein allergens. Thus, important considerations contributing to the allergenicity of proteins ingested orally includes exposure and an assessment of the factors that contribute to exposure, such as stability to digestion, prevalence in the food, and consumption pattern (amount) of the specific food (Metcalf *et al.*, 1996; Kimber *et al.*, 1999).

A key parameter contributing to the systemic allergenicity of certain food proteins appears to be stability to the peptic and acidic conditions of the digestive system (Astwood *et al.*, 1996; Astwood and Fuchs, 1996; Fuchs and Astwood, 1996; FAO, 1996; Kimber *et al.*, 1999). Important protein allergens tend to be stable to peptic digestion and the acidic conditions of the stomach if they are to reach the intestinal mucosa where an adverse immune response can be initiated. As noted above, the *in vitro* assessment of the CP4 EPSPS protein digestibility

indicates that the protein, like other food-derived proteins, is very labile to digestion when compared to many clinically important food allergens.

Another significant factor contributing to the allergenicity of certain food proteins is their high concentrations in foods (Taylor, 1992; Taylor *et al.*, 1987; Fuchs and Astwood, 1996). Most food allergens are present as major protein components in the specific food, in amounts ranging from 1% up to 80% of total protein (Fuchs and Astwood, 1996). In contrast, the CP4 EPSPS protein is present at low levels, approximately 0.08% of the total protein, in whole Roundup Ready soybean seed (Table 1; Padgett *et al.*, 1995). Furthermore, it has been shown that the processing steps used in the production of soybean oil, one of the main sources of soybean in the human diet, reduce the vast majority of protein such that refined soybean oil did not trigger allergic reactions in humans who were sensitive to soybean (Bush *et al.*, 1985).

It is also important to establish that the protein does not represent a previously described allergen and does not share potentially immunologically relevant amino acid sequence segments or structure with a known allergen. An efficient way to determine whether the added protein is an allergen, or is likely to contain cross-reactive structures, is to compare the amino acid sequence of the introduced protein with those of all known allergens. A database of protein sequences associated with allergy and coeliac disease has been assembled from publicly available genetic databases (GenBank, EMBL, PIR and SwissProt). The amino acid sequence of the CP4 EPSPS protein was compared to these sequences and shown to have no meaningful amino acid sequence similarity with any of the known allergens (Fuchs and Astwood, 1996).

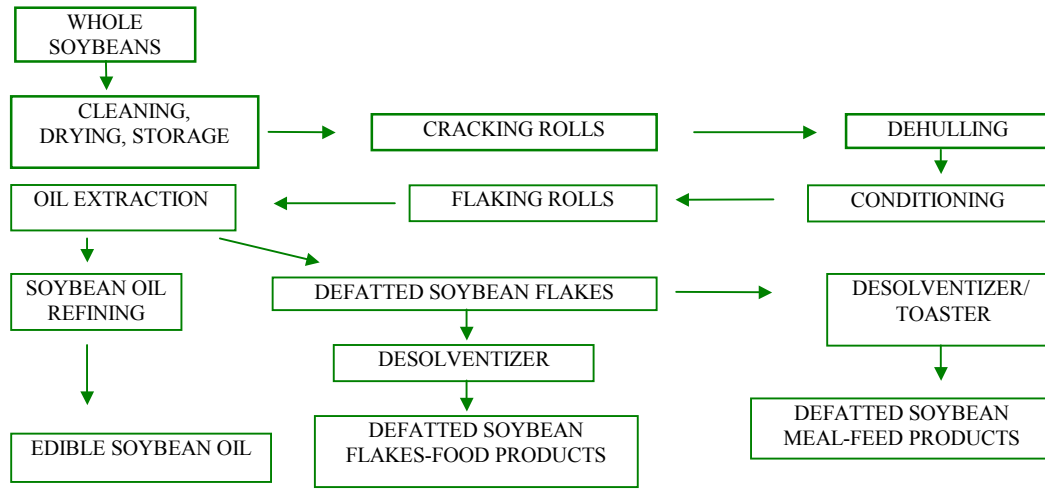
Finally, an assessment of the endogenous allergens in conventional and Roundup Ready soybeans has been made using sera from patients confirmed to be sensitive to soybean protein. This study demonstrated that the introduction of the CP4 EPSPS protein did not cause any discernible changes, either qualitatively or quantitatively, in the composition of the allergenic proteins endogenous to soybean (Burks and Fuchs, 1995).

In summary, the known function and ubiquity of EPSPS proteins and direct studies with CP4 EPSPS protein demonstrate that this protein does not represent a new risk in the food supply. Results showed that there was no indication of toxicity of the CP4 EPSPS protein as measured by treatment-related adverse effects in mice administered the CP4 EPSPS protein by oral gavage. This lack of toxicity was expected based on the rapid degradation of the CP4 EPSPS protein and its enzymatic activity in simulated human gastric and intestinal fluids. In addition, CP4 EPSPS protein is not homologous to known protein toxins or allergens and is present at very low levels in Roundup Ready soybeans. Furthermore, this protein is from a family of proteins with a long history of safe consumption.

Compositional Analysis and Nutritional Assessment of Roundup Ready Soybeans

Soybean and Its Uses

The design of a food and feed safety assessment program for a biotechnology crop requires a detailed understanding of the uses of the crop and crop products in animal and human nutrition. Soybean, *Glycine max*, is one of the world's largest sources of plant protein and oil. The following diagram depicts how soybean seed is processed into oil and meal:



Typically, a 60-pound bushel of soybeans yields about 48 pounds of protein-rich meal and 11 pounds of oil (American Soybean Association, 1999). The primary use of the defatted toasted soybean meal is in animal feed (97%). The principle fraction used by the food industry is processed soybean oil, which is utilized in margarines, shortenings and cooking and salad oils. Lecithin, a phosphatide removed from crude soybean oil, is used as a natural emulsifier, lubricant, and stabilizing agent. Soybean flakes are also used in various food products, including tofu, soya sauce, and simulated milk and meat products. There are few food uses of unprocessed soybeans, since they naturally contain trypsin inhibitors, which may act as anti-nutrients if the soybeans are not properly heated during preparation. Industrial uses of soybeans range from the production of yeasts and antibodies to the manufacture of soaps and disinfectants.

Compositional Analysis

Compositional analyses are a critical component of the safety assessment process that integrates with the evaluation of the trait described above (Astwood and Fuchs, 2000). Each of the measured parameters provides an assessment of the cumulative result of numerous biochemical pathways and hence provides an assessment of a wide range of metabolic pathways within the plant. Comparisons of various nutrients and anti-nutrients are made to both a closely related conventional counterpart as well as to the established range for the

specific component within that crop, to compare the observed levels to the natural variation of that component in current plant varieties.

Extensive compositional analyses of Roundup Ready soybean event 40-3-2 have been conducted and the results of these studies have been published (Padgett *et al.*, 1995; Padgett *et al.*, 1996b; Taylor *et al.*, 1999; List *et al.*, 1999). Over 1700 individual analyses have been conducted and establish that the composition of Roundup Ready soybeans is substantially equivalent to the non-transgenic parental soybean variety and other commercial soybean varieties. These analyses included:

- *Proximate analysis*: protein, fat, fiber, ash, carbohydrates, and moisture
- *Anti-nutrients*: trypsin inhibitors, lectins, stachyose, raffinose and phytate
- *Isoflavones*: genistein and daidzein
- *Fatty acid profile*: percentage of individual fatty acids
- *Amino acid composition*: levels of individual amino acids

The results of proximate analyses conducted on seed from Roundup Ready soybean and the non-transgenic parental control are presented in Table 2. These analyses were conducted on soybeans grown at six locations across the U.S. in 1992 and four locations in 1993; the Roundup Ready soybean plants were treated with the original Roundup herbicide. No statistical differences were observed between the proximate values (protein, ash, moisture, oil, fiber and carbohydrates) for Roundup Ready soybeans and the A5403 control at the 5 percent confidence level, confirming that the levels of these components in Roundup Ready soybeans are comparable to those of conventional soybeans (Taylor *et al.*, 1999).

Amino acid analysis was performed on Roundup Ready soybean and the A5403 soybean seed from the 1993 U.S. field trials (Table 3). The levels of amino acids, including those of the aromatic amino acids (phenylalanine, tyrosine, and tryptophan) were comparable between the two lines. There were no significant differences observed at the 5% confidence level for any amino acid analyzed (Taylor *et al.*, 1999).

A summary of isoflavone (also referred to as phytoestrogens) levels from soybean seed harvested from the 1993 U.S. field trials is provided in Table 4. The range of total genistein and total diadzein was variable across sites and is reflected in the ranges for both Roundup Ready soybean and the A5403 control soybean seed. However, the mean levels of total genistein and total diadzein in the Roundup Ready soybean seed are comparable to those in the A5403 control seed, and are comparable to isoflavone levels reported for field-grown conventional soybeans (Wang *et al.*, 1990). There were no significant statistical differences observed at the 5% confidence level (Taylor *et al.*, 1999).

Compositional analyses have also been conducted on various processed fractions of soybeans, including toasted meal, defatted non-toasted meal, protein isolate, protein concentrate and oil (Padgett *et al.*, 1996b). Roundup Ready and A5403 control soybeans from the 1992 U.S. field trials were processed into the various fractions using procedures that mimic commercial processing procedures as closely as possible, although the scale was much

smaller. The results of proximate analyses conducted on defatted non-toasted soybean meal, isolate and concentrate are presented in Table 5. The levels of macronutrients (protein, ash, fat, fiber and carbohydrates) in these fractions made from Roundup Ready soybeans were comparable to the levels in the fractions made from the parental soybean control cultivar. The fatty acid composition of the refined, bleached, deodorized oil (RBDO) processed from Roundup Ready oil was comparable to RBDO made from the control soybean line (Table 6).

A detailed characterization of the phospholipid composition of Roundup Ready soybean seed was performed by List *et al.* (1999). Lecithin derived from Roundup Ready soybeans was compared to commercial lecithin samples and the levels of phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidic acid were found to be comparable.

The following table summarizes the analyses that were conducted on whole soybean seed and various processed soy fractions (Padgett *et al.*, 1996b; Taylor *et al.*, 1999; List *et al.*, 1999):

Whole soybeans	Proximate analysis Amino acid composition Fatty acid composition Trypsin inhibitor Lectins Isoflavones Urease activity Phospholipid composition
Toasted meal	Proximate analysis Trypsin inhibitor Lectins Isoflavones Urease activity Stachyose, raffinose Phytate Nitrogen solubility
Defatted flour	Proximate analysis Trypsin inhibitor Urease activity
Protein isolate	Proximate analysis
Protein concentrate	Proximate analysis
Refined, bleached deodorized oil	Fatty acid composition

In all cases, the results of the analyses demonstrate that soybean seed and food components from Roundup Ready soybean event 40-3-2 are substantially equivalent to soybean seed and food components from conventional soybean varieties.

Nutritional Assessment

A series of animal feeding studies have been completed using diets incorporating raw or processed soybeans from Roundup Ready soybean event 40-3-2. These studies confirm the nutritional equivalence of event 40-3-2 when used as animal feed and address potential pleiotropic effects caused by the insertion process or site of insertion.

The animal feeding studies included two separate four-week studies in rats, a four-week dairy cow study, an approximately fourteen-week growing-finishing pig study, a six-week chicken study, a ten-week catfish study and a five-day quail study (Hammond *et al.*, 1996; Rogers, 1998; Cromwell *et al.*, 2001). Animals were fed either raw cracked soybean, unprocessed soybean meal or processed soybean meal (dehulled, defatted, toasted). Included in these studies were control groups fed meal from the non-modified parental soybean line A5403. Results from all groups were compared using conventional statistical methods to detect differences between groups in measured parameters.

Samples of both Roundup Ready soybeans and the conventional counterpart produced similar growth and feed efficiency for rats, chickens, catfish and quail (Hammond *et al.*, 1996) and swine (Cromwell *et al.*, 2001). Milk production, composition and rumen fermentation parameters for dairy cows were also comparable across all groups (Hammond *et al.*, 1996). Results for other parameters measured in each feeding study were also similar across all groups. When compared to the US population as a whole, the levels of soybean consumption (in mg/kg of body weight) in these animal feeding studies were 100-fold or more higher than the average human daily consumption of soybean-derived foods in the U.S. (USDA, 1993).

A 15-week study in rats and mice compared the feeding value of Roundup Ready soybeans to that of the parental control (Teshima *et al.*, 2000). No remarkable compositional differences in fatty acids or amino acids were observed between Roundup Ready soybeans and the non-transgenic control. No significant differences in growth, feeding value and the histopathology of immune-related organs were observed between animals fed Roundup Ready soybean meal and meal from the non-transgenic control. The production of soybean-specific IgE was not detected in sera of either group of animals, and the increase in soybean-specific IgG was comparable in both groups. No immunotoxic activity was observed in the rats or mice fed Roundup Ready soybeans.

These studies all confirm the food and feed safety and nutritional equivalence of Roundup Ready soybean event 40-3-2 compared to conventional soybean varieties. The nutritional value or wholesomeness of Roundup Ready soybean event 40-3-2, even when fed to animals at levels much higher than humans would encounter in the diet, was the same as conventional varieties of soybeans.

Environmental Assessment

Soybean

Cultivated soybean, *Glycine max* (L.), is a diploidized tetraploid ($2n=40$). It is an erect, bushy herbaceous annual that can reach a height of 1.5 meters. The genus *Glycine* is of Asian and Australian origin (Lackey, 1981) and is divided into two subgenera, *Glycine* and *Soja*. The first consists of twelve wild perennial species (Hymowitz *et al.*, 1992) that are primarily distributed in Australia, South Pacific Islands, Philippines, and Taiwan (Newell and Hymowitz, 1978). The subgenus *Soja* consists of three annual species from Asia, *G. max*, *G. soja*, and *G. gracilis*. The first species is the cultivated soybean, the second species is the wild form of the soybean, and the third species is referred to as the "weedy" form of the soybean (Lackey, 1981). The cultivated soybean is native to north and central China and is commonly considered one of the oldest cultivated crops (Hymowitz, 1970). Historical and geographical evidence suggests that soybeans were first domesticated in the eastern half of China between the 17th and 11th century B.C. (Hymowitz, 1970). Soybeans were first introduced into the United States in 1765, primarily as a forage crop (Hymowitz and Harlan, 1983). Successful use of soybean as an oilseed in Europe from 1900 to 1910 promoted interest in its use in the United States.

Cultivated soybean is essentially self-pollinated (Carlson and Lersten, 1987; McGregor, 1976). The anthers mature in the bud and shed their pollen directly onto the stigma of the same flower, ensuring a high degree of self-pollination. Cross-pollination is generally very low and various studies have shown it to be from 0.03 to 3.62% between adjacent rows (Woodworth, 1922; Caviness, 1966; McGregor, 1976; Ahrent and Caviness, 1994). At distances of more than 4.5 meters from the pollen source, natural cross-pollination in soybean is rare (less than 0.02%) and most often not detectable (Caviness, 1966). Caviness (1970) showed that honeybees are responsible for the occasional cross-pollination, and that thrips are ineffective pollinators. As a result, soybean plants are generally considered pure breeding homozygous lines.

Cultivated soybean is sexually compatible only with members of the genus *Glycine*. Soybean crosses with members of subgenus *Glycine* only with extreme technical assistance. Soybean does not cross with any extrageneric relatives (Hymowitz and Singh, 1987). Cultivated soybean is the only member of the genus *Glycine* that grows in the United States and its territories. In addition, cultivated soybean is not sexually compatible with any other *Glycine* species found in the United States or its territories, with the exception of specialized research collections maintained under scientific care and scrutiny.

Soybean plants are annuals and do not survive vegetatively in the United States from one growing season to the next (Hymowitz and Singh, 1987). Survival from one season to the next is by seed; however, volunteers are seldom seen when cultivated soybean is grown in the United States. Since soybeans do not retain high germination rates and vigor for long

periods, fresh, properly grown and handled seed is required for commercial varieties each growing season (TeKrony *et al.*, 1987).

Assessment of Agronomic Performance

Agronomic evaluations are an important component of assessing the potential ecological risk of a genetically modified crop. Roundup Ready soybean event 40-3-2 has been tested in field trials in the United States, Central and South America, Europe, Central Europe and Canada since 1991. Data collected from over 150 field trials conducted over a three-year period prior to commercialization in the U.S. demonstrate that Roundup Ready event 40-3-2 does not differ significantly from conventional soybeans in morphology, seed production (yield), agronomic characteristics such as time to flowering and pod set, or vigor (emergence or persistence) (Re *et al.*, 1993). In addition, Roundup Ready soybean event 40-3-2 was monitored for its susceptibility to diseases and insects and there were no differences observed in disease severity or insect infestations between Roundup Ready soybeans and the control plants (Re *et al.*, 1993; USDA, 1994).

Reports published more recently include data on yield and glyphosate tolerance (Delannay *et al.*, 1995), fungal resistance (Sanogo *et al.*, 2000) and weed control (Nelson and Renner, 1999). These studies confirm the conclusion that tolerance to glyphosate is stably inherited and that there are no unexpected plant pest risks or other risks posed to the environment. Furthermore, the Roundup Ready soybean trait has been introgressed into conventional soybean varieties by most of the major soybean seed suppliers and has led to the development of over one thousand Roundup Ready soybean varieties in North America. Roundup Ready soybean varieties have been in commercial production on over 240 million acres (100 million hectares) globally since 1996. No unusual plant pest characteristics or unintended environmental effects have been observed that are attributed to the inserted event, as confirmed by the extensive studies developed prior to, and subsequent to, approval and market introduction. Agronomic performance has been as expected and tolerance to glyphosate has been uniform and consistent in these varieties.

Assessment of Potential Weediness

The introduction of herbicide tolerance genes to a cultivar should not increase the “weediness” of the plant. A general consensus of the traits common to many weeds was developed by Baker (1965, 1974) and soybean possesses few of the characteristics of plants that are successful weeds. Soybean is an annual crop that is considered to be a highly domesticated, well-characterized crop plant that does not persist in undisturbed environments without human intervention. The A5403 variety, which is the parental variety of Roundup Ready soybean event 40-3-2, is not considered to be a weed and introduction of the glyphosate tolerance trait into this cultivar has not imparted any new “weedy” characteristics. No differences were noted between the transformed and non-transformed cultivars with respect to the number of seeds produced, the germination characteristics of seeds, final stands, and disease or insect susceptibility (USDA, 1994). Based on the assessment of agronomic performance, observations of no differences in terms of interactions

with diseases and insects, and the fact the CP4 EPSPS is a member of a family of enzymes that are ubiquitous in plants, fungi and bacteria affording no selective advantage to organisms possessing them, it is concluded that Roundup Ready soybean plants have no greater weediness potential than their conventional counterparts. In addition, conventional and Roundup Ready soybean plants can be effectively controlled by herbicides other than glyphosate and by cultivation.

Assessment of Effects on Non-Target Organisms

Roundup Ready soybean event 40-3-2 encodes the CP4 EPSPS enzyme. EPSPS is an enzyme of the shikimate pathway for aromatic amino acid biosynthesis in plants and microorganisms (Levin and Springson, 1964), and is thus ordinarily present in food and feed derived from plant sources. EPSPS proteins from a number of bacteria exhibit tolerance to glyphosate (Schulz *et al.*, 1985). CP4 EPSPS protein thus represents one of many different EPSPS proteins found in nature. EPSPS is considered to be ubiquitous in nature since it is present in all plants and microorganisms. Therefore, all organisms that presently feed on plants and/or microbes have historically been exposed to EPSPS protein.

Since birds may feed on soybean seeds left in the field after harvest, the wholesomeness of Roundup Ready soybeans was evaluated in bobwhite quail, which are traditionally used in bird feeding studies. No differences in food consumption, body weight gain or behavior occurred between birds fed 200,000 ppm (20% w/w) of raw soybean meal from Roundup Ready soybeans and birds fed raw soybean meal from the parental A5403 soybean variety. Based on the parameters measured, the wholesomeness of Roundup Ready soybean meal was comparable to that of the non-transgenic parental line when fed in the diet to quail.

Potential Impact on Biodiversity

Since the naturally-occurring EPSPS proteins are ubiquitous among plants and fungi in nature and non-toxic to fish, avian species, insects, mammals and other species, and exposure to these species is not likely due to their feeding preferences, the effects on wildlife from the commercialization of Roundup Ready soybean plants are not expected to be any different from those experienced in the production of conventional soybeans. In addition, the agronomic and compositional data obtained on Roundup Ready soybeans support the assertion that the impact on biodiversity of the modified soybeans will be equivalent to or less than conventional soybeans.

Assessment of tolerance to Glyphosate

More than 100 herbicide-resistant weed biotypes have been identified to date; over half of them are resistant to the triazine family of herbicides (Holt and Le Baron, 1990; Shaner, 1995). Resistance has usually developed because of the selection pressure exerted by the repeated use of herbicides with a single target site and a specific mode of action, long residual activity of the herbicide with the capacity to control weeds yearlong, and frequent applications of the same herbicide without rotation to the other herbicides or cultural control

practices. Using these criteria, and based on current use data, glyphosate is considered to be a herbicide with a low risk for weed resistance (Benbrook, 1991). Nonetheless, questions have been raised as to whether the introduction of crops tolerant to a specific herbicide, such as glyphosate, may lead to the occurrence of weeds resistant to that particular herbicide. This concern is based on the assumptions that the use of the herbicide will increase significantly, and possibly that it will be used repeatedly in the same location. However, increases in other uses of glyphosate over the previous years have been more significant than the projected increase associated with the introduction of Roundup Ready crops. Although it cannot be stated that evolution of resistance to glyphosate will not occur, the development of weed resistance to glyphosate is expected to be a rare event because:

1. Generally, weeds and crops are inherently not tolerant to glyphosate, and the long history of extensive use of glyphosate has resulted in few instances of resistant weeds (Bradshaw *et al.*, 1997);
2. Glyphosate has many unique properties, such as its mode of action, chemical structure, limited metabolism in plants, and lack of residual activity in soil, which make the development of resistance unlikely;
3. Selection for glyphosate resistance using whole plant and cell/tissue culture techniques was unsuccessful, and would, therefore, be expected to occur rarely in nature under normal field conditions.

In 1996 in Australia, it was reported that a biotype of annual rye-grass (*Lolium rigidum*) was surviving application of label recommended rates of glyphosate (Pratley *et al.*, 1996). To date, after examination of thousands of samples, only three locations have been confirmed as having the resistant population, indicating that the phenomenon is not widespread. A large body of biochemical and molecular biology experiments to determine the cause of observed weed control differences between Australian rye-grass biotypes resistant and susceptible to glyphosate indicate that the observed resistance is due to a combination of factors. Conclusions drawn to date are that the resistant biotype is easily controlled by conventional practices (tillage, other herbicides), and is caused by a complex inheritance pattern, unlikely to occur across a wide range of other species. Results of these studies have been presented (Pratley, 1999).

Additional reports of resistant ryegrass in northern California and South Africa are being investigated. Similar to the Australian locations, these fields are small and isolated. Again, the use of mowing and other herbicides have been very effective in controlling the ryegrass. Weed management recommendations are also in place and have successfully controlled the ryegrass. Research continues in an effort to better understand the resistance mechanism.

A population of *Elusine indica* (goosegrass) was reported to survive labelled rates of glyphosate in Malaysia. The fields from which these biotypes were collected had been treated an average of eight times per year with glyphosate for the past ten years. The glyphosate resistance observed in the field trials was confirmed in dose/pot greenhouse experiments. The analyses found that the resistant goosegrass has a modified EPSPS protein that is two- to four-fold less sensitive to glyphosate than in more sensitive biotypes.

Research is underway to investigate the resistance mechanism genetics and biology of the resistant biotype.

Most recently, observations of a resistant biotype of maretail (*Conyza canadensis*) were made in southern New Jersey, Delaware and western Tennessee. Maretail has a long history of being difficult to control with glyphosate, so these isolated incidences were assumed to be weather related. An increase in reports prompted field visits and research was conducted to confirm that higher than labelled rates were necessary to control this biotype versus susceptible maretail plants. With this particular biotype, the most effective weed management plan to control this resistant population includes the use of herbicides with different modes of action.

Historically, the onset of resistance to glyphosate has been far less than with other products (HRAC, *et al.*, 2002). After 20 years of world wide use, confirmed resistance exists in only three plant species. Monsanto continues to aggressively monitor and investigate any such reports from customers. Weed management recommendations for Roundup Ready crops will continue to be based on specific local needs and follow basic weed management principles. Weed management practices shall be structured to include a glyphosate herbicide alone, or in combination with other herbicides and/or cultural practices to deliver effective and economic weed control.

Environmental Assessment Conclusions

In summary, the evidence collected to date show that Roundup Ready soybean plants offer growers new opportunities to produce a soybean crop in a manner that is environmentally superior to traditional methods. Assessments indicate that the environmental risks present with Roundup Ready soybean are equivalent to or less than those already present with conventional soybean. Agronomic evaluations consisting of plant vigor, growth habit characteristics and disease susceptibility have shown Roundup Ready soybeans pose no increased pest potential compared to the parental A5403 soybean variety. In addition, the CP4 EPSPS protein introduced affords no significant potential for toxicity to wildlife and non-target organisms broadly. Furthermore, data generated to support the registrations of Roundup agricultural herbicides and almost 30 years of use experience with glyphosate demonstrate that these herbicides will not cause unreasonable adverse effects to humans, mammals or other non-target organisms under normal use conditions. In addition, the data demonstrate that the use of these herbicides in soybeans will not cause unreasonable adverse effects to the environment.

Summary

The introduction of Roundup Ready soybeans has reduced the number and cost of herbicide applications, and offers considerable environmental benefits due to its fit with conservation tillage systems. Detailed food, feed and environmental safety assessments confirm the safety of this product based on: the safety of the genetic elements contained in the transformation vector used to produce Roundup Ready soybean event 40-3-2; the detailed molecular characterization of the inserted DNA and conclusion that only the CP4 EPSPS protein is produced from the inserted DNA; the known metabolic function and history of safe use (*i.e.*, consumption) of the EPSPS family of proteins present in all plants, fungi and bacteria; the direct assessment of the CP4 EPSPS protein (*i.e.*, lack of toxicity and allergenicity concerns); the assessment of compositional and nutritional equivalence of event 40-3-2 (comparing the key nutrients, anti-nutrients and allergens to the parental event and conventional soybeans); a comparison of crop agronomic characteristics of event 40-3-2 to the parental and conventional soybeans; and a comparison of the safety and nutritional properties of event 40-3-2 to parental and conventional soybean varieties in animal feeding studies. All studies completed with soybean materials contained the inserted DNA and hence confirm the safety of the inserted DNA and any products derived from them. These assessments confirm that Roundup Ready soybeans are as safe and nutritious as conventional soybean varieties, do not pose a plant pest risk and pose no greater environmental impact than conventional soybean varieties.

Information and data contained within this document have been provided to regulatory authorities for review. Regulatory review continues as we update regulatory files and make submissions to additional countries globally.

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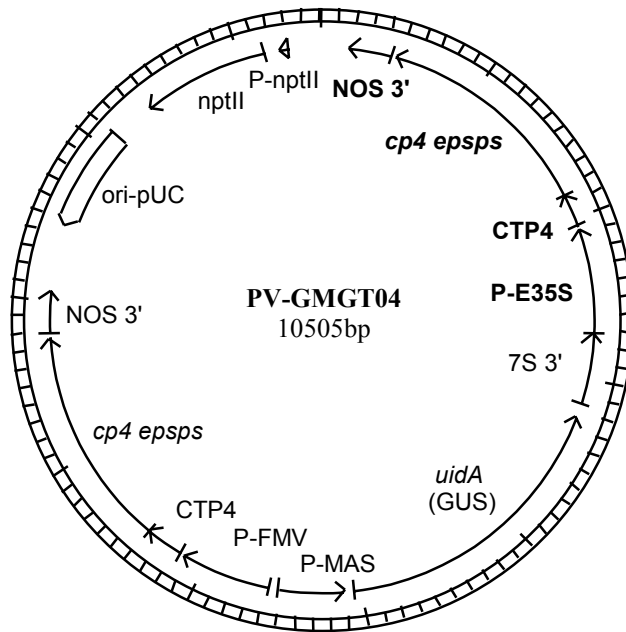


Figure 1. Plasmid Map of PV-GMGT04 Used to Produce Roundup Ready Soybean Event 40-3-2.

Key to genetic elements:

P-E35S: Enhanced cauliflower mosaic virus (CaMV) promoter

CTP4: Chloroplast transit peptide sequence from the *Petunia hybrida* EPSPS gene

cp4 epsps: 5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp. strain CP4

NOS 3': 3' nontranslated region of the *Agrobacterium tumefaciens* nopaline synthase gene

P-nptII: Promoter from *nptII* gene obtained from Tn5

nptII: Neomycin phosphotransferase type II gene from transposon Tn5

ori-pUC: The origin of replication from the high copy *E.coli* plasmid pUC119

P-FMV: 35S promoter from the figwort mosaic virus

P-MAS: Mannopine synthase promoter region from *Agrobacterium tumefaciens*

uidA (GUS): β -glucuronidase gene from *E. coli*

7S 3': 3' nontranslated region of the soybean 7S seed storage protein alpha' subunit

Table 1. CP4 EPSPS Protein Levels (μg of protein/mg of fresh weight of tissue) in Roundup Ready Soybean (40-3-2) and Parental (A5403) Soybean Seed and Leaf Tissue.

Year / Tissue		A5403	40-3-2 Treated		A5403	40-3-2 Untreated	
1992 (6 sites)				1992 (9 sites)			
Seed	mean	ND ²	0.301	Seed	mean	ND	0.288
	range	NA ³	0.258-0.378		range	NA	0.186-0.395
	SD	NA	0.03		SD	NA	0.066
1993(4 sites)				1993(4 sites)			
Seed	mean	ND	0.218	Seed	mean	ND	0.201
	range	NA	0.166-0.287		range	NA	0.127-0.277
	SD	NA	0.042		SD	NA	0.017
Leaf ¹	mean	ND	0.489	Leaf	mean	ND	0.415
	range	NA	0.308-0.856		range	NA	0.299-0.601
	SD	NA	0.239		SD	NA	0.153

¹ Leaf samples were collected from three of the four sites.

² ND, not detectable. The mean and standard deviation (SD) were not calculated because the CP4 EPSPS protein was not detected in the A5403 extracts.

³ NA, not applicable.

Table 2. Summary of Proximate Analyses from Glyphosate-Treated Roundup Ready (40-3-2) and Parental Control (A5403) Soybean Seed¹. Results of 1992 and 1993 Field Trials.

Characteristic ²	A5403 Mean	A5403 Range ³	A5403 SEM ⁴	40-3-2 Mean	40-3-2 Range	40-3-2 SEM	Literature Range
1992 (6 sites) ⁵							
Protein	41.01	37.46-44.90	0.37	40.35	36.42-44.71	0.37	36.9-46.4 ⁷
Ash	5.18	4.61-5.52	0.07	5.34	4.73-5.91	0.07	4.61-5.37 ⁷
Moisture	12.68	11.10-14.30	1.28	10.56	7.67-22.65	1.28	7-11 ^{7,8}
Oil	19.8	17.40-21.84	0.23	20.41	18.19-22.19	0.23	13.2-22.5 ^{7,9}
Fiber	6.35	5.86-6.52	0.15	6.44	6.13-7.11	0.15	4.7-6.48 ^{7,10}
Carbohydrates	34.01	32.36-35.26	0.24	33.86	32.11-35.73	0.24	30.9-34.0 ^{7,11}
1993 (4 sites) ⁶							
Protein	41.4	40.39-42.32	0.451	41.43	39.35-44.14	0.451	36.9-46.4 ⁷
Ash	5.31	5.01-5.94	0.052	5.35	5.04-5.81	0.052	4.61-5.37 ⁷
Moisture	5.73	5.18-6.19	0.137	5.74	5.32-6.20	0.137	7-11 ^{7,8}
Oil	19.89	18.67-20.57	0.353	20.53	19.01-22.17	0.353	13.2-22.5 ^{7,9}
Fiber	7.36	6.63-8.10	0.145	6.86	5.59-7.66	0.147	4.7-6.48 ^{7,10}
Carbohydrates	33.38	31.57-35.08	0.712	32.67	27.86-35.32	0.711	30.9-34.0 ^{7,11}

¹ Means reported are from single assays on single samples from six sites in 1992 and four sites in 1993.

² All values reported as percent dry weight, except for moisture.

³ Range denotes the lowest and highest value reported for each assay.

⁴ SEM – Standard error of the mean

⁵ The 1992 Roundup Ready soybean field plots were treated with a pre-emergence application of 17.8 L/ha and an early postemergence application of 1.75 L/ha of the original Roundup herbicide.

⁶ The 1993 Roundup Ready soybean field plots were treated with a pre-emergence application of 17.8 L/ha and early and late postemergence applications of 2.34 L/ha of the original Roundup herbicide.

⁷ Smith and Circle, 1972

⁸ Perkins, 1995

⁹ Wilcox, 1985

¹⁰ Mounts *et al.*, 1987

¹¹ Orthofer, 1978

Table 3. Summary of Amino Acid Analyses of Glyphosate-Treated Roundup Ready and Parental Control Soybean Seed^{1,2,3} (g/100 g dry weight). 1993 U.S. Field Trials.

Amino Acid	A5403 Mean	A5403 Range	A5403 SEM ⁴	40-3-2 Mean	40-3-2 Range	40-3-2 SEM ⁴	Literature Range ³
Aspartic Acid	4.50	4.30-4.59	0.063	4.51	4.21-4.75	0.063	3.87-4.98
Threonine	1.57	1.54-1.60	0.013	1.57	1.52-1.63	0.013	1.33-1.79
Serine	2.03	1.95-2.06	0.024	2.03	1.92-2.11	0.024	1.81-2.32
Glutamic Acid	7.48	7.06-7.81	0.112	7.44	6.84-7.97	0.112	6.10-8.72
Proline	2.07	1.97-2.16	0.023	2.06	1.91-2.16	0.023	1.88-2.61
Glycine	1.73	1.65-1.83	0.013	1.71	1.61-1.78	0.013	1.88-2.02
Alanine	1.73	1.66-1.79	0.012	1.72	1.67-1.76	0.012	1.49-1.87
Valine	1.94	1.87-1.97	0.019	1.93	1.83-2.00	0.019	1.52-2.24
Isoleucine	1.83	1.75-1.88	0.022	1.82	1.71-1.91	0.022	1.46-2.12
Leucine	3.09	2.96-3.16	0.037	3.06	2.90-3.19	0.037	2.71-3.20
Tyrosine	1.39	1.34-1.41	0.012	1.38	1.32-1.44	0.012	1.12-1.62
Phenylalanine	2.01	1.92-2.06	0.015	1.98	1.86-2.08	0.015	1.70-2.08
Histadine	1.10	1.07-1.12	0.015	1.10	1.05-1.15	0.015	0.89-1.08
Lysine	2.63	2.53-2.69	0.021	2.64	2.53-2.76	0.021	2.35-2.86
Argenine	2.88	2.70-2.97	0.051	2.89	2.64-3.09	0.051	2.45-3.49
Cysteine	0.57	0.50-0.61	0.008	0.59	0.54-0.60	0.008	0.56-0.66
Methionine	0.54	0.48-0.57	0.009	0.54	0.51-0.55	0.009	0.49-0.66
Tryptophan	0.49	0.48-0.50	0.012	0.49	0.47-0.53	0.012	0.53-0.54

¹ Means are from single assays on single samples from four U.S. sites in 1993. A5403 is the control line and 40-3-2 is the Roundup Ready soybean line. The 1993 Roundup Ready soybean field plots were treated with a pre-emergence application of 17.8 L/ha and early and late post-emergence applications of 2.34 L/ha of the original Roundup herbicide.

² No significant differences between Roundup Ready soybeans (40-3-2) and the A5403 control line were observed at the 5% level (SAS GLM procedure).

³ Han *et al.*, 1991; Orthofer, 1978.

⁴ SEM – Standard error of the mean

Table 4. Summary of Isoflavone Analyses of Glyphosate-Treated Roundup Ready and Parental Control Soybean Seed¹ ($\mu\text{g/g}$ dry weight). 1993 U.S. Field Trials.

Isoflavone	A5403	A5403	A5403	40-3-2	40-3-2	40-3-2	Literature Range ⁸
	Mean	Range	SEM ²	Mean	Range	SEM ²	
Total genistein ³	681	230-1086	38.152	742	196-1231	38.152	461.1-1000
Free genistein ⁴	20	14-28	NT	23	12-38	NT	
Bound genistein ⁴	662	210-1058	NT	719	172-1193	NT	
Total daidzein ³	521	161-931	36.323	578	106-1064	36.323	330.6-706
Free daidzein ⁴	42	19-70	NT	42	20-86	NT	
Bound daidzein ⁴	479	142-862	NT	536	85-978	NT	
Total coumestrol ⁵	ND	NA ⁶	NT ⁷	ND	NA	NT	
Total biochanin ⁵	ND	NA	NT	ND	NA	NT	

¹ Means are from single assays on single samples from four U.S. sites in 1993. A5403 is the control line and 40-3-2 is the Roundup Ready soybean line. The 1993 Roundup Ready soybean field plots were treated with a pre-emergence application of 17.8 L/ha and early and late post-emergence applications of 2.34 L/ha of the original Roundup herbicide.

² SEM – Standard error of the mean

³ No statistical differences were observed between Roundup Ready soybeans (40-3-2) and A5403 control line total genistein and daidzein concentrations at the 5% confidence level (SAS GLM procedure).

⁴ No statistical comparisons were made between Roundup Ready soybeans (40-3-2) and A5403 control line free and bound genistein and daidzein.

⁵ Coumestrol and biochanin levels below the limit of quantitation (10 ppm) of the assay; not detectable (ND).

⁶ NA, not applicable.

⁷ NT, not tested.

⁸ Pettersson and Kiessling, 1984; Wang *et al.*, 1990.

Table 5. Proximate Analysis of Defatted Non-Toasted Soybean Meal, Isolate and Concentrate from the 1992 U.S. Processing Study¹ (g/100 g dry weight, unless noted).

Component	A5403	40-3-2	Literature Range
Defatted meal (non-toasted)			
Protein	53.2	53.6	40.0-59.0 ²
Ash	6.53	6.89	6.0-6.4 ³
Moisture, g/100 g fresh wt	6.55	11.90	
Fat	2.30	0.73	0.9-1.0 ⁴
Fiber	4.52	4.23	2.5-4.5 ⁵
Carbohydrates	38.0	38.8	34.0-38.0 ⁶
Urease, Δ pH	2.30	2.45	
Trypsin inhibitor, TIU ⁷ /mg dry wt	65.9	83.5	
Protein Isolate			
Protein	84.6	82.2	85.2-92.0 ⁸
Ash	3.30	3.89	2.3-7.6 ⁹
Moisture, g/100 g fresh wt	4.77	5.26	
Fat	1.06	1.68	0.1-2.5 ¹⁰
Fiber	<0.2	<0.2	0.1-0.4 ¹¹
Carbohydrates	11.0	12.2	0.3-0.6 ¹²
Protein Concentrate			
Protein	65.1	67.2	66.2-78.1 ¹³
Ash	4.91	5.91	4.7-6.5 ¹⁴
Moisture, g/100 g fresh wt	9.36	5.31	
Fat	5.27	4.47	0.9-2.0 ¹⁵
Fiber	4.60	2.80	2.8-5.0 ¹⁶
Carbohydrates	24.7	22.5	17.1-25.0 ¹⁷

¹ Values are from single assays on single samples. A5403 is the control line and 40-3-2 is the Roundup Ready soybean line.

² Smith and Circle, 1972; Wolf, 1983.

³ Fulmer, 1988; Smith and Circle, 1972.

⁴ Horan, 1974; Smith and Circle, 1972.

⁵ Fulmer, 1988; Sipos, 1988.

⁶ Sipos, 1988; Waggle and Kolar, 1979.

⁷ TIU = trypsin inhibitor units

⁸ Torun, 1979; Waggle and Kolar, 1979.

⁹ Smith and Circle, 1972; Wolf, 1983.

¹⁰ Horan, 1974; Wolf, 1983.

¹¹ Smith and Circle, 1972; Wolf, 1983.

¹² Waggle and Kolar, 1979; Wolf, 1983.

¹³ Bookwalter, 1978; Smith and Circle, 1972.

¹⁴ O'Dell, 1979; Wolf, 1983.

¹⁵ Mattil, 1974; Wolf, 1983.

¹⁶ Mattil, 1974; Smith and Circle, 1972.

¹⁷ Rackis, 1974; Wolf, 1983.

Table 6. Fatty Acid Analysis of Refined, Bleached, Deodorized Soybean Oil from the 1992 U.S. Processing Study¹ (g/100 g)

Fatty Acid ²	A5403	40-3-2	Literature Values ³
6:0 (caproic)	0.16	0.20	
7:0 (heptanoic)	0.39	0.46	
16:0 (palmitic)	10.46	10.50	7-12, 10.7
17:0(margaric)	0.12	0.14	
18:0 (stearic)	4.09	4.19	2-5.5, 3.9
18:1 cis (oleic)	21.13	21.41	20-50, 22.8
18:2 (linoleic)	52.20	51.71	35-60, 50.8
18:3 (linolenic)	7.41	7.51	2-13,6.8
19:0 (nonadecanoic)	0.13	<0.10	
20:0 (arachidic)	0.13	0.27	0.2, <1.0
20:1 (eicosenoic)	0.17	0.17	<1.0
22:0 (behenic)	0.55	0.52	<0.5
24:0 (lignoceric)	0.15	0.16	
Unknowns	2.68	2.47	

¹ Values are from single assays on a single sample. A5403 is the control line and 40-3-2 is the Roundup Ready soybean line.

² Fatty acids 8:0, 9:0, 10:0, 11:0, 12:0, 13:0, 14:1, 15:0,16:1, 17:1, 18:1 *trans*, 20:2, 20:3, 20:4, 20:5, 22:1, 22:2, 22:6 and 24:1 were <0.1 g/100g for both lines.

³ Pryde, 1990.