Safety Assessment of Roundup Ready® Corn Event GA21

Executive Summary

Glyphosate, the active ingredient in Roundup® agricultural herbicides, kills plants by inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). This enzyme is a critical step in the shikimic acid pathway for the biosynthesis of aromatic amino acids in plants and microorganisms, and its inhibition leads to the 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® corn event GA21 contains a modified *epsps* gene from corn (*Zea mays*, L.). The modified maize EPSPS (mEPSPS) protein differs from wild-type maize EPSPS by two amino acids. This results in an EPSPS protein with greater than 99.3% sequence identity to that produced naturally in corn. The mEPSPS protein has a low affinity for glyphosate compared to the wild-type EPSPS enzyme. Thus, when corn plants expressing the mEPSPS protein are treated with glyphosate, the plants are unaffected. The continued action of the tolerant mEPSPS enzyme provides the plant's need for aromatic acids.

The Roundup Ready corn plant was made using particle acceleration technology with a linear DNA fragment of a plasmid carrying the *mepsps* gene.

The food and feed safety of Roundup Ready corn was established based upon: the evaluation of mEPSPS activity and homology to EPSPS proteins present in a diversity of plants, including those used for foods; the low dietary exposure to mEPSPS, the rapid digestibility of mEPSPS, and the lack of toxicity or allergenicity of EPSPSs generally and by direct studies of this specific mEPSPS. The nutritional and compositional equivalence of Roundup Ready corn to conventional corn was demonstrated by analyses of key nutrients including protein, fat, carbohydrates, moisture, calories, amino acids, fatty acids, and minerals. Nutritional equivalence of Roundup Ready corn to conventional corn was confirmed by evaluation in the performance of broiler chickens. The environmental impact of Roundup Ready corn is the same as that of conventional corn. Glyphosate-tolerant volunteer corn is infrequent and easily managed in the farmer's field. The results of all these studies demonstrate that Roundup Ready corn is equivalent to traditional corn with respect to food, feed and environmental safety.

Introduction

Roundup Ready corn was developed by Monsanto Company and DEKALB Genetics Corporation. The genetically modified corn plants are tolerant to glyphosate, the active ingredient in Roundup agricultural herbicides. Roundup Ready corn event GA21 was produced by the introduction of a modified maize 5-enolpyruvylshikimate-3-phosphate synthase (*mepsps*) gene from corn (*Zea mays*).

The use of Roundup Ready corn provides:

- *Broad-spectrum weed control.* Roundup agricultural herbicides control both broadleaf weeds and grasses, including difficult to control weed species (Franz et al., 1996).
- *Excellent crop safety.* When Roundup agricultural herbicides are used according to label recommendations in fields of Roundup Ready corn, weeds are controlled without injury to the Roundup Ready corn.
- *Favorable environmental properties.* Roundup agricultural herbicides have been used for almost 30 years in various applications. Glyphosate, the active ingredient in Roundup agricultural herbicides, has favorable environmental characteristics, including that it binds tightly to soil, making it unlikely to move to groundwater or reach non-target plants, and that it degrades over time into naturally occurring materials. In addition, glyphosate will not cause unreasonable adverse effects to the environment under normal use conditions (US EPA, 1993; WHO, 1994; Geisy *et al*., 2000).
- *Flexibility in treating for weed control.* Since Roundup agricultural herbicides are applied onto the foliage of weeds after crop emergence, applications are only necessary if weed infestation reaches the threshold level for yield reductions.
- *Excellent fit with reduced-tillage systems.* Benefits of conservation tillage include improved soil quality, improved water infiltration, reduced soil erosion and sedimentation of water resources, reduced runoff of nutrients and pesticides to surface water, improved wildlife habitat, increased carbon retention in soil, reduced fuel usage, and encourages sustainable agricultural practices (Warburton and Klimstra, 1984; Edwards *et al.,* 1988; Hebblethwaite, 1995; Reicosky *et al.,* 1995).
- *Cost effective weed control.* The cost of weed control with Roundup agricultural herbicides is competitive with the cost of alternative weed control options, especially in view of the high weed control efficacy of Roundup. Both large and small-scale farmers can benefit equally from use of this technology.
- *A new herbicidal mode of action for in-season corn weed control .* Roundup agricultural herbicides can only be used as a pre-plant herbicide, in all but a few pre-harvest uses without the Roundup Ready genetic modification in the crop.
- *Use of a herbicide with low risk to human health.* Under present conditions of use, Roundup agricultural herbicides will not cause unreasonable adverse effects on human health (U.S. EPA, 1993; WHO, 1994; Williams *et al*., 2000). Glyphosate has been classified by the U.S. EPA as Category E (evidence of non-carcinogenicity for humans) (U.S. EPA, 1992). Additionally, the World Health Organization stated in 1994 that glyphosate is not carcinogenic, mutagenic, or teratogenic (WHO, 1994).

Glyphosate works by inhibiting the enzyme 5-enolpyruvyl shikimate-3-phosphate synthase (EPSPS). This enzyme is involved in the shikimic acid pathway for aromatic amino acid biosynthesis in plants and microorganisms (Steinrucken and Amrhein, 1980). The shikimate pathway is not present in animals, which contributes to the selective toxicity of glyphosate to plants. The modified maize *epsps (mepsps*) gene is completely sequenced and encodes a 47.4 kD protein consisting of 445 amino acids. It differs from wild-type maize EPSPS by two amino acid substitutions. This results in a protein with greater than 99.3% sequence identity to that of the maize protein. The mEPSPS protein also shows high amino acid sequence homology to other EPSPSs found in crops such as soybean (82%) and tomato (83%) and in microorganisms such as baker's yeast.

The mEPSPS protein and the wild-type EPSPS from corn are immunologically and functionally equivalent, except for their affinity to glyphosate, as anticipated by their high sequence similarity.

The mEPSPS protein has a low affinity for glyphosate. The concentrations of glyphosate required to attain 50% inhibition of EPSPS activity were determined to be 5 mM and 300 mM for the wild-type maize EPSPS and the mEPSPS, respectively. This establishes that the mEPSPS enzyme has significantly reduced affinity for glyphosate when compared with the wild-type enzyme. The plants expressing the mEPSPS protein are unaffected by exposure to glyphosate, since the continued action of the tolerant mEPSPS enzyme provides for the plant's need for aromatic amino acids.

Roundup Ready corn has been field tested in the United States since 1994 and in the European Union since 1996. Roundup Ready corn was commercialized in the U.S. in 1998 and in Canada in 1999. The extensive testing demonstrate that Roundup Ready corn is equivalent to conventionally produced corn in safety, nutrition, composition and environmental impact (Sidhu *et al*., 2000).

Molecular Characterization of Roundup Ready Corn

Corn genetics has been extensively studied for over 100 years. As a result, it is one of the best characterized crop plants. Recently, more complete genetic maps of corn have been developed using molecular genetics. Corn has been used in tissue culture research, molecular marker assisted plant breeding, in the study of transposons for gene tagging and generating genetic variability.

The plant transformation vector used to produce Roundup Ready corn event GA21 was plasmid pDPG434 (Figure 1). The DNA segment used in the transformation contained the *mepsps* gene and its expression components. The NotI restriction fragment (shown in Figure 1) contains the *mepsps* gene under regulation of the rice actin promoter and rice actin intron and contains the *nos* 3' polyadenylation sequence. The *mepsps* coding sequence is fused to the optimized transit peptide (OTP) coding sequence (*otp*). The OTP directs the mEPSPS protein to the chloroplast, the location of native EPSPS in plants and the site of aromatic amino acid biosynthesis (Kishore *et al.,* 1988). Transit peptides are typically cleaved from the "mature" protein following delivery to the plastid (della-Cioppa *et al.,* 1986).

The DNA fragment used for transformation, which was derived from plasmid pDPG434, was incorporated into the corn chromosome using particle acceleration technology. In this procedure, DNA is precipitated onto microscopic gold particles that are then placed onto a plastic macrocarrier and accelerated at a high velocity into the target plant cells, where the DNA is deposited and incorporated into the chromosome.

The corn plant tissue that was the recipient of the introduced DNA was a cell culture designated AT824, initiated from immature embryos of an inbred corn line. Transformants were selected by their ability to survive and grow in the presence of glyphosate. R0 plants were generated from the embryonic callus by placing the callus on media that stimulates the production of shoots and roots.

Molecular studies demonstrated that Roundup Ready corn plants contain a single insert of DNA. The single insert in maize line GA21 contains:

- a *mepsps* gene cassette, truncated at the 5' end of the rice actin promoter sequence;
- three complete internal *mepsps* gene cassettes;
- a partial *mepsps* gene cassette containing the promoter, intron, *otp*, and a partial *mepsps* coding sequence terminating in a stop codon; and
- an additional partial gene cassette at the 3' end containing only the rice actin promoter and 5' mRNA leader sequence, but truncating before the start of the rice actin intron, followed by maize genomic DNA.

Northern blot analysis showed that the truncated sequence at the 3' end was not transcribed into a detectable mRNA product. Western blot analyses demonstrated that only a single, immunoreactive band corresponding to the full-length mEPSPS protein was produced.

Based on the maize DNA sequence downstream from the 3' rice actin promoter (minus the r-act intron), two putative open reading frames, -one potentially coding for 97 animo acids and the other potentially coding for 19 amino acids were identified (encoded solely from maize DNA). The northern blot analysis using flanking maize DNA sequence showed no specific RNA transcript was produced. Neither of the two potential polypeptide sequences was homologous to known allergens or toxins when evaluated using bioinformatics tools and public domain sequence databases.

The *mepsps* insert is inherited in the expected Mendelian pattern and the stability of the insert has been demonstrated through five generations of crossing and one generation of self-pollination. In addition, Roundup Ready corn event GA21 has been planted commercially in the U.S. since 1998 on over 6 million acres with no reported instability.

mEPSPS Protein Levels in Roundup Ready Corn Plants

The expression of *mepsps* gene is expected to occur throughout the corn plant because the rice actin promoter drives constitutive gene expression in corn (Zhong *et al.*, 1996). Table 1 shows the levels of resulting mEPSPS protein in both grain and forage of Roundup Ready corn using enzyme linked immunosorbent assays (ELISA). The mean level of mEPSPS protein in corn forage was 119 µg/g fresh weight, with a range of 47 to 210 µg/g fresh weight. In corn grain, the mean level of mEPSPS protein was 3 µg/g fresh weight, with a range of 1 to 5 µg/g fresh weight.

Safety Assessment of the mEPSPS Protein in Roundup Ready Corn

The safety assessment of the mEPSPS protein produced in Roundup Ready corn event GA21 included an enzymatic characterization - functional and structural similarity to ubiquitous plant EPSPSs, digestibility *in vitro*, acute oral toxicity in mice, and sequence similarity to known toxins and allergens.

mEPSPS Protein Characterization

The mEPSPS protein shows high amino acid sequence identity to the wild-type maize EPSPS enzyme (>99.3%), as well as to other EPSPS proteins found in crops that have long a history of safe human consumption (*e.g.,* soybean and tomato) or in fungal and microbial food sources such as baker's yeast. Since the mEPSPS protein is derived from corn and has only two amino acid changes (Thr102 replaced by Ile and Pro106 replaced by Ser), the safety profile of the mEPSPS protein relative to the wild-type EPSPS protein was expected to be unchanged. In addition, the mEPSPS protein is present at low levels in Roundup Ready corn event GA21. Thus, human dietary exposure to mEPSPS protein is expected to be very low.

Digestion of mEPSPS Protein in Simulated Gastric and Intestinal Fluids

The mEPSPS protein was found to be highly digestible in vitro, using both simulated gastric and simulated intestinal environments. Based on western blot analysis, the mEPSPS protein was no longer detectable after 15 seconds in the gastric system and less than one minute in the intestinal system. If the mEPSPS protein did survive the gastric system, it would be rapidly degraded in the intestine. Rapidly digested proteins represent a minimal risk of conferring novel toxicity or allergy and are comparable to other safe dietary proteins (Astwood *et al*., 1996b, Astwood and Fuchs, 2000).

Acute Oral Toxicity of mEPSPS in Mice

An acute oral toxicity study with the mEPSPS protein was performed in mice. Since proteins that are toxic act via acute mechanisms (Pariza and Foster, 1983; Jones and Maryanski, 1991; Sjoblad *et al.*, 1992), acute oral administration was considered appropriate to assess the safety of the mEPSPS protein. No statistically significant differences in body weight, cumulative body weight or food consumption were observed between the vehicle or BSA (bovine serum albumin) protein groups and the mEPSPS protein-treated groups up to a single dose of 45.6 mg/kg. In addition, gross necropsy revealed no obvious findings. Based on the highest anticipated worst-case human exposure of maize containing the mEPSPS protein, assuming all corn contains this protein, 0.02 mg mEPSPS/kg body weight/day would be consumed. The highest dose in these studies represents a level that is at least 2280 times higher than the maximum level of anticipated consumption.

Sequence Similarity of the mEPSPS Protein to Known Protein Toxins

One method to predict potential toxicity of proteins introduced into plants is to compare the amino acid sequence of the protein to known toxic proteins. Homologous proteins derived from a common ancestor are likely to share function (e.g. toxicity). Therefore, it is undesirable to introduce DNA that encodes for proteins that are homologous to toxins. Homology is determined by comparing the degree of amino acid similarity between proteins using published criteria (Doolittle, 1990). These criteria were used to assess whether the mEPSPS protein is homologous to known toxins. The mEPSPS protein does not show meaningful amino acid sequence similarity when compared to known protein toxins present in the PIR, EMBL, SwissProt and GenBank protein databases.

Sequence Similarity of mEPSPS Protein to Known Allergens

It is recognized that most food allergens are naturally occurring proteins. Although large quantities of a range of proteins are consumed in human diets each day, rarely do any of these tens of thousands of proteins elicit an allergenic response (Taylor, 1992). Although there are no predictive bioassays available to assess the allergenic potential of proteins in humans (U.S. 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 (Metcalfe *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.,* 1996a; Astwood *et al.,* 1996b; Metcalfe *et al.,* 1996). As noted above, the *in vitro* assessment of the mEPSPS protein digestibility indicates that the protein was very labile to digestion relative to many clinically important food allergens.

Another significant factor contributing to the allergenicity of certain food proteins is their high concentrations in foods (Taylor *et al.,* 1987; Taylor, 1992; Fuchs and Astwood, 1996). Most food allergens are present as major protein components in the specific food (*e.g.,* egg, milk, soybean peanut), present from as low as 1% up to 80% of total protein (Fuchs and Astwood, 1996). In contrast, the mEPSPS protein is present at extremely low levels -- approximately 0.01% of the total protein found in the grain of Roundup Ready corn.

It is also important to establish that the protein does not represent a previously described allergen, and further, does not share potentially immunologically-relevant amino acid sequences. A comparison of the characteristics of the mEPSPS protein to known allergenic proteins showed no allergenic concern. This was done by comparing the amino acid sequence of the mEPSPS protein to known allergens using a series of public domain genetic databases. No biologically significant sequence similarities were observed between the mEPSPS protein and any of the allergens described in the databases.

The low level of the mEPSPS protein in corn grain combined with the digestibility of this protein relative to that for known food allergens establishes an extremely low probability that mEPSPS protein would become an allergen when consumed in food.

In summary, the known function and ubiquity of EPSPS proteins and direct studies of the mEPSPS protein demonstrated that the mEPSPS protein does not represent a new risk in the food supply. Results showed that there were no indications of toxicity of the mEPSPS protein as measured by treatment-related adverse effects in mice administered mEPSPS protein by oral gavage. Furthermore, the mEPSPS protein and its enzymatic activity was rapidly degraded in simulated human gastric and intestinal fluids. Also, the mEPSPS protein is not homologous to known protein toxins or allergens present in public genetic databases. And finally, the mEPSPS protein is present at very low levels in Roundup Ready corn.

Compositional Analysis and Nutritional Assessment of Roundup Ready Corn

Although an ideal source of energy, relatively low levels of whole kernel or processed corn is consumed by humans worldwide when compared to corn based food ingredients (Hodge, 1982 and Watson, 1988). Corn is an excellent raw material for the manufacture of starch, not only because of price and availability, but also because the starch is easily recovered in high yield and purity (Anderson and Watson, 1982). Nearly 25% of corn starch is sold as starch products; more than 75% of the starch is converted to a variety of sweetener and fermentation products, including high fructose corn syrup and ethanol (Watson, 1988; National Corn Growers Association, 1995). Additionally, corn oil is commercially processed from the germ and accounts for approximately nine percent of domestic vegetable oil production (Orthoefer and Sinram, 1987). Each of these materials is a component of many foods, including bakery and dairy goods, beverages, confections and meat products.

Feed for animals is by far the largest use of corn in the United States, with more than half (50-60%) of annual production fed to cattle, chickens and swine (Hodge, 1982; U.S. Feed Grains Council, 1999; Watson, 1988). Corn is readily consumed by livestock and because of its high starch-low fiber content; it is one of the most concentrated sources of energy, containing more total digestible nutrients than any other feed grain.

Compositional analyses are a critical component of the safety assessment process. To assess whether the composition of Roundup Ready corn is substantially equivalent to conventional corn present in the marketplace – with the exception of the introduced trait – the grain composition was measured. In this assessment, corn grain measurements included proximate analysis (protein, fat, ash, fiber, carbohydrates, and moisture), amino acid composition, fatty acid profile, and measurement of the minerals calcium and phosphorus. In corn forage, proximates were measured, as well as calcium and phosphorus. Results of these studies are compiled in published work (Sidhu *et al*., 2000). In 1996 corn tissues for compositional analyses were produced in five locations across Illinois and Connecticut. Similarly, in 1997 tissues for compositional analyses were produced from 11 locations across the U.S., Spain and Italy. Tables 2-5 show the results of these analyses.

Proximate, Fiber and Mineral Composition

Compositional analysis results for corn grain and corn forage are presented in Tables 2 and 3, respectively. These results show that the levels of proximate components (protein, fat, ash, carbohydrate, moisture), acid detergent fiber (ADF) and neutral detergent fiber (NDF) and phosphorus in the grain and forage of Roundup Ready corn line GA21 were comparable to those in the grain and forage of the control, conventional line. In addition, these values were either within published literature ranges, within the range determined for commercial varieties evaluated in the 1997 field trials, or within the range of historical conventional control values determined from previous studies. No statistically significant differences were observed for the content of calcium in forage in data from either 1996 or 1997 field trials. Similarly, the content of calcium in the grain of corn line GA21 was not statistically significantly different from the control line in data from 1996 field trials. However, the content of calcium in the grain of corn line GA21 was statistically significantly lower (~9%) than the control line in data from 1997 field trials. This small difference is unlikely to be of biological significance as there were no statistically significant differences observed for the content of calcium in grain in data from 1996 field trials and in comparisons of corn line GA21 with commercial lines in data from 1997 field trials.

Amino Acid Composition

These results are presented in Table 4. The content of the 18 amino acids in the grain of Roundup Ready corn line GA21 were comparable to those in the grain of the control line. In addition, these values were either within published literature ranges, within the range determined for commercial varieties evaluated in 1997 field trials, or within the range of historical conventional control values determined from previous studies. The content of serine and tyrosine in corn line GA21 were 1.1% higher and 3.5% lower, respectively, than the control line in the data from 1996 field trials. However, these small differences are unlikely to be of biological significance as the levels are within literature ranges and these statistically significant differences were not observed in data from 1997 field trials.

Since the EPSPS protein catalyzes a step in the aromatic amino acid biosynthetic pathway, it was important to assess if expression of the mEPSPS protein influenced the levels of the aromatic amino acids in corn line GA21. EPSPS is not the rate-limiting step in aromatic amino acid biosynthesis (Weiss and Edwards, 1980; Herrmann, 1983). Therefore, increased EPSPS activity would not be expected to increase the levels of aromatic compounds in plants. The data from corn line GA21 grown at 16 sites over two years establish that there were no statistically significant differences in the levels of the aromatic amino acids phenylalanine and tryptophan in comparisons of corn line GA21 with the control line (Table 4). The statistically significant decrease in tyrosine levels in the grain of corn line GA21 versus the control line in data from the 1996 field trials is unlikely to be of biological significance as the level is within literature ranges and no statistically significant difference in tyrosine levels was observed in data from 1997 field trials.

Fatty Acid Composition

These results, presented in Table 5, show that the content of the fatty acids in the grain of Roundup Ready corn line GA21 were comparable to those observed in the grain of the control line. In addition, these values were either within published literature ranges, within the range determined for commercial varieties evaluated in 1997 field trials, or within the range of historical conventional control values determined from previous studies. No statistically significant differences in the levels of fatty acids between corn line GA21 and the control line were observed in either the data from the 1996 or 1997 field trials. Also, no statistically significant differences were observed in comparisons of corn line GA21 with commercial lines in the data from 1997 field trials.

Based on the compositional data presented for grain (protein, fat, ash, carbohydrates, moisture, acid detergent fiber and neutral detergent fiber, amino acids composition, fatty acids profile, calcium and phosphorus) and forage (protein, ash, acid detergent fiber, neutral detergent fiber, fat, calcium, phosphorus, carbohydrates and moisture), it was concluded that the composition of Roundup Ready corn event GA21 is substantially equivalent to that of the conventional control, as well as to corn varieties in commerce.

Nutritional Assessment in Broiler Chickens

To further confirm the nutritional equivalence of Roundup Ready corn hybrids, a broiler chicken performance study was conducted to compare the wholesomeness of Roundup Ready corn event GA21 to commercial corn varieties, including the parental line (Sidhu *et al.*, 2000). The results of this study showed no differences in growth, feed efficiency, adjusted feed efficiency and fat pad weights between birds fed corn event GA21 and the parental line. Similarly, Roundup Ready corn line GA21 did not differ from five commercial varieties tested for these same parameters. It is therefore concluded that Roundup Ready corn event GA21 is as wholesome and nutritious as the parental line and five commercial lines in its ability to support the rapid growth of broiler chickens.

The compositional analyses and poultry feeding studies taken together demonstrate that the composition and feeding value of Roundup Ready corn line GA21 is equivalent to conventional corn. Together with safety data of the introduced protein, mEPSPS, it is concluded that Roundup Ready corn is as safe and nutritious as conventional corn for food and feed use.

Environmental Assessment

Corn

Corn (*Zea mays* L.), or maize, is one of the few major crop species indigenous to the Western Hemisphere. Corn is grown in nearly all areas of the world and ranks third behind rice (*Oryza sativa* L.) and wheat (*Triticum* sp.) in total production. The origin of corn has been studied extensively, and it seems its probable domestication was in southern Mexico more than 7,000 - 10,000 years ago. Several hypotheses for the origin and parentage of corn have been advanced (Mangelsdorf, 1974).

Evidence has been reported to support the various hypotheses, but the preponderance of evidence supports the hypothesis that corn descended from teosinte (Galinat, 1988). The teosinte genome is similar to corn, teosinte easily crosses with corn, and teosinte has several plant morphological traits similar to corn. Teosinte has a more weedy appearance and more tillers than modern corn varieties. The one major distinguishing difference between corn and teosinte is the female inflorescence, or ear. Modern corn varieties have 1 to 3 lateral branches that terminate in an ear with 8 to 24 kernel rows of 50 seeds, and the ear is enclosed in modified leaves or husks. Teosinte also has lateral branches, but they terminate in two-rowed spikes of perhaps 12 fruit cases, with each fruit case having one seed enclosed by an indurated glume.

Teosinte, however, is not present in Europe and in the U.S. “Corn Belt”. The natural distribution of teosinte is limited to the seasonally dry, subtropical zone with summer rain along the western escarpment of Mexico and Guatemala and the Central Plateau of Mexico (Wilkes, 1972).

Assessment of Agronomic Performance

Based on the observation in field trials conducted in the United States at more than 150 locations from 1994-1997 and in Europe at eight locations from 1996-1998, Roundup Ready corn event GA21 did not differ significantly from conventional corn plants in seedling emergence, phenotypic characteristics (ear height, plant height, percentage of roots lodged, percentage of stalks lodged, percentage of dropped ears, stay green), growth (seedling vigor, plant height), tasseling, pollen and silk growing degree units, and yield (USDA, 1997).

In addition, Roundup Ready corn event GA21 was also monitored for its susceptibility to diseases and insects in field trials conducted in the United States over three years. There were no differences in disease severity or insect infestations between Roundup Ready corn event GA21 and the control plants (USDA, 1997).

Assessment of Weediness

Modern corn cannot survive as a weed because of the past selection in the evolution of corn for domestication. In contrast with weedy plants, the corn ear is enclosed with its husk. Consequently, seed dispersal of individual kernels does not occur naturally because of the structure of ears of corn. However, even if individual kernels of corn were distributed in the fields and main avenues of travel from the fields to storage facilities, volunteer corn is not found growing in fence rows, ditches, or roadsides as a weed. Corn cannot survive without human assistance and is not capable of surviving as a weed (Galinat, 1988). Further, although corn seed can overwinter into a crop rotation of soybeans, mechanical and chemical measures can control volunteers. Even in the case of a rotation involving Roundup Ready soybeans, it should be considered that a) no-till cultivation, which is used on 35% of the soybean acreage, dramatically reduces the occurrence of volunteer corn and b) control of Roundup Ready corn volunteers is possible with an application of grass-killer herbicides such as fluazifop-p, clethodim, quizalofop-ethyl, sethoxydim, all of which are currently used in soybean crops for volunteer corn control.

Assessment of Horizontal Gene Transfer

Gene exchange between cultivated corn and genetically modified corn is similar to what occurs naturally. Wind-blown pollen moves about among plants within the same field and among plants in nearby fields. Free flow of genes would occur similarly to what occurs in cultivated corn. The production of the mEPSPS protein in resulting seed would not be of concern since the protein is closely related (more than 99.3% identical) to the endogenous corn EPSPS. EPSPS is an enzyme of the shikimate pathway for aromatic amino acid biosynthesis in plants (including corn) and microorganisms. It is ordinarily present in plants and in food derived from plant sources (Levin and Sprinson, 1964).

Based on limited DNA homologies, transfer of DNA from plants to microorganisms may have occurred in evolutionary time over many millennia. Even if such transfer were to take place, transfer of an EPSPS gene to a microbe would not pose any plant pest or environmental risk. Based on these considerations, transfer to microbes or other living species in nature is extremely unlikely and in terms of plant pests or the environment is of no significant consequence.

Potential Impact on Non-target Organisms

On the basis of the characterization of the introduced protein and the compositional analyses, no specific interactions of the mEPSPS protein with non-target organisms are expected beyond those that occur with other corn varieties. Extensive observations in the field have also confirmed that there are no differences between Roundup Ready corn event GA21 and conventional plants in their agronomic traits, susceptibility to diseases and insects, and yield (USDA, 1997). This indicates that there are no alterations in the interactions with predatory and beneficial non-target organisms.

The Roundup Ready phenotype of corn event GA21 would not extend the use of corn beyond areas where corn is typically grown and the potential impact on biodiversity by Roundup Ready corn event GA21 is equivalent to that of existing commercial corn varieties.

Assessment of Resistance to Glyphosate

Today, some 109 herbicide-resistant weed biotypes have been identified; over half of them are resistant to the triazine family of herbicides (Holt and Le Baron, 1990; Le Baron, 1991; 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 year-long, and frequent applications of the same herbicide without rotation to 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, other increases in glyphosate use 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 in corn to glyphosate will not occur, the development of weed resistance to glyphosate is expected to be a very rare event because:

1. 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; and
3. selection for glyphosate resistance using whole plant and cell/tissue culture techniques was unsuccessful, and as a result would be expected to occur rarely in nature under normal field conditions (Padgette *et al.*, 1996).

Summary

Weed control in corn is essential to protect against yield losses and to maintain grain and forage quality. In developed countries, this weed control is predominately achieved by chemical methods. This development of Roundup Ready corn enables the farmer to utilize Roundup agricultural herbicides for effective control of weeds during the corn-growing season and to take advantage of the herbicide's favorable environmental and safety characteristics. This in turn provides environmental benefits as well as significant value to the corn grower.

Extensive compositional analyses of corn seed and forage establish that Roundup Ready corn is substantially similar to other commercial varieties of corn that are currently available, except for the tolerance to glyphosate. The additional mEPSPS protein is present in Roundup Ready corn event GA21 at very low levels and it shares more than 99.3% homology with the wild-type corn EPSPS protein already found in foods derived from corn. The mEPSPS protein is rapidly degraded in simulated digestive fluids and lacks properties common to known allergenic and

toxic proteins. Data demonstrate that Roundup Ready corn event GA21 is not likely to pose a greater plant pest risk than conventional corn. This conclusion is based on evaluation of phenotypic characteristics, homology of the expressed protein to endogenous corn EPSPS protein and the lack of any deleterious environmental fate or effects.

The inserted genes in Roundup Ready corn are stable and the line is phenotypically and genetically stable over generations and across varied environments. Field trials established across a broad geographic range of environments have shown no phenotypic differences, except for the expected tolerance to glyphosate. This indicates that corn event GA21 and its progeny will not have impacts any different from corn varieties developed through traditional breeding methods. Such an outcome is expected, based upon the ubiquitous nature of EPSPS proteins in the environment and the safety of the mEPSPS protein.

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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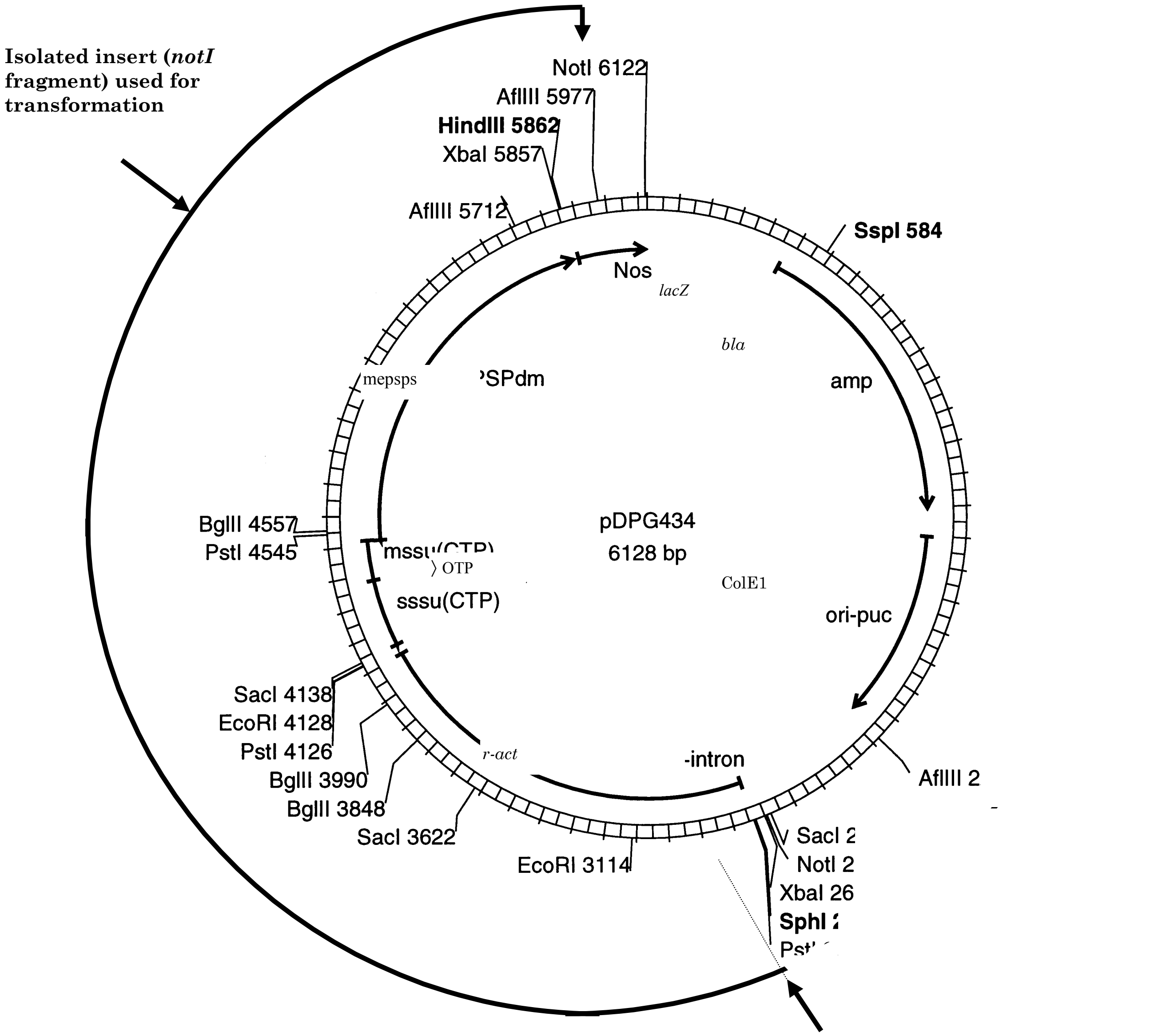

Figure 1. Plasmid Map of pDPG434

Table 1. Summary of mEPSPS[1] Protein Levels Measured by the ELISA in Tissues of Roundup Ready Corn Event GA21 Plants (µg/g fresh weight)

	mean[2]	range[3]
Forage[4]	119	47-210
Grain[4]	3.2	1.4-4.9

[1] Values represent the sum of the endogenous and modified corn EPSPS protein expression levels.
[2] The mean was calculated from the analyses of plant samples, one from each of five sites.
[3] Minimum and maximum values from the analyses of samples across sites.
[4] The quantitation limit of the EPSPS ELISA assay was ca. 0.003 µg/g.

Table 2. Fiber, Mineral and Proximate Composition of Grain from Roundup Ready Corn Line GA21

Component[c]	1996[a] GA21 Mean (Range)[h]	1996[a] Control[d] Mean (Range)[h]	1997[b] GA21 Mean (Range)[h]	1997[b] Control[e] Mean (Range)[h]	1997[b] Comm. Lines Mean (Range)[h]	Literature (Range)[h]	Historical[g] (Range)[h]
Protein	10.05 (9.39-11.00)	10.05 (9.17-11.19)	11.05 (9.48-14.06)	10.54 (9.70-12.92)	10.87 (7.8-14.20)	(6.0-12.0)[k] (9.7-16.1)[l]	(9.0-13.6)
Total fat	3.51 (2.94-3.72)	3.55 (2.76-3.93)	3.90 (3.04-4.63)	3.98 (3.30-4.81)	3.69 (2.48-4.81)	(3.1-5.7)[k] (2.9-6.1)[l]	(2.4-4.2)
Ash	1.27 (1.06-1.45)	1.27 (1.21-1.40)	1.38 (1.06-1.80)	1.56 (1.07-3.09)	1.79 (0.89-6.28)	(1.1-3.9)[k]	(1.2-1.8)
ADF[i]	3.73 (3.35-3.99)	3.72 (3.52-4.05)	6.35 (2.73-9.47)	6.35 (3.00-9.33)	6.06 (2.75-11.34)	(3.3-4.3)[k]	(3.1-5.3)
NDF[i]	10.82 (10.06-11.88)	11.70 (9.40-13.58)	9.33 (7.51-11.57)	9.8 (8.03-11.58)	10.12 (7.58-15.91)	(8.3-11.9)[k]	(9.6-15.3)
Carbohydrates	85.15 (84.00-86.11)	85.15 (83.71-86.14)	83.66 (80.57-84.97)	83.79 (81.69-85.26)	83.68 (77.41-87.16)	Not reported in this form	(81.7-86.3)
Calcium	0.0026 (0.0020-0.0031)	0.0027 (0.0024-0.0033)	0.0039[j] (0.0027-0.0056)	0.0043 (0.0033-0.0058)	0.0040 (0.0022-0.0208)	(0.01-0.1)[k]	(0.0029-0.006)
Phosphorus	0.299 (0.28-0.32)	0.299 (0.28-0.31)	0.326 (0.303-0.350)	0.326 (0.292-0.349)	0.330 (0.208-0.411)	(0.26-0.75)[k]	(0.288-0.363)
Moisture	14.15 (7.44-22.60)	14.40 (7.24-23.00)	16.86 (9.57-23.10)	16.21 (8.67-24.70)	16.30 (8.18-26.20)	(7-23)[k]	(9.4-15.8)

[a] Data from five U.S. sites; GA21 grain harvested from plants not treated with a glyphosate herbicide.
[b] Combined data from four non-replicated E.U. sites, six U.S. non-replicated sites and one U.S. replicated site; GA21 grain harvested from plants treated with Roundup Ultra®.
[c] Percent dry weight of sample, except for moisture.
[d] Non-transgenic negative segregant.
[e] Parental control line.
[f] Commercial lines; local hybrids planted at each site.
[g] Range for control lines planted in Monsanto Company field trials conducted between 1993-5.
[h] Range denotes the lowest and highest individual value across sites for each line.
[i] ADF = acid detergent fiber; NDF = neutral detergent fiber.
[j] Statistically significantly different from the control at the 5% level ($p<0.05$).
[k] Watson, 1987.
[l] Jugenheimer, 1976.

Table 3. Fiber, Mineral and Proximate Composition of Forage from Roundup Ready Corn Line GA21

Component[c]	1996[a] GA21 Mean (Range)[h]	1996[a] Control[d] Mean (Range)[h]	1997[b] GA21 Mean (Range)[h]	1997[b] Control[e] Mean (Range)[h]	1997[b] Comm. Lines[f] Mean (Range)[h]	Historical[g] (Range)
Protein	7.91 (5.70-10.37)	7.58 (6.11-8.61)	7.49 (6.40-8.67)	7.45 (5.88-8.76)	7.20 (5.11-10.27)	(4.8-8.4)
Ash	4.22 (3.20-4.67)	3.85 (2.64-5.28)	4.29 (2.12-5.29)	4.26 (2.94-5.91)	4.19 (2.00-6.60)	(2.9-5.1)
ADF[i]	25.04 (23.06-27.96)	25.89 (22.72-28.62)	23.85 (20.08-30.21)	25.55 (21.13-34.20)	25.56 (18.32-40.99)	(21.4-29.2)
NDF[i]	39.47 (35.94-44.48)	40.85 (36.97-44.31)	37.91 (31.47-46.29)	38.92 (33.99-49.28)	39.54 (26.37-54.45)	(39.9-46.6)
Total Fat	1.73 (1.27-2.30)	1.50 (1.24-1.93)	1.88 (0.71-2.98)	2.21 (1.16-3.22)	2.04 (0.35-3.62)	(1.4-2.1)
Carbohydrates	86.14 (82.94-89.57)	87.04 (84.83-89.88)	86.35 (85.06-89.96)	86.06 (83.58-87.85)	86.62 (83.16-91.55)	(84.6-89.1)
Calcium	0.1934 (0.0965-0.2488)	0.1766 (0.0866-0.2172)	0.2304 (0.1420-0.3173)	0.2177 (0.1515-0.2754)	0.1948 (0.0969-0.3184)	(not available)
Phosphorus	0. 2288 (0.1822-0.2622)	0.2124 (0.2016-0.2365)	0.2178 (0.1419-0.3475)	0.2179 (0.1602-0.2914)	0.1992 (0.1367-0.2914)	(not available)
Moisture	72.30 (69.5-77.0)	65.52 (42.0-75.3)	68.83 (62.20-74.10)	68.73 (64.60-73.80)	68.31 (55.30-75.30)	(68.7-73.5)

[a] Data from five U.S. sites; GA21 forage harvested from plants not treated with a glyphosate herbicide.
[b] Combined data from four non-replicated E.U. sites, six U.S. non-replicated sites and one U.S. replicated site; GA21 forage harvested from plants treated with Roundup Ultra®.
[c] Percent dry weight of sample, except for moisture.
[d] Non-transgenic negative segregant.
[e] Parental control line.
[f] Commercial lines; local hybrids grown at each site.
[g] Range for control lines planted in Monsanto Company field trials conducted between 1993-5.
[h] Range denotes the lowest and highest individual value across sites for each line.
[i] ADF = acid detergent fiber; NDF = neutral detergent fiber.

Table 4. Amino Acid Composition of Corn Grain from Roundup Ready Corn Line GA21

Amino acid[a]	1996[b]		1997[c]				
	GA21 Mean (Range)[i]	Control[d] Mean (Range)[i]	GA21 Mean (Range)[i]	Control[e] Mean (Range)[i]	Comm. Lines[f] Mean (Range)[i]	Literature[g] (Range)[i]	Historical[h] (Range)[i]
Alanine	7.62 (7.34-7.81)	7.64 (7.45-7.84)	7.64 (7.49-7.86)	7.62 (7.50-7.97)	7.78 (7.44-8.98)	(6.4-9.9)	(7.2-8.8)
Arginine	4.13 (3.72-4.34)	4.30 (4.05-4.51)	4.48 (3.74-4.93)	4.51 (4.11-4.90)	4.36 (3.67-5.34)	(2.9-5.9)	(3.5-5.0)
Aspartic Acid	6.71 (6.46-6.87)	6.78 (6.35-6.83)	6.63 (6.17-7.05)	6.65 (6.22-7.08)	6.57 (6.14-7.35)	(5.8-7.2)	(6.3-7.5)
Cystine	2.10 (1.85-2.36)	2.11 (1.91-2.24)	2.22 (1.73-2.49)	2.28 (2.06-2.57)	2.19 (1.63-2.62)	(1.2-1.6)	(1.8-2.7)
Glutamic Acid	19.27 (18.70-19.71)	19.06 (18.61-19.64)	18.78 (18.12-19.45)	18.70 (18.04-19.43)	19.17 (17.83-20.53)	(12.4-19.6)	(18.6-22.8)
Glycine	3.72 (3.44-3.95)	3.78 (3.48-3.96)	3.83 (3.44-4.27)	3.89 (3.52-4.14)	3.71 (3.05-4.29)	(2.6-4.7)	(3.2-4.2)
Histidine	2.81 (2.72-2.99)	2.84 (2.75-2.93)	2.67 (2.36-2.87)	2.74 (2.46-2.86)	2.80 (2.36-3.20)	(2.0-2.8)	(2.8-3.4)
Isoleucine	3.60 (3.48-3.66)	3.58 (3.44-3.70)	3.53 (3.06-3.85)	3.57 (3.13-3.92)	3.75 (3.13-4.14)	(2.6-4.0)	(3.2-4.3)
Leucine	13.11 (12.32-13.71)	12.90 (12.37-13.49)	12.98 (12.33-13.96)	12.87 (12.26-13.69)	13.32 (11.99-15.19)	(7.8-15.2)	(12.0-15.8)
Lysine	3.02 (2.68-3.30)	3.09 (2.69-3.27)	3.11 (2.59-4.04)	3.02 (2.66-3.33)	2.96 (2.20-3.50)	(2.0-3.8)	(2.6-3.5)
Methionine	1.98 (1.78-2.24)	2.03 (1.85-2.28)	2.16 (1.80-2.34)	2.17 (1.67-2.44)	2.02 (1.53-2.44)	(1.0-2.1)	(1.3-2.6)

Phenylalanine	5.15 (4.88-5.31)	5.17 (4.98-5.30)	5.31 (5.03-5.63)	5.33 (4.96-5.76)	5.36 (4.88-6.10)	(2.9-5.7)	(4.9-6.1)
Proline	8.69 (8.41-8.92)	8.69 (8.49-9.10)	8.98 (8.22-9.38)	9.00 (8.62-9.23)	9.16 (8.08-9.94)	(6.6-10.3)	(8.7-10.1)
Serine	5.33[j] (5.25-5.49)	5.27 (5.17-5.43)	5.17 (4.43-5.60)	5.03 (3.82-5.63)	4.64 (2.87-5.63)	(4.2-5.5)	(4.9-6.0)
Threonine	3.77 (3.64-3.88)	3.73 (3.58-3.85)	3.59 (3.33-3.74)	3.54 (3.08-3.71)	3.43 (2.61-3.89)	(2.9-3.9)	(3.3-4.2)
Tryptophan	0.62 (0.55-0.66)	0.57 (0.53-0.61)	0.61 (0.52-0.75)	0.61 (0.43-1.04)	0.59 (0.41-1.04)	(0.5-1.2)	(0.4-1.0)
Tyrosine	3.81[j] (3.68-3.99)	3.95 (3.88-4.10)	3.73 (3.06-4.20)	3.77 (2.78-4.32)	3.48 (2.37-4.32)	(2.9-4.7)	(3.7-4.3)
Valine	4.58 (4.40-4.74)	4.64 (4.45-4.73)	4.57 (4.15-5.18)	4.62 (4.00-5.00)	4.79 (3.93-5.40)	(2.1-5.2)	(4.2-5.3)

[a] Values expressed as percent of total amino acids for statistical comparisons. These values are slightly higher when expressed as percent of total protein, e.g., alanine = 7.8% for GA21 (1996).
[b] Data from five U.S. sites; GA21 grain harvested from plants not treated with a glyphosate herbicide.
[c] Combined data from four non-replicated E.U. sites, six U.S. non-replicated sites and one U.S. replicated site; GA21 grain harvested from plants treated with Roundup Ultra®.
[d] Non-transgenic negative segregant.
[e] Parental control line.
[f] Commercial lines; local hybrids planted at each site.
[g] Watson, 1982. Values are percent of total protein [10.1% total protein (N x 6.25)].
[h] Range for control lines planted in Monsanto Company field trials conducted between 1993-5; values are percent of total protein.
[i] Range denotes the lowest and highest individual values across sites.
[j] Value statistically significantly different than the control at the 5% level ($p<0.05$).

Table 5. Fatty Acid Composition of Corn Grain from Roundup Ready Corn Line GA21

Fatty acid[a]	1996[b]		1997[c]				
	GA21 Mean (Range)[i]	Control[d] Mean (Range)[i]	GA21 Mean (Range)[i]	Control[e] Mean (Range)[i]	Comm. Lines[f] Mean (Range)[i]	Literature[g] (Range)[i]	Historical[h] (Range)[i]
Arachidic (20:0)	0.40 (0.36-0.48)	0.41 (0.39-0.46)	0.37 (0.32-0.44)	0.36 (0.33-0.41)	0.40 (0.31-0.57)	(0.1-2)	(0.3-0.5)
Behenic (22:0)	0.16 (0.14-0.18)	0.17 (0.16-0.18)	0.16 (0.12-0.24)	0.15 (0.13-0.16)	0.18 (0.13-0.24)	(not reported)	(0.1-0.3)
Eicosenoic (20:1)	0.28 (0.27-0.31)	0.29 (0.28-0.30)	0.30 (0.28-0.34)	0.30 (0.28-0.36)	0.30 (0.19-0.45)	(not reported)	(0.2-0.3)
Linoleic (18:2)	58.56 (54.20-64.70)	58.72 (53.40-65.60)	61.40 (58.2-63.4)	61.51 (59.7-63.0)	59.18 (46.9-64.3)	(35-70)	(55.9-66.1)
Linolenic (18:3)	1.10 (1.07-1.13)	1.08 (0.98-1.16)	1.14 (0.92-1.24)	1.14 (1.04-1.20)	1.11 (0.77-1.55)	(0.8-2)	(0.8-1.1)
Oleic (18:1)	27.50 (22.10-31.30)	27.40 (21.40-32.40)	24.2 (22.4-26.0)	24.1 (22.9-26.0)	26.2 (21.3-39.2)	(20-46)	(20.6-27.5)
Palmitic (16:0)	9.94 (9.59-10.40)	9.92 (9.60-10.40)	10.70 (10.30-11.40)	10.72 (10.40-11.40)	10.58 (8.75-13.30)	(7-19)	(9.9-12.0)
Stearic (18:0)	1.87 (1.52-2.11)	1.86 (1.46-2.11)	1.68 (1.44-2.04)	1.67 (1.59-1.86)	1.88 (1.36-2.65)	(1-3)	(1.4-2.2)

[a] Value of fatty acids expressed as % of total fatty acid. The method included the analysis of the following fatty acids which were not detected in the majority of samples analyzed: caprylic acid (8:0), capric acid (10:0), lauric acid (12:0), myristic acid (14:0), myristoleic acid (14:1), pentadecanoic acid (15:0), pentadecenoic acid (15:1), heptadecanoic acid (17:0), heptadecenoic acid (17:1), gamma linolenic (18:3), eicosadienoic acid (20:2), eicosatrienoic acid (20:3), and arachidonic acid (20:4). Palmitoleic acid (16:1) was observed at levels of ~0.17% of total fatty acids in grain samples collected in 1996 but was not detected in the majority of grain samples collected in 1997.
[b] Data from five U.S. sites; GA21 grain harvested from plants not treated with glyphosate herbicide.
[c] Combined data from four non-replicated E.U. sites, six U.S. non-replicated sites and one U.S. replicated site; GA21 grain harvested from plants treated with Roundup Ultra®.
[d] Non-transgenic negative segregant.
[e] Parental control line.
[f] Commercial lines; local hybrids planted at each site.
[g] Watson, 1982. Values expressed as % of total fat except for palmitic acid (16:1) which is expressed as % of triglyceride fatty acids.
[h] Range for control lines planted in Monsanto Company field trials conducted between 1993-5; values are expressed as % of total fatty acids.
[i] Range denotes the lowest and highest individual values across sites.