U.S. Food and Drug Administration
Department of Health and Human Services
CENTER FOR FOOD SAFETY AND APPLIED NUTRITION

CFSAN/Office of Food Additive Safety
July 22, 2004

Biotechnology Consultation Note to the File BNF No. 000080

Date: July 22, 2004

Subject: Monsanto Roundup Ready® Wheat Event MON 71800

Keywords: Wheat, Roundup Ready®, *Triticum aestivum*, Glyphosate (N-phosphonomethyl-glycine), EPSPS (5-enolpyruvylshikimate-3-phosphate synthase), *cp4 epsps* gene, *Agrobacterium* sp. CP4 strain, Herbicide-Tolerant, Glyphosate-Tolerant, event MON 71800, Bobwhite

1. Introduction

In a submission dated June 28, 2002, Monsanto provided information to support the safety and nutritional assessment of their glyphosate-tolerant (Roundup Ready®) wheat (*Triticum aestivum*) containing a transformation event designated MON 71800. The company provided additional information in a submission dated April 25, 2003. Monsanto concluded that their Roundup Ready® wheat event MON 71800 and the foods and feed derived from it are as safe and nutritious as current commercial varieties of wheat and the comparable foods and feed derived from them. Monsanto has previously completed consultations for other Roundup Ready® crops which are also tolerant to glyphosate. These other crops include bioengineered soybean, canola, corn, and cotton (BNFs 01, 20, 71, and 86, respectively). In a letter to FDA dated June 9, 2004, Monsanto states that the firm is deferring all further commercial development efforts to introduce Roundup Ready® wheat until such time that other wheat biotechnology traits are introduced. In its letter, Monsanto requests that FDA complete the consultation process for Roundup Ready® wheat event MON 71800.

2. Intended Effect

The intended effect of the genetic modification is to confer tolerance to the herbicidal compound glyphosate (N-phosphonomethyl-glycine) which is the active ingredient in Roundup® agricultural herbicides. In glyphosate-sensitive plants, glyphosate binds to the plant 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) and prevents the synthesis of aromatic amino acids that are necessary for plant growth. Roundup Ready® wheat MON 71800 contains

the *epsps* gene from *Agrobacterium* sp. strain CP4 (*cp4 epsps* gene) encoding the CP4 EPSPS enzyme. This enzyme has a reduced affinity for glyphosate when compared to the native plant EPSPS enzyme. As a result, wheat plants expressing the CP4 EPSPS enzyme are tolerant to glyphosate and survive spraying with Roundup®

3. Development of Roundup Ready® Wheat Event MON 71800

3.1. The Parent Plant Wheat

Monsanto describes the history and biology of wheat. The scientific name for common bread wheat is *Triticum aestivum* L. Wheat belongs to the order Poales (*Glumiflorae*), family *Poaceae* (*Graminae*), tribe *Triticeae*, genus *Triticum*. *T. aestivum* is hexaploid with a total of 42 chromosomes. Modern wheat cultivars are either tetraploid (durum) or hexaploid (common and club types). Wheat is predominantly self-pollinating.

3.2. Genetic Modifications and Characterization of the Introduced DNA

Monsanto used Bobwhite spring wheat (designated as MON 71900) as the parental variety to produce Roundup Ready® wheat event MON 71800. Event MON 71800 was developed through *Agrobacterium*-mediated transformation using the double border, binary vector PV-TXGT10. The vector consists of the T-DNA segment intended for transformation, as well as the backbone DNA which is not expected to be incorporated into the transformed wheat.

The T-DNA contains two *cp4 epsps* gene cassettes and the left and right T-DNA border sequences. The segment is approximately 7.0 Kb in size. The *cp4 epsps* coding sequence in both cassettes is derived from *Agrobacterium* sp. strain CP4 and begins with a leader chloroplast transit peptide coding sequence (*Arab*TP) derived from the *Arabidopsis thaliana epsps* gene. The ArabTP transit peptide directs the CP4 EPSPS protein expressed in event MON 71800 to chloroplasts. The *Arab*TP-*cp4 epsps* fusion coding sequence in each cassette is followed by the 3' non-translated region of the nopaline synthase gene (*nos 3'*) from *Agrobacterium tumefaciens* that provides the transcriptional termination signal. The upstream regulatory sequence of the first cassette consists of the 5' region of the rice *actin1* gene (P-*ract*1-*ract*1I) which contains the promoter, transcription start site and the first intron. The upstream regulatory sequence of the second cassette consists of an enhanced 35S promoter from the cauliflower mosaic virus (CMV) (P-e35S) and the intron of the corn heat shock protein gene (*hsp*70I). The two different promoters were used to drive expression of the gene product in both vegetative and reproductive tissues.

The vector backbone contains the origin of DNA replication *ori-V* that allows maintenance of PV-TXGT10 in *Agrobacterium* as well as the origin of replication *ori-322/rop* that allows the replication of PV-TXGT10 in the intermediate host *E. coli*. The vector backbone also contains the *aad* gene encoding the selectable marker enzyme streptomycin adenyltransferase that allows selection of bacteria containing PV-TXGT10.

Monsanto used Southern blot analysis to characterize the DNA introduced in the transformation event MON 71800. Genomic DNA isolated from event MON 71800 and the parent line MON 71900 was digested with restriction enzymes and subjected to Southern blot analysis using radiolabeled DNA probes corresponding to different segments of the T-DNA and plasmid backbone. Based on this analysis, Monsanto concludes that: 1) event MON 71800 contains a

single T-DNA insert of the expected size (approximately 7.0 Kb) comprising one intact copy of each *cp4 epsps* expression cassette; 2) event MON 71800 does not contain the plasmid backbone sequences, including *ori-V*, *ori-322/rop*, and *aad* coding sequence; and 3) all genetic elements present in the expression cassettes before transformation are also present in event MON 71800.

Monsanto performed polymerase chain reaction (PCR) on event MON 71800 genomic DNA to verify the presence of the unique T-DNA insert-to-plant junction sequences. This analysis yielded PCR products of the expected size, thereby confirming the presence of these unique sequences in event MON 71800.

3.3. Stability of the Introduced DNA

Monsanto describes the experiments it conducted to evaluate the stability of the DNA insert. Monsanto used Southern blot analysis to test the insert stability across several generations of wheat plants containing event MON 71800. The tested plants included R2, R3, R4, and R5 progeny derived from the initial MON 71800 transformant by self-fertilization and three pre-commercial wheat varieties (Westbred 926 BC4F1, HJ-98 BC4F1, and BW251 BC5F4) containing event MON 71800 introduced by traditional breeding. These seed materials represent the 2nd, 3rd, 4th, 5th, 8th, 8th, and 12th generations, respectively. The non-transgenic wheat lines MON 71900, Westbred 926, HJ-98, and BW251 were used as control lines. Genomic DNA isolated from the seeds of all of these lines was digested with the restriction enzyme *Bam* HI and probed with the *cp4 epsps* coding sequence. The DNA from the non-transgenic control lines did not contain detectable hybridization bands. The digested transformation plasmid used as a positive control produced two bands of the expected length (3.7 and 8.6 Kb). The DNA from the test lines containing event MON 71800 produced two DNA bands - the expected 3.7 Kb band (which represents the internal segment of the insert) and a 9.0 Kb fragment (which represents a border segment containing the 3' end of the insert and a portion of the flanking wheat DNA sequence). Monsanto concludes that these experiments demonstrate the stability of the inserted DNA spanning six different seed generations.

3.4 Inheritance of the Roundup Ready® Trait

Monsanto tested the inheritance of the Roundup Ready® trait as follows: the original event MON 71800 R0 plant was selected on a glyphosate medium. Initial R1 plants were sprayed with Roundup® herbicide and evaluated for their resistance and sensitivity. Homozygous glyphosate-resistant R1 plants were identified by a PCR-based homozygosity assay and confirmed by spray testing their R2 progeny with Roundup® herbicide. One of the homozygous glyphosate-resistant plants from the R1 generation was used to increase the number of generations to R18 by self-pollination. These generations showed no decrease in vegetative or reproductive tolerance and no sensitivity to Roundup® confirming the stability of the Roundup Ready®trait.

Monsanto also described a Mendelian segregation study for the Roundup Ready® trait in event MON 71800. Monsanto selected heterozygous R1 plants and self pollinated them to produce progeny. The R2 plants were sprayed with Roundup® herbicide and scored for their resistance or sensitivity to the herbicide. The ratio of resistant to sensitive plants was found to be in conformance with the expected 3:1 ratio by the chi square test. Monsanto concludes that this result is consistent with a single insertion site of the Roundup Ready® trait in the wheat

genome.

4. Expressed Proteins: the CP4 EPSPS Enzymes

4.1. Identity, Function and Characterization

Monsanto notes that the CP4 EPSPS enzyme is structurally and functionally similar to native plant EPSPS enzymes, which are involved in the biosynthesis of aromatic amino acids that are necessary for growth and development of the plant.

In order for EPSPS to function in plants, it must be transported to the chloroplast. In event MON 71800, the *A. thaliana* chloroplast transit peptide coding sequence was joined to the *cp4 epsps* coding sequence so that a fusion protein of CP4 EPSPS (47.6 kilodaltons (kDa), 455 amino acids) and the *A. thaliana* chloroplast transit peptide (1.6 kDa, 76 amino acids) would be expressed. The transit peptide directs the protein to the chloroplast. Typically, transit peptides are completely cleaved from the protein following delivery to the chloroplast. However, there are examples in the literature of alternatively processed forms where the transit peptide is only partially cleaved.

Monsanto isolated and purified CP4 EPSPS expressed in the grain of event MON 71800 and used the following techniques for characterization: sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE); western blot analysis; glycosylation analysis; enzymatic assay; N-terminal amino acid sequence analysis; and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry analysis. Monsanto identified two forms of the CP4 EPSPS protein in the grain of event MON 71800, i.e., a "mature form" and an "alternatively processed form". The mature form is the full length CP4 EPSPS, where the transit peptide was fully cleaved; the alternatively processed form is the full length CP4 EPSPS protein plus seven amino acids from the transit peptide. Monsanto used the mature form of CP4 EPSPS produced in *E. coli* for comparison in the characterization analyses.

Based on quantitative image analysis of western blots, Monsanto reports that the relative percentages of the CP4 EPSPS proteins produced in event MON 71800 are 80% mature form and 20% alternatively processed form. Glycosylation analysis (ECL glycoprotein detection system) indicated that neither form of the protein is glycosylated. In addition, Monsanto concludes from enzyme activity assays that the average specific activity of both plant-produced CP4 EPSPS proteins is comparable to the specific activity of the protein produced in *E. coli.*

4.2. Expression Level and Human Exposure

Monsanto used a direct double antibody sandwich enzyme-linked immunosorbent assay (ELISA) analysis to estimate the levels of the CP4 EPSPS proteins in forage and grain tissues collected from event MON 71800. The forage and grain tissues from the parental line MON 71900 were used as control samples. Monsanto provides the results of the assay which show that the average level of CP4 EPSPS proteins are 106 μg/g in forage and 13 μg/g in grain on a fresh weight basis. Monsanto estimates a per capita exposure to the CP4 EPSPS proteins to be 0.039 mg/kg body weight/day assuming no loss due to food processing.

4.3. Presence in Food Crops

Monsanto discusses the similarity of the CP4 EPSPS proteins present in event MON 71800 to native EPSPS proteins that occur in plants and microorganisms and to CP4 EPSPS proteins present in bioengineered crops. Monsanto states that the CP4 EPSPS proteins expressed in event MON 71800 are similar to naturally occurring EPSPS proteins present in a variety of food and feed sources, such as, soybean, corn, and Baker's yeast. Monsanto also states that the mature CP4 EPSPS protein expressed in event MON 71800 grain has been consumed by humans and animals since 1996, through the consumption of Roundup Ready® crops, such as soybean, corn and canola. The mature protein is identical to or shares greater than 99% amino acid sequence identity with the CP4 EPSPS proteins produced in these food crops. The alternatively processed form of CP4 EPSPS shares greater than 98% amino acid sequence identity with the CP4 EPSPS protein produced in other Roundup Ready® food crops.

4.4. Assessment of Potential Allergenicity

4.4.1. Donor

Agrobacterium species, the source of the CP4 EPSPS gene, are not known to be allergenic.

4.4.2. Amino Acid Sequence Homology

Monsanto searched the ALLPEPTIDES database for amino acid sequence homology to the CP4 EPSPS proteins found in event MON 71800 using the FASTA algorithm. The ALLPEPTIDES database is comprised of publically available protein sequences in SwissProt version 39+, TrEMBL (updated weekly), and GenPept version 124. Monsanto concluded that the CP4 EPSPS proteins do not demonstrate sequence similarity to proteins known to pose human health risks.

In addition, Monsanto screened all overlapping peptides of eight or more contiguous amino acids of the CP4 EPSPS proteins against the ALLERGEN3 database using a pairwise comparison algorithm. ALLERGEN3 is an allergen and gliadin protein sequence database compiled by Monsanto. Neither of the two forms of CP4 EPSPS contained sequences of eight or more contiguous amino acids identical to those from proteins in the allergen database.

4.4.3. Stability in Simulated Digestive Fluid

Monsanto examined the *in vitro* stability of both forms of CP4 EPSPS in simulated gastric fluids (SGF) prepared according to U.S. Pharmacopeia (1990). Stability was assessed by colloidal blue staining of SDS-PAGE gels, western blot analysis, and EPSPS enzyme activity assay.

In experiments performed with the purified *E. coli*-produced mature form of CP4 EPSPS, 95-98% of the protein was digested in SGF within 15 seconds, with no detectable degradation products present. The enzymatic activity of this form of CP4 EPSPS decreased by greater than 90% following SGF treatment for 15 seconds. Monsanto also refers to previous experiments which show that purified *E. coli*-produced mature form of CP4 EPSPS was digested in less than ten minutes in simulated intestinal fluid (SIF) (Harrison *et al*., 1996).

In experiments performed with the purified *E. coli*-produced alternatively processed form of CP4 EPSPS, 98% of the protein was digested in SGF within 15 seconds. The enzymatic activity

of this form of CP4 EPSPS was lost following SGF treatment for 15 seconds. In addition, purified *E. coli*-produced alternatively processed form of CP4 EPSPS was degraded within four to eight hours of SIF treatment.

Monsanto performed additional experiments examining the *in vitro* stability of the CP4 EPSPS proteins produced in event MON 71800 grain in SGF. These experiments were performed to assess the stability of the CP4 EPSPS proteins within the matrix of other wheat proteins. The results indicated that these proteins were rapidly digested within a matrix of wheat grain proteins, with greater than 95% of CP4 EPSPS digested within 15 seconds.

Finally, Monsanto calculated that the amount of CP4 EPSPS proteins present in event MON 71800 grain is very low and represents only a small portion of the total protein. From these data and information, Monsanto concluded that the CP4 EPSPS proteins in event MON 71800 do not pose a significant allergenic risk.

4.5. Assessment of Potential Toxicity

Monsanto provided information about *Agrobacterium* sp. strain CP4, the donor of the *cp4 epsps* gene. *Agrobacterium* species are non-pathogenic and non-toxigenic. The safety of the *Agrobacterium* sp. strain CP4 has been previously evaluated during Monsanto's consultations with FDA on other Roundup Ready&174; crops.

In two separate studies, Monsanto assessed the acute oral toxicity of the CP4 EPSPS proteins in mice. They used both the mature and alternatively processed CP4 EPSPS proteins, each expressed in *E. coli*. Each protein was administered by oral gavage. The highest doses tested were 572 mg/kg body weight for the mature protein and 1028 mg/kg body weight for the alternatively processed protein. Monsanto reports that no acute toxicity resulted from the oral administration of either protein in male or female mice at any of the doses administered in the experiments.

Monsanto compared the amino acid sequences of the CP4 EPSPS proteins expressed in the grain of event MON 71800 to the amino acid sequences of proteins (including toxins) available in the database ALLPEPTIDES using FASTA algorithm. Monsanto concluded that its search showed no relevant similarities between the CP4 EPSPS proteins present in event MON 71800 and proteins that are known to cause adverse health effects in humans or animals.

5. Food and Feed Uses of Wheat

Monsanto describes historical and current uses of wheat in food and animal feed. Wheat has been cultivated for use in food for several millennia. In the U.S., wheat is one of the most abundant crops in terms of planted acreage. Wheat grain contains approximately 60% carbohydrate, 10-16% protein, 2% fat, and 13% water. It is mainly used for the production of flour which is used in baked goods or other flour-based foods. U.S. consumers currently consume approximately 143 pounds of wheat flour per capita per year.

The use of wheat as animal feed is minor when compared to its use as food. Wheat grain is used as feed for poultry, swine, and cattle. It is estimated that on average 13 percent of total U.S. wheat grain has been utilized for animal feed in recent years. Wheat forage is also used as an animal feed source and is used for grazing cattle over the winter months in certain regions of

the U.S.

Monsanto is not aware of any food or feed uses of standard spring wheat varieties that are not also applicable to spring wheat varieties containing event MON 71800.

6. Compositional Analysis of Roundup Ready® Wheat

Monsanto compared MON 71800 wheat to the parent line (Bobwhite MON 71900) and to commercially available non-transgenic spring wheat varieties.

6.1 Overview of the Approach to Compositional Analysis

To assess whether Roundup Ready® wheat event MON 71800 is as safe and nutritious as wheat varieties currently consumed, Monsanto conducted compositional analyses of grain and forage from event MON 71800, the non-transgenic parent line MON 71900 (Bobwhite), and several commercial wheat varieties. To conduct these compositional analyses, Monsanto collected both grain and forage samples from field trials and purchased commercially available grain produced from certified seed.

Monsanto planted event MON 71800, the parental line MON 71900, and 18 different commercial lines at five North American sites (three sites in the U.S. and two sites in Canada). Monsanto considered that these five sites provided a variety of environmental conditions representative of regions where Roundup Ready® wheat can be grown as a commercial product. Monsanto analyzed 76 components in grain collected from event MON 71800, the parental line MON 71900, the 18 different commercial spring wheat lines that Monsanto grew as part of its field trials, and grain from 4 spring wheat varieties purchased from certified seed producers. Monsanto also analyzed 9 components in forage tissues collected from event MON 71800, the parental line MON 71900, and the 18 different commercial spring wheat lines that Monsanto grew as part of its field trials.

Monsanto used standard analytical methods or other suitable methods for compositional analyses and provides references and descriptions for all analytical methods used. Monsanto provided the analytical results for the five test sites individually, and for all the sites combined. Monsanto reported the level of each analyzed component (mean and range) in the transgenic line MON 71800 and non-transgenic control line MON 71900; the difference between the level of each component (mean and range) obtained for line MON 71800 and line MON 71900; the range of levels obtained for 22 commercial lines; and the historical range based on values in the literature.

Monsanto subjected all of the compositional data obtained for Roundup Ready® wheat event MON 71800 and the parental control line MON 71900 to statistical analysis using a mixed model analysis of variance. Monsanto conducted the statistical analysis on data from each of the five replicated trials, as well as on data combined from all five trials.

6.2 Grain

Monsanto determined the levels of the following components of wheat grain:

- Proximates: protein, fat, carbohydrate (by calculation), ash and moisture

- Sugars: arabinose, fructose, galactose, glucose, maltose, raffinose, sucrose and xylose
- Total dietary fiber (TDF)
- Amino acid composition
- Fatty acid composition (16:0 palmitic, 16:1 palmitoleic, 18:0 stearic, 18:1 oleic, 18:2 linoleic, 18:3 linolenic, 20:0 arachidic, and 20:1 eicosenoic)
- B vitamins: niacin, riboflavin (vitamin B_2), thiamin (B_1), and vitamin B_6
- Vitamin E
- Minerals: cadmium, calcium, copper, iron, magnesium, manganese, phosphorus, potassium, selenium, and zinc
- Starch

Monsanto also analyzed wheat grain for 14 other fatty acids, sodium, and the sugars mannose and stachyose; however, these substances were not detected in any samples tested (i.e., either from line 71800, line 71900, or commercial varieties).

Based on the combined statistical analysis of data from all test sites, Monsanto did not identify any statistically significant differences in grain composition between the event MON 71800 line and the parental MON 71900 line. Monsanto did identify some statistically significant differences ($p<0.05$) in composition between MON 71800 and MON 71900 at individual sites. In total, Monsanto found small differences in the levels of 16 components. However, these differences were found at only one or two test sites, and were not consistent over all trials. Monsanto also reported that for grain, all measured parameters fell within the range detected by Monsanto for the commercial varieties. The values also fell within the range found in the literature, except for the two amino acids, serine and valine; however, there were no differences in the levels of these amino acids when comparing MON 71800, MON 71900, or the commercial wheat varieties. Monsanto concludes that grain from event MON 71800 is compositionally equivalent to that of the non-transgenic parental control wheat and other wheat varieties grown commercially.

6.3 Forage

Monsanto determined the levels of the following components of wheat forage:

- Proximates: protein, fat, carbohydrate (by calculation), ash and moisture
- Fiber: acid detergent fiber, neutral detergent fiber
- Minerals: calcium and phosphorus

Based on the combined statistical analyses from individual test sites, Monsanto identified no statistically significant differences ($p<0.05$) in composition between forage samples from MON 71800 and MON 71900. In the individual tests, Monsanto found significant ($p<0.05$) differences in the levels of five components from two out of five test sites. However, the differences were small and were not consistent over all locations as indicated by the combined analysis. In addition, the range of values for those components fell within the interval of values determined for the commercial varieties and within values reported in the literature.

Monsanto concludes that the forage from event MON 71800 is compositionally equivalent to that of the non-transgenic parental control wheat and other wheat varieties grown commercially.

6.4 Antinutrients

Phytic acid is an antinutrient that occurs naturally in wheat. Monsanto measured phytic acid in the grain of all test and control lines. The mean level of phytic acid in event MON 71800 was comparable to that of the parental line MON 71900 and fell within the range established for the 22 commercial lines and within the historical range for wheat based on values in the literature.

6.5 Endogenous Allergens

Because wheat is known to cause allergic reactions in sensitive individuals, Monsanto performed an evaluation to assess whether the transformation process may have increased the overall allergenicity of wheat grain from event MON 71800. Monsanto conducted IgE-inhibition ELISAs and IgE-immunoblot analysis with extracts from event MON 71800, parental MON 71900, and seven other commercial varieties of wheat. Sera used in these assays were obtained from ten human subjects with IgE-mediated allergic response to wheat consumption as evidenced by clinical history and response in single or double blind oral food challenge. From these experiments, Monsanto concluded that the transformation process did not significantly alter endogenous allergens in event MON 71800, because IgE binding properties were similar to those of other commercial wheat varieties.

6.6 Gluten

Celiac disease (gluten-sensitive enteropathy) is caused by a specific immune response to antigens present in gluten in susceptible individuals. Gluten refers to a mixture of glutenin and gliadin proteins present in wheat, barley and rye. Monsanto measured gliadin levels and calculated gluten levels in event MON 71800, parental MON 71900, and 22 other commercial wheat varieties. Gliadin and gluten levels in event MON 71800 are comparable to those of the parental and other commercial varieties tested.

6.7 Summary of Compositional Analysis

Monsanto concludes that the results of its compositional analysis established that the levels of nutrients and other components of the grain and forage derived from event MON 71800 fall within the ranges found for commercially available wheat varieties. Monsanto considered that the few minor differences in the levels of certain components seen at individual test sites are unlikely to be meaningful, and that grain and forage from event MON 71800 are compositionally equivalent to that of the non-transgenic parental control wheat and other wheat varieties grown commercially.

7. Conclusions

Monsanto has concluded that Roundup Ready® wheat event MON 71800 is not materially different in composition, safety, or any other relevant parameter from wheat now grown, marketed, and consumed. At this time, based on Monsanto's data and information, the agency considers Monsanto's consultation on Roundup Ready® event MON 71800 wheat to be complete.

/s/
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Hypertext updated by jmf/rxm September 22, 2004