# GM Crop Database

## Database Product Description

# MON71800

| | |
|---|---|
| **Host Organism** | *Triticum aestivum* (Wheat) |
| **Trait** | Herbicide tolerant, glyphosate. |
| **Trait Introduction** | Agrobacterium tumefaciens-mediated plant transformation. |
| **Proposed Use** | Production for human consumption and livestock feed. |
| **Product Developer** | Monsanto Company |

## Summary of Regulatory Approvals

| Country | Food | Feed | Env | Notes |
|---|---|---|---|---|
| Colombia | 2004 | | | |
| United States | 2004 | 2004 | | |

## Introduction

The spring wheat variety MON 71800 (Roundup Ready® wheat) was developed to allow the use of glyphosate, the active ingredient in the herbicide Roundup®, as a weed control option in spring wheat. This genetically engineered wheat variety contains a glyphosate-tolerant form of the plant enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), isolated from the soil bacterium *Agrobacterium tumefaciens* strain CP4. The novel form of this enzyme is termed hereafter CP4 EPSPS.

The EPSPS 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 herbicide binds to EPSPS, thereby preventing the synthesis of aromatic amino acids needed for plant growth. The CP4 EPSPS enzyme in MON 71800 spring wheat has a reduced affinity for glyphosate; its enzymatic activity is therefore not hindered by the herbicide.

EPSPS is present in all plants, bacteria, fungi, but not in animals, which do not synthesize their own aromatic amino acids. Because the aromatic amino acid biosynthetic pathway is not present in mammalian, avian or aquatic life forms, glyphosate has little if any toxicity for these organisms (U.S. EPA, 1993; WHO, 1994; Williams et al. 2000). The EPSPS enzyme is normally present in food derived from plant and microbial sources.

MON 71800 was developed by introducing the CP4 EPSPS coding sequences into the spring wheat variety ‘Bobwhite’ using *Agrobacterium*-mediated transformation.

## Summary of Introduced Genetic Elements

| Code | Name | Type | Promoter, other | Terminator | Copies | Form |
|---|---|---|---|---|---|---|
| CP4 epsps | 5-enolpyruvyl shikimate-3-phosphate synthase | HT | enhanced CaMV 35S | *A. tumefaciens* nopaline synthase (*nos*) 3'-untranslated region | | |
| CP4 epsps | 5-enolpyruvyl shikimate-3-phosphate synthase | HT | rice actin I promoter and intron sequences | *A. tumefaciens* nopaline synthase (*nos*) 3'-untranslated region | | |

## Characteristics of *Triticum aestivum* L. (Wheat)

| Center of Origin | Reproduction | Toxins | Allergenicity |
|---|---|---|---|
| Asia Minor, Tigris-Euphrates drainage basin of the Middle East, as well as the regions of southern Caucasus and Crimea. | Primarily self-pollinated (autogamous). Some outcrossing by wind-pollination of less than 10%. Seed does not display dormancy. | Phytic acid, trypsin inhibitor, lectins. Gliadins responsible for celiac enteropathy. | Glutenins and gliadins (*e.g.*, the IgE-inducing alpha-gliadin). |

## Donor Organism Characteristics

| Latin Name | Gene | Pathogenicity |
|---|---|---|
| Agrobacterium tumefaciens strain CP4 | CP4 epsps | *Agrobacterium tumefaciens* is a common soil bacterium that is responsible for causing crown gall disease in susceptible plants. There have been no reports of adverse effects on humans or animals. |

## Modification Method

The wheat line MON 71800 was produced by *Agrobacterium*-mediated transformation of plant cells from ‘Bobwhite’ spring wheat. The plasmid vector PV-TXGT10 used for the transformation contained two *cp4 epsps* gene cassettes coding for glyphosate tolerance. Each gene cassette consisted of chloroplast transit peptide coding sequences from the *Arabidopsis thaliana epsps* gene (*Arab*TP) associated with the sequences of the *cp4 epsps* gene. Two different promoters were used to regulate the expression of each *cp4 epsps* gene: 1) the enhanced 35S promoter from the cauliflower mosaic virus and 2), the promoter, transcription start site, and first intron of the 5’ region of the rice *actin*1 gene. Terminator sequences in each gene casette consisted of the 3’ non-translated region of the nopaline synthase gene (nos 3’). The PV-TXGT10 vector backbone contained the origin of replication sequences *ori-V* and *ori-322/rop*. The vector backbone also contained the *aad* gene, which codes for streptomycin adenyltransferase, to allow the selection of bacteria containing the PV-TXGT10 vector.

## Characteristics of the Modification

### The Introduced DNA

Southern blot analysis and Polymerase Chain Reaction (PCR) amplification of the genomic DNA of MON 71800 wheat demonstrated one site of

integration of a single copy of the T-DNA insert of PV-TXGT10. Southern blot analysis also confirmed the insertion of one intact copy of each *cp4 epsps* gene cassette, including the promoter, terminator and chlorophyll transit peptide sequences. None of the vector backbone sequences were integrated into the genome of MON 71800 wheat.

**Genetic Stability of the Introduced Trait**

The stability of the inserted DNA was evaluated, across several generations of wheat plants, using Southern blot analysis. The plants tested were progeny from several generations of self-fertilization, as well as from crosses with commercial varieties. The results of the genomic DNA blot analysis confirmed the stable inheritance of the inserted *cp4 epsps* gene cassettes. The stability of the introduced trait was also demonstrated after 18 generations of selfing of the original homozygous glyphosate-resistant plants. These generations showed no decrease in tolerance to glyphosate.

Mendelian segregation studies for the inheritance of the glyphosate tolerant trait were conducted with heterozygous first generation plants: these were selfed and the progeny were sprayed with glyphosate. The resulting 3:1 ratio of tolerant to sensitive plants was statistically significant and confirmed the inheritance of a single insertion site of the glyphosate tolerant trait.

**Expressed material**

In plant cells, the EPSPS enzyme is transported to the chloroplast by a transit peptide, which then cleaves from the enzyme. The introduced gene sequences in MON 71800 included a gene for a chloroplast transit peptide from *Arabidopsis thaliana*. Thorough analyses were conducted to investigate whether the same mechanism of binding, transporting, and cleaving of the chloroplast transit peptide to the CP4 EPSPS enzyme exists in MON 71800. The analyses revealed two forms of CP4 EPSPS expressed in MON 71800: one in which the chloroplast transit peptide has completely cleaved from the full length enzyme, and the other in which only part of the transit peptide is bound to the enzyme. Further analysis of Western blots showed that of the total amount of CP4 EPSPS expressed in MON 71800, 80% is in the form where the transit peptide is fully cleaved and 20%, where part of the transit peptide is still bound to the enzyme.

An enzyme-linked immunosorbent assay (ELISA) analysis was used to quantify the levels of the CP4 EPSPS proteins in forage and grain from MON 71800. The mean levels of both CP4 EPSPS proteins, on a fresh weight basis, were 106 ?g/g in forage and 13 ?g/g in grain. Assuming 14.5% moisture in stored grain, the concentration of both CP4 EPSPS proteins in the grain is approximately 0.0015%, on a dry matter basis.

## Food and/or Feed Safety Considerations

**Dietary exposure**

The genetic modification of MON 71800 spring wheat will not result in any change in the consumption pattern of wheat and wheat-based products. MON 71800 is expected to be used in similar applications as other spring wheat cultivars by the food industry. MON 71800 did not express any novel compositional characteristics, as confirmed by the similarity in composition of the modified line to its parental counterpart, and other conventional spring wheat varieties. Furthermore, the availability of many spring wheat cultivars for cultivation, and the normal variation in wheat composition due to differences in grade and growing conditions,

result in a wide variation in the composition of conventional wheat grain. Consequently, the dietary exposure of consumers in the United States to MON 71800 is anticipated to be the same as for other varieties of commercially available spring wheat.

**Nutritional and Compositional Data**

The nutritional components of MON 71800 grain and forage were determined analytically and compared to those of the parental line 'Bobwhite' and several commercial varieties grown at five locations in the United States and Canada. For the grain, these components included proximates (crude protein, crude fat, ash, moisture, total carbohydrates), total dietary fibre, sugars, starch, amino acids, fatty acids, B vitamins, vitamin E and minerals. Forage samples were analyzed for proximates, acid detergent fibre, neutral detergent fibre, calcium and phosphorus. At some of the locations, for both the grain and the forage, there were statistically significant differences in the levels of certain components between MON 71800 and its parental line. These differences within locations could be attributed to environmental effects rather than to any unintended effect of the genetic modification in MON 71800. However, the combined data from all test locations demonstrated that the nutritional composition of MON 71800 grain and forage was comparable to that of the parental line 'Bobwhite,' and other commercial spring wheat varieties.
Phytic acid occurs naturally in wheat and other cereals. It is indigestible by humans and non-ruminant livestock, and inhibits the absorption of iron and other minerals. Grain samples of MON 71800, the parental line 'Bobwhite,' other commercial wheat varieties were analyzed to determine levels of phytic acid. The concentration of phytic acid in MON 71800 was comparable to that in the parental line, and was within the range of values determined for the commercial varieties and those found in the literature.

**Toxicity and Allergenicity**

The potential for toxicity and allergenicity of MON 71800 wheat was investigated using the following data and information: results from the determination of amino acid sequence similarity between the CP4 EPSPS proteins and known toxins and allergens; analysis for possible glycosylation of the CP4 EPSPS proteins; analysis of the stability the novel proteins in simulated gastric fluids; results from an acute oral toxicity study in mice using the CP4 EPSPS proteins; and, information on the safety of the *cp4 epsps* gene donor, *A. tumefaciens* strain CP4. The potential for increased allergenicity of the grain from MON 71800 was investigated, specifically with regard to endogenous wheat allergens that induce an IgE reaction in susceptible humans. Various immunilogical assays, using sera from humans with an IgE-mediated wheat allergy, were performed with extracts from MON 71800, the parental line, and several other commercial wheat varieties. The possibility that the genetic modification would have also altered the levels of gliadin, proteins that cause celiac enteropathy in susceptible persons, was also investigated. Gliadin levels were measured, and gluten levels calculated in MON 71800, its parental line, and other commercial varieties.

The CP4 EPSPS proteins in MON 71800 showed no amino acid sequence similarity with known toxins and allergens and neither of the two forms of the protein are glycosylated. Both forms of the CP4 EPSPS protein were rapidly digested under simulated gastric fluid conditions, and the enzyme activity of each protein was substantially diminished within the same period as for the digestion. The results of the acute oral toxicity study on mice, at the highest administered dose, showed no adverse

effects of the CP4 EPSPS proteins. Gluten levels were not significantly altered by the genetic modification of MON 71800, neither were the endogenous allergens, as demonstrated by the results of the immunological studies.

Both forms of the CP4 EPSPS protein were expressed at very low levels in MON 71800 grain and forage. This fact, along with negative results of the various safety studies, sequence homology investigations, and immunological assays led to the conclusion that MON 71800 spring wheat did not demonstrate any potential for toxicity and novel allergenicity, nor any altered endogenous allergenicity, compared to conventional spring wheat varieties.

## Abstract

Commercial wheat is comprised mainly of two species: common, or bread wheat (*T. aestivum* L.) and durum wheat (*T. durum* Desf.). Bread wheat is classified into several types, based on vernalisation requirement (winter and spring types) and kernel hardness. The hard types of bread wheat are high in protein, especially gliadins and glutenins. The high levels of these protein fractions in the flour impart elasticity to bread dough and allow it to expand during leavening and baking. Soft wheats are low in protein, and have low levels of gliadin and glutenin. These wheats are milled into flour for use in bakery products such as cakes, pastries, and unleavened breads. Durum wheat produces very hard, almost vitreous kernels due to its high protein content. This wheat is milled into semolina for the production of pasta and couscous.

Harvested wheat consists of a naked kernel, unlike other cereals such as rice, barley or oats that retain their hull (*i.e.*, the palea and lemma) after harvest. The wheat kernel is loosely enclosed within the palea and lemma of each spikelet; these are eliminated as chaff during threshing. The wheat kernel is milled into white flour by removing the bran, aleurone layers and the germ prior to grinding; whole-wheat flour retains these fractions. By-products of wheat milling include: bran, germ, shorts and middlings. Some of these by-products are used as human food (*i.e.*, bran, germ), and others, as livestock feed. Grain that does not meet the grade for food use can be used as animal feed, mainly for poultry and swine, but also for cattle. Wheat can also be fed as forage, either as pasture prior to stem elongation, or as ensilage. Wheat is also used in the brewing and distilling industries.

Weeds are a major production problem in wheat cultivation. Weeds compete for light, water and nutrients, and can also cause lodging and problems with harvesting. The seeds of several weed species are almost impossible to clean out of harvested wheat (*e.g.*, *Avena fatua* L. wild oats), causing loss of quality and downgrading of the crop. Weeds can be managed using a combination of cultural practices (*e.g.*, seed bed preparation, use of clean [certified] seed, narrow row spacing, fertilizer banding), integrated weed management (*e.g.*, weed scouting, economic thresholds) and the use of herbicides. Depending on the weed species present, herbicides can be applied before the crop emerges (*e.g.*, amitrole, glyphosate, trifluralin), or after (*e.g.*, 2-4D, bromoxynil, dicamba, fenoxaprop-p-ethyl, MCPA, metsulfuron methyl). The build-up of weed populations can be stemmed by applying herbicides on summer-fallowed fields, and by practicing crop rotation, which allows the use of different herbicides. Rotating among herbicide groups also prevents the development of herbicide-resistant biotypes.

Roundup Ready® wheat (MON 71800) was developed to allow the use of glyphosate, the active ingredient in the herbicide Roundup®, as a weed

control option in spring wheat production. This genetically engineered spring wheat contains a novel form of the plant enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) that allows MON 71800 to survive an otherwise lethal application of glyphosate. The EPSPS gene introduced into MON 71800 was isolated from a strain of the common soil bacterium *Agrobacterium tumefaciens* strain CP4, and the novel form of the EPSPS enzyme produced by this gene is tolerant to glyphosate.

The EPSPS enzyme is part of the shikimate pathway, an important biochemical pathway in plants involved in the production of aromatic amino acids and other aromatic compounds. When conventional plants are treated with glyphosate, the plants cannot produce the aromatic amino acids needed for growth and survival. EPSPS is present in all plants, bacteria, and fungi. It is not present in animals, since these organisms are unable to synthesize their own aromatic amino acids. Because the aromatic amino acid pathway is not present in mammals, birds, or aquatic life forms, glyphosate has little, if any, toxicity for these organisms. The EPSPS enzyme is naturally present in foods derived from plant and microbial sources. MON 71800 was developed by introducing two CP4 EPSPS genes into the spring wheat variety 'Bobwhite' using *Agrobacterium*-mediated transformation.

The food and livestock safety of MON 71800 wheat was based on the safety assessment of the CP4 EPSPS protein and the level of expression of the protein in the grain. The CP4 EPSPS proteins constitutes a small amount of the total protein in MON 71800 so there is little dietary exposure. The lack of toxicity or allergenicity of CP4 EPSPS was demonstrated from the results of laboratory and safety studies. The nutritional equivalence and wholesomeness of MON 71800 wheat compared to conventional wheat was demonstrated by the analysis of key nutrients in the grain including proximates (*e.g.*, crude protein, crude fat, crude fibre, ash, moisture), total dietary fibre, sugars, starch, amino acid and fatty acid composition, B vitamins and vitamin E, minerals, as well the composition in the anti-nutrient phytic acid.

## Links to Further Information

**Food Standards Australia New Zealand**

Initial Assessment Report: Application A524 - Food derived from herbicide - tolerant wheat MON 71800 (http://www.cera-gmc.org/files/cera/GmCropDatabase/docs/decdocs/05-245-006.pdf)
[PDF Size: 289.13K bytes]

**U.S. Food and Drug Administration**

Biotechnology Consultation Note to the File BNF No. 000080 (http://www.cera-gmc.org/files/cera/GmCropDatabase/docs/decdocs/04-300-008.pdf)
[PDF Size: 160.69K bytes]