A Review of the Environmental Safety of the CP4 EPSPS Protein

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INTRODUCTION

This document provides a comprehensive review of information and data relevant to the environmental risk assessment of the protein 5-enolpyruvylshikimate-3-phosphate synthase isolated from *Agrobacterium* sp. strain CP4 (CP4 EPSPS) and presents a summary statement about the environmental safety of this protein. All sources of information reviewed herein were publically available and included: dossiers presented to regulatory authorities; decision summaries prepared by regulatory authorities; peer reviewed literature; and product summaries prepared by product developers.

Environmental risk assessments related to the introduction of genetically engineered (GE) plants are conducted on a case-by-case basis taking into account the biology of the plant, the nature of the transgene and the protein or gene product it produces, the phenotype conferred by the transgene, as well as the intended use of the plant and the environment where it will be introduced (i.e., the receiving environment). These assessments typically involve comparisons of the transgenic event to an untransformed parent line and/or closely related isoline, and also use baseline knowledge of the relevant plant species (CBD 2000b, Codex 2003a, b, EFSA 2006a, NRC 1989, OECD 1992). The objective of these comparisons is to identify potential risks that the GE plant might present beyond what is already accepted for similar plants in the environment by identifying meaningful differences between the GE crop and its conventional counterpart. Any identified differences that have the potential to affect assessment endpoints can subsequently be evaluated for likelihood and consequence.

To date, regulatory authorities in twelve countries have approved the environmental (commercial) release of at least one of 30 plant lines[1] expressing the protein CP4 EPSPS (Table 1). This represents a total of seven plant species: *Beta vulgaris* L. (sugarbeet), *Brassica napus* L. and *Brassica rapa* L. (oilseed rape and turnip rape, respectively, although both can be referred to as canola) *Glycine max* L. (soybean), *Gossypium hirsutum* L. (cotton), *Medicago sativa* L. (alfalfa) and *Zea mays* L. (maize)[2]. Environmental risk assessments by regulatory authorities in these countries have considered risk hypotheses related to the following three categories of potential harms: (1) the CP4 EPSPS protein may have an adverse environmental impact on non-target organisms; (2) transformation of the host plant and subsequent expression of CP4 EPSPS may alter the characteristics of the plant resulting in adverse environmental impacts (e.g., increased weediness); and (3) introgression of the *cp4 epsps* gene into a sexually compatible plant species may alter that species resulting in adverse environmental impacts (e.g., establishment of new weedy populations) (ANZFA 2000a, 2000b, 2001, 2002; CFIA 1995, 1998, 2005; FSANZ 2005; USDA APHIS 1994, 1995b, 1995d, 1996b, 1997a, 1998b, 1999, 2000b, 2002, 20004b, 2004d, 2005a, 2005b, 2007a).

Note that environmental effects that may be associated with the use of the herbicide glyphosate in association with CP4 EPSPS-transformed plants are outside the purview of this review.

Key words

CP4 EPSPS, glyphosate, herbicide tolerant, genetically engineered, environmental risk assessment

1 Lines means primary events developed through genetic engineering and stacked events derived through conventional crossing of primary events.

2 One line of potato (*Solanum tuberosum*) has also been approved that contains CP4 EPSPS as a selectable marker for tissue culture and it is included in Table 1 as an eighth species. Anecdotal evidence suggests this line is not functionally glyphosate resistant as a crop plant, however, and information related to this event is not further considered here.

Table 1. Regulatory approvals for the environmental release of GE plants containing CP4EPSPS and functionally similar EPSPS modifications.

Species	Event Name	Also Known As	United States	Canada	Mexico	Argentina	Brazil	Colombia	Paraguay	Uruguay	South Africa	Australia	Japan	Korea	Philippines
Beta Vulgaris (sugarbeet)	GTSB77		X												
	H7-1		X	X									X		
Brassica napus (oilseed rape)	GT200		X	X									X		
	GT73 (RT73 synonym)		X	X								X	X		
Brassica rapa (turnip rape)	ZSR500/502		*1	X											
Glycine max L. (soybean)	GTS 40-3-2		X	X	X	X	X		X	X	X		X		
	MON-889788-1		X	X									X		
Gossypium hirsutum L. (cotton)	MON-01445-2		X			X	X	X			X	X	X		
	MON1698 (grouped with MON1445 in approvals		X				X	X				X	X		
	MON-15985-7 x MON-01445-2		*1									X			
	MON-00531-6 x MON-01445-2		*1			X	X				X	X			
	MON88913		X								X	X			
	MON-15985-7 x MON88913										X	X			
	DAS-24236-5 X DAS-21023-5 X MON88913	DAS-24236-5 X DAS-21023-5 X MON-88913-8	*1												
	DAS-21023-5 x DAS-24236-5 x MON-01445-2		*1												
Medicago sativa (alfalfa)	MON-00101-8 (J101)			X									X		
	MON-00163-7 (J163)			X									X		
Solanum tuberosum L. (potato)[2]	RBMT22-082		X	X											
Zea mays (corn)	MON-00603-6	NK603	X	X		X	X				X		X		X
	MON80100		X												
	MON00603-6 x MON-00810-6	NK603 x MON810	*1	*1		X	X				X		X		X
	DAS-01507-1 x MON-00603-6	TC1507 x NK603	*1	*1		X	X						X		
	MON-89034-3 x DAS-01507-1 x MON88017 x DAS-59122-7	MON89034 x TC1507 x MON88017 x DAS-59122-7	X	X									X		
	MON-00863-5 x MON-00603-6	MON863 x NK603	*1	*1									X		
	MON-00863-5 x MON-00810-6 x MON-00603-6	MON863 x MON810 x NK603	*1	*1									X		
	MON809		X	X									X		
	MON-88017-3	MON88017	X	X									X		
	MON802		X	X									X		
	DAS-59122-7 x DAS-01507-1 x MON-00603-6	DAS-59122-7 x TC1507 x NK603	*1	*1									X		
	DAS-59122-7 x DAS-01507-1 x MON-00603-6	DAS-59122-7 x NK603	*	*1									X		
EPSPS mutants (not CP4)															
Zea mays (maize)	MON-00021-9	GA21	X	X		X	X						X		X
	MON-00021-9 x MON-00810-6	GA21 x MON810	*1	*1											
	SYN-IR604-5 x MON-00021-9	MIR604 x GA21	*1	*1									X	X	
	SYN-BT011-1 x SYN-IR604-5 x MON-00021-9	BT11 x MIR604 x GA21	*1	*1										X	
	SYN-BT011-1 x MON-00021-9	BT11 x GA21	*1	*1			X						X		
Gossypium hirsutum L. (cotton)	BCS-GH002-5	GHB614	X												

X = Approved for environmental (commercial) release.
1 Stacked events that may be considered approved for environmental release based on existing approvals for the GE parent lines from which they are derived.
2 Contains CP4 EPSPS as a marker for transformation selection. Lines generated from this event may not be functionally resistant to glyphosate.

THE ORIGIN AND FUNCTION OF CP4 EPSPS

The CP4EPSPS Enzyme Family and CP4 EPSPS

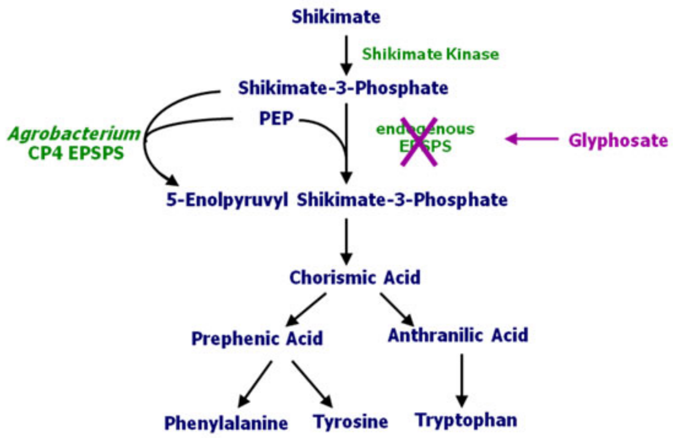
The 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS: EC 2.5.1.19) family of enzymes is ubiquitous in plants and microorganisms. EPSPS enzymes have been isolated from both sources, and their properties have been extensively studied. The bacterial and plant enzymes are mono-functional with a molecular mass of 44-48 kD (Kishore *et al.* 1988). EPSPS proteins catalyze the transfer of the enolpyruvyl group from phosphoenol pyruvate (PEP) to the 5-hydroxyl of shikimate-3-phosphate (S3P), thereby yielding inorganic phosphate and 5-enolpyruvylshikimate-3-phosphate (Alibhai and Stallings, 2001). This is the only known metabolic product and 5-enolpyruvly shikimate-3-phosphate is the penultimate product of the shikimic acid pathway. Shikimic acid is a substrate for the biosynthesis of aromatic amino acids (phenylalanine, tryptophan and tyrosine) as well as many secondary metabolites, such as tetrahydrofolate, ubiquinone, and vitamin K. Importantly, the shikimate pathway and, hence, EPSPS proteins, are absent in mammals, fish, birds, reptiles and insects (Alibhai and Stallings, 2001). In contrast, it has been estimated that aromatic molecules, all of which are derived from shikimic acid, represent 35% or more of the dry weight of a plant (Franz *et al.* 1997).

The *cp4 epsps* gene was isolated from *Agrobacterium* sp. strain CP4, a common soil-borne bacterium. It has been sequenced and encodes a 47.6 kD EPSPS protein consisting of a single polypeptide of 455 amino acids. The CP4 EPSPS protein expressed in GE glyphosate tolerant plants is functionally equivalent to endogenous plant EPSPS enzymes with the exception that CP4 EPSPS displays reduced affinity for glyphosate (Franz *et al.* 1997).

Mechanism of Glyphosate Tolerance

In plants that are not glyphosate tolerant, glyphosate binds to the endogenous plant EPSPS enzyme and blocks the biosynthesis of 5-enolpyruvyl-shikimate-3-phosphate, thereby starving plants of essential amino acids and secondary metabolites (Steinrücken and Amrhein, 1980). Inhibition of EPSPS enzyme activity has been shown to proceed through the formation of a ternary complex of EPSPS-S3P-glyphosate. Formation of the complex occurs in an ordered fashion with glyphosate binding occurring only after the formation of a binary EPSPS-S3P complex. Glyphosate binding effectively blocks the binding of PEP and prevents EPSPS catalysis of S3P and PEP. In CP4 EPSPS however, affinity for PEP is much higher than affinity for glyphosate, so the CP4 EPSPS preferentially binds PEP even in the presence of glyphosate and catalysis proceeds just as in the absence of glyphosate (Franz *et al.* 1997). This difference in the glyphosate binding affinity is the basis for glyphosate tolerance in CP4 EPSPS-transformed plants. The CP4 EPSPS enzyme continues to function in the presence of glyphosate, producing the aromatic amino acids and other metabolites that are necessary for normal plant growth and development (Figure 1).

Figure 1. Schematic representation of glyphosate mode of action and mechanism of CP4 EPSPS mediated tolerance.



EXPRESSION OF CP4 EPSPS IN GLYPHOSATE TOLERANT GE PLANTS

Data for the level of expression of CP4 EPSPS in glyphosate tolerant GE plants that have obtained regulatory approvals are available in publicly accessible regulatory submissions and decision documents (ANZFA 2000a, 2000b, 2001, 2002, CFIA 1995, 1998, 2005, FSANZ 2005, USDA APHIS 1993, 1995a, 1995c, 1996a, 1996b, 1997b, 1998a, 1998c, 2000a, 2001, 2003, 2004a, 2004c, 2004e, 2006). Tissue types and collection methods differed between studies but all of them used an enzyme-linked immunosorbent assay (ELISA) to quantify the amount of CP4 EPSPS (or other EPSPS) present in samples.

Typically, one or more samples were taken at one or more field trial sites and pooled for analysis. Samples were usually collected from several tissue types and at multiple growth stages providing data from plants over time and from multiple locations. The amount of CP4 EPSPS was calculated in comparison to the total fresh weight of the sample and represented in a ratio (e.g., micrograms of CP4 EPSPS protein per gram of fresh weight). In most cases the data were presented as a mean value (normally a mean of means as values were averaged within a field trial and across trials as well) and a range (normally also a range of means representing the average amount of protein present in the sampled tissues at a trial site, although this also varied depending on the individual example).

Variations in methodology for sample collection makes direct statistical cross-comparisons of the data inappropriate but the weight of evidence suggests that GE plants express CP4 EPSPS at very low levels (see Annex I and references therein). The highest reported level of expression was for soybean leaves (798 ug/g fresh weight) and typically values were much lower (see Table 2 for summary data and Annex I for comprehensive data).

Table 2. Highest reported expression levels of CP4 EPSPS in plant tissues from representative approved events.

Species	Transformation Event	Tissue	Highest Reported Expression (ug/g fresh weight)
Beta vulgaris	GTSB77	Top	370
Brassica napus	GT73	Leaf	70
Brassica rapa	ZSR500/502	Seed	53
Glycine max	GTS-40-3-2	Leaf	798
Medicago sativa	J101 x J163	Forage	390
Gossypium hirsutum	MON88913	Seed	550
Zea mays	MON88017	Pollen	280

ESTABLISHMENT AND PERSISTENCE OF CP4 EPSPS-EXPRESSING PLANTS IN THE ENVIRONMENT

Biology of the Plant Species

Familiarity with the biology of the nontransformed or host plant species in the receiving environment is typically the starting point for environmental risk assessments of GE plants (OECD 2006). Information about the biology of the host plant can be used to identify species-specific characteristics that may be affected by the novel trait so as to permit the transgenic plant to become "weedy", invasive of natural habitats, or to be otherwise harmful to the environment. It can also provide details on significant interactions between the plant and other organisms that may be important when considering potential harms. By considering the biology of the host plant, a risk assessor can identify potential hazards that may be associated with the expression of the novel protein (e.g., CP4 EPSPS) and then be able to assess the likelihood of these hazards being realized. For example, if the plant species is highly domesticated and requires significant human intervention to grow or reproduce, the assessor can take that into account when assessing the likelihood of the GE plant establishing outside of cultivation.

Phenotypic Data

Information about the phenotype of GE plants expressing CP4 EPSPS was collected from laboratory, greenhouse and field trial studies and was presented in regulatory submissions to: (1) identify any intentional changes to the phenotype that might impact the environmental safety of the plant; and (2) to identify any unintended changes to the biology of the plant that might impact environmental safety. Phenotypic data in regulatory submissions and peer reviewed publications have focused on characteristics of the plant that might contribute to its survival or persistence (i.e., potential weediness), or that negatively affect agronomic performance (e.g., disease susceptibility and yield data) (ANZFA 2000a, 2000b, 2001, 2002, CFIA 1995, 1998, 2005, FSANZ 2005, USDA APHIS 1993, 1995a, 1995c, 1996a, 1996b, 1997b, 1998a, 1998c, 2000a, 2001, 2003, 2004a, 2004c, 2004e, 2006). Additional agronomic data, especially yield data representing different environmental or management conditions, have also been collected for the purpose of product characterization (Delannay *et al.* 1995, Ellmore *et al.* 2001, Light *et al.* 2003). Phenotypic data presented were either quantitative (e.g., yields and seed counts, days to maturity) or qualitative (e.g., survey data for disease or insect susceptibility).

Direct comparisons between phenotypic observations of different CP4 EPSPS events could not be made because differences in the biology of host plant species make different phenotypic characteristics relevant for each species and because data were variably collected and presented. Table 3 provides a summary of available information on phenotypic characteristics for representative events. Statistically significant differences between CP4 EPSPS plants and their controls were reported in seven instances out of the 59 observations summarized in Table 3. These differences were subsequently determined to fall within the range of observed values for that crop species under cultivation, and risk assessors did not consider the differences to be biologically meaningful (see also Annex I) (ANZFA 2000a, 2000b, 2001, 2002, CFIA 1995, 1998, 2005, FSANZ 2005, USDA APHIS 1994, 1995b, 1995d, 1996b, 1997a, 1998b, 1999, 2000b, 2002, 20004b, 2004d, 2005a, 2005b, 2007a). These observations support the conclusion that expression of CP4 EPSPS in these events did not alter plant phenotype with the exception of the intended trait of glyphosate tolerance.

Weediness in Agricultural Environments

All of the plant species that have been engineered to express CP4 EPSPS have some potential to "volunteer" as weeds in subsequent growing seasons and demonstrate varying degrees of ability to persist in an agricultural environment (OECD 1997, 2000, 2001, 2003a, 2008, OGTR 2008, USDA APHIS 2004d). The characteristics that influence the ability of a plant to volunteer are largely the same as those for weediness in general, such as seed dormancy, shattering, and competitiveness (Baker 1974). The data available indicate there is no linkage between CP4 EPSPS protein expression and any increased survival or over-wintering capacity that would alter the prevalence of volunteer plants in the subsequent growing season (USDA APHIS 1993, 1995a, 1995c, 1996a, 1996b, 1997b, 1998a, 1998c, 2000a, 2001, 2003, 2004a, 2004c, 2004e, 2006). Following-season volunteers expressing CP4 EPSPS may complicate volunteer management programs, particularly if different crop species expressing glyphosate tolerance are planted in consecutive rotations (e.g., glyphosate-tolerant soybean and glyphosate-tolerant maize in rotation). Alternative options are available for managing glyphosate tolerant volunteers, including the use of other herbicides and mechanical weed control (Beckie *et al.* 2004, Deen *et al.* 2006, OECD 1997, OECD 2000, OECD 2001, OECD 2003a, OECD 2008, OGTR 2008, USDA APHIS 2004d).

Weediness in Non-Agricultural Environments

The primary mechanisms by which CP4 EPSPS may be introduced into a non-agricultural environment are: (1) seed or propagule movement (which may include incidental release during transportation of commodities) and establishment of the GE plant outside of cultivated areas, and; (2) gene flow from the GE plant to a natu-

Table 3. Summary of available phenotypic data reported for representative events expressing CP4 EPSPS[1].

Species Event	Germination / Emergence	Dormancy	Competetiveness/ Volunteerism / Overwintering	Vegetative vigor	Morphology	Time to Maturity	Time to Flowering	Number of flowers	Shattering/ seed dispersal	Yield/ seed production	Abiotic stress susceptibility	Insect and other pest susceptibility	Disease Susceptibility	Susceptibility to Other Herbicides
B. vulgaris GTSB77	X	X	X	X	X							X	X	X
B. napus GT73	X	X				X	X		X	X	X	X	X	
B. rapa ZR500/502			X			X	X			X	X			
G. max GTS 40-3-2	X			X	X[2]	X	X			X	X	X	X	
G. hirsutum MON1445	X	X	X	X		X[3]	X	X		X[3]		X	X	
M. sativa[5] J101, J163	X[4]	X[4]	X	X	X			X	X	X	X	X	X	
Z. mays NK603				X	X[6]	X[6]			X			X	X	X

1 An "X" indicates that this phenotypic comparison was explicitly represented in a regulatory dossier or publication. The characteristic was not significantly different between GE and control unless marked.
2 Difference in plant height were reported for 2 of 4 test locations (16%) but this was within the observed range of other soybean cultivars.
3 Differences in time to maturity and productivity were observed in some lines at some field trial locations, but these were reported to fall within the expected range for cotton germplasm.
4 Significant differences in "hard" seed and germination rates were observed in one test year, but not others. These results were within the normal range for alfalfa cultivars.
5 The USDA Petition for these events contains voluminous data from agronomic studies. Only a subset of this was used to prepare this table.
6 Significant statistical difference in ear height (38.3 inches (97.3 cm) mean for control versus 40.3 inches (102 cm) mean for NK603) and days to 50% silking (61.8 days for NK603 compared to 60.2 for control) were observed, but these were within the range of expected values for maize germplasm.

ralized (or feral) population of the same crop species or other sexually compatible relatives (Mallory-Smith and Zapiola, 2008). Risk assessments for GE plants expressing CP4 EPSPS have considered the potential impacts associated with both types of introduction (ANZFA 2000a, 2000b, 2001, 2002, CFIA 1995, 1998, 2005, EFSA 2003, 2004a, 2004b, 2005a, 2005b, 2006a, 2006b, 2006c, 2008a, 2008b, 2009a, 2009b, FSANZ 2005, Japan BCH 2003, 2004, USDA APHIS 1994, 1995b, 1995d, 1996b, 1997a, 1998b, 1999, 2000b, 2002, 2004b, 2004d, 2005a, 2005b, 2007a).

While all plants can be considered weeds in certain contexts, none of the crops for which glyphosate tolerant GE lines are available are considered to be invasive or problematic weeds outside of agricultural systems. Most can persist under favorable conditions and they may at times require management, particularly when they volunteer in subsequent crops (OECD 1997, OECD 2000, OECD 2001, OECD 2003a, OECD 2008, OGTR 2008, USDA APHIS 2004d). Based on agronomic and compositional data showing that CP4 EPSPS does not have a significant impact on agronomic or compositional traits (including those that are related to weediness) there is no evidence to date that expression of the CP4 EPSPS protein has resulted in any altered potential for weediness for those GE plant events subjected to a pre-commercial environmental risk assessment. CP4 EPSPS expression only affects the ability of the plant to survive if treated with glyphosate. Just as in agricultural environments, other management options to control glyphosate tolerant plants in non-agricultural environments are available (Beckie *et al*. 2004, Deen *et al*. 2006, OECD 1997, OECD 2000, OECD 2001, OECD 2003a, OECD 2008, OGTR 2008, USDA APHIS 2004d).

Movement of the Transgene to Wild Relatives

The movement of transgenes to wild relatives is pollen mediated and the production of reproductively viable hybrids depends on the physical proximity and flowering synchrony of the GE plants to sexually compatible species. As with the presence of CP4 EPSPS in transformed events, there is no evidence that expression of the CP4 EPSPS protein in a range of plant species has resulted in any alteration to anticipated gene flow. However, introgression of glyphosate tolerance into sexually compatible, weedy populations in agricultural or peri-agricultural ecosystems has the potential to raise management issues (Mallory-Smith and Zapiola, 2008, Warwick e*t al.* 2007). In at least one instance, a regulatory decision has geographically limited the release of a glyphosate tolerant GE plant: the environmental approval of *B. rapa* event ZSR500/502 was limited to the western region of Canada due to the presence of feral populations of *B. rapa* in eastern Canada where it is considered a weed of agriculture (CFIA 1998).

ADVERSE IMPACTS ON OTHER ORGANISMS IN THE RECEIVING ENVIRONMENT

The potential for the CP4 EPSPS protein to have adverse impacts on organisms in the receiving environment has been considered in regulatory risk assessments using a weight of evidence approach

(CFIA 1995, 1998, OGTR 2003a, 2003b, 2006, USDA APHIS 1994, 1995b, 1995d, 1997a, 1997c, 1998b, 1999, 2000a, 2000b, 2004b, 2004d, 2005b, 2007a). Toxic proteins are known to act acutely (Sjoblad *et al.* 1992), and experiments in mice show that CP4 EPSPS has no adverse affect on acutely gavaged mice (Harrison *et al.* 1996). Further, CP4 EPSPS is rapidly degraded in mammalian digestive systems, reducing exposure, and has no significant sequence or structural homology to known toxins or allergens (Harrison *et al.* 1996, Nickson and Hammond, 2002). In addition, CP4 EPSPS is not known to be toxic to any other organisms (CFIA 1995, 1998, EFSA 2003, 2004a, 2004b, 2005a, 2005b, 2006b, 2006c, 2008a, 2008b, 2009a, 2009b, OGTR 2003a, 2003b, 2006, USDA APHIS 1994, 1995b, 1995d, 1997a, 1997c, 1998b, 1999, 2000a, 2000b, 2004b, 2004d, 2005b, 2007a). The isolation of the *cp4 epsps* gene from the common soil bacterium *Agrobacterium tumefaciens* suggests that there will be no novel exposure in soil, and risk assessors have also considered the similarity in structure and function of CP4 EPSPS to other EPSPS enzymes endogenous to the plant and present throughout the environment (CFIA 1995, 1998, EFSA 2003, 2004a, 2004b, 2005a, 2005b, 2006b, 2006c, 2008a, 2008b, 2009a, 2009b, OGTR 2003a, 2003b, 2006, USDA APHIS 1994, 1995b, 1995d, 1997a, 1997c, 1998b, 1999, 2000a, 2000b, 2004b, 2004d, 2005b, 2007a). The enzymatic activity of CP4 EPSPS is highly specific and equivalent to other EPSPS proteins in plants and microorganisms, making it unlikely that organisms in the receiving environment would have altered exposure to the metabolic products of CP4 EPSPS (CFIA 1995, 1998, OGTR 2003a, 2003b, 2006, USDA APHIS 1994, 1995b, 1995d, 1997a, 1997c, 1998b, 1999, 2000a, 2000b, 2004b, 2004d, 2005b, 2007a).

Risk assessors have considered whether the introduction of CP4 EPSPS into a GE plant would lead to changes in the plant that might have an adverse impact on other organisms. Phenotypic characterization of the GE plant (see above) as well as compositional analyses (see below) and nutritional analyses suggest that the introduction of CP4 EPSPS has not had any unanticipated effects on characteristics of GE plants that might impact other organisms (CFIA 1995, 1998, EFSA 2003, 2004a, 2004b, 2005a, 2005b, 2006b, 2006c, 2008a, 2008b, 2009a, 2009b, Nickson and Hammond 2002, Nida *et al.* 1996, OGTR 2003a, 2003b, 2006, Padgette *et al.* 1996, Ridley *et al.* 2002, USDA APHIS 1994, 1995b, 1995d, 1997a, 1997c, 1998b, 1999, 2000a, 2000b, 2004b, 2004d, 2005b, 2007a). Observations of CP4 EPSPS expressing plants during field trial evaluations have indicated no adverse impacts on other organisms (OGTR 2003a, 2003b, 2006, USDA APHIS 1994, 1995b, 1995d, 1997a, 1997c, 1998b, 1999, 2000a, 2000b, 2004b, 2004d, 2005b, 2007a). These observations, together with information on the lack of evidence for direct toxicity or novel exposure to the CP4 EPSPS protein, have lead regulatory authorities to conclude that GE plants expressing CP4 EPSPS have no more potential to adversely affect other organisms than their non-transformed counterparts (CFIA 1995, 1998, EFSA 2003, 2004a, 2004b, 2005a, 2005b, 2006b, 2006c, 2008a, 2008b, 2009a, 2009b, OGTR 2003a, 2003b, 2006, USDA APHIS 1994, 1995b, 1995d, 1997a, 1997c, 1998b, 1999, 2000a, 2000b, 2004b, 2004d, 2005b, 2007a).

COMPOSITIONAL ANALYSIS OF CP4 EPSPS PLANTS

Detailed compositional analysis is a scientifically rigorous component of the characterization of GE plants and is a regulatory requirement for GE food and feed safety approvals (OECD 1992; WHO 1995, FAO/WHO 1996, EFSA 2006A, Codex 2003a, 2003b). The choice of analyses conducted depends on the nature of the product and its intended uses. Glyphosate tolerant GE crops have all undergone proximate analysis (crude protein, crude fat, fiber, moisture and ash). Detailed analyses of fatty acid and amino acid composition have also been conducted, as well as analyses of important secondary metabolites that have toxic or anti-nutritional properties (e.g., glucosinolates and erucic acid in canola, trypsin inhibitors in soybean). The data collected are useful as indicators of the presence or absence of any unintended changes to the transformed plant (Codex 2003a, 2003b, Nickson and Hammond 2002, Nida *et al.* 1996, Padgette *et al.* 1996, Ridley *et al.* 2002, Taylor *et al.* 1999).

Summary data from proximate analyses are presented for representative transformation events in Table 4 (see Annex II for additional data). Proximate analysis was selected here as a compositional indictor of unintended effects because it was performed for all events regardless of the properties of the transformed plants or their intended uses.

The results of the proximate analyses considered here show that the plants transformed with CP4 EPSPS are largely equivalent to their conventional comparators in terms of these compositional parameters. In 80% of the proximate comparisons summarized in Table 4 there were no statistical differences between the GE plants and their comparators. In 20% of comparisons, where statistically significant differences were observed, these differences all fell within the range of known values for the crop species (when reference ranges are available). In six instances where statistically significant compositional differences were reported, they were not repeated in replicate trials, suggesting the differences may not be due to true genetic differences rather may reflect the role of random environmetal variation or experimental artifacts. In all cases, the subsequent regulatory analyses did not consider these differences to be meaningful in the context of environmental safety (see Annex II and the references therein).

Considering data across species and events, there were no patterns of consistent or reliable changes in proximate composition. This indicates that the expression of CP4 EPSPS did not have any biologically significant effect on the gross metabolism of the transformed plants.

CONCLUSION

The CP4 EPSPS protein expressed in approved GE events is functionally equivalent to endogenous plant EPSPS enzymes with the exception of its reduced affinity for the glyphosate molecule. The *cp4 epsps* gene, which encodes CP4 EPSPS, was isolated from a

Table 4. Summary of proximate analyses for representative CP4 EPSPS events (see Annex II for additional information and references).

Species	Event	Reference	Oil/Fat	Protein	Ash	Fiber	Carb.	Moist./ Dry matter
Beta vulgaris	GTSB77	USDA APHIS 1998b, ANZFA 2001		X	X	X	X	X
				X	X	X	X	X
				X	X	X	X	X
Brassica napus	GT73	CFIA 1995	X	X	X	X	X	X
			X	X	X	X	X	X
Brassica rapa	ZSR500/502/503	CFIA 1998	X	-	X	+		
			-	X	X	+		
			-	-	-	+		
Glycine max	GTS 40-3-2	Taylor *et al*. 1999	X	X	X	X	X	X
			X	X	X	X	X	X
		Padgette *et al*. 1996	+	X	+	X	-	X
			X	X	X	X	X	X
Gossypium hirsutum	MON1445.1698	Nida *et al*. 1996, USDA APHIS 1995b	X	+	X		-	X
			+	+	+		-	X
			X	+	X		-	X
			X	X	X		-	X
Medicago sativa	J101 and J163	USDA APHIS 2004c	X	X	X	X	X	X
			X	X	X	+	X	X
			X	X	+	+	-	X
Zea mays	NK603	Ridley *et al*. 2002	X	X	X	X	X	X
			+	X	X	X	X	X
			X	X	X	X	X	X
			X	X	X	X	X	X

X indicates no significant difference between the GE event and its comparator.
+ indicates the proximate was higher in the GE plant than control.
- indicates the proximate was lower than control.

common soil bacterium. EPSPS proteins are universally present in plants and microorganisms and, although their sequences are variable, their chemical function is highly specific and conserved. Data from regulatory submissions and peer reviewed publications provide a weight of evidence that CP4 EPSPS, as expressed in GE plants, has negligible impact on the phenotypes of plants beyond conferring the trait of glyphosate tolerance. After numerous environmental risk assessments on a range of plant species expressing the CP4 EPSPS protein, data indicate no correlation between CP4 EPSPS protein expression and any increased tendency for persistence or spread in the environment, alterations in reproductive biology affecting gene flow, or negative impacts on other organisms in the environment. Although the introduction of glyphosate-tolerant crop plants has the potential to complicate the management of herbicide-tolerant volunteers or weeds, there is no evidence to indicate that expression of the CP4 EPSPS protein has negatively impacted the effectiveness of other non-glyphosate-containing herbicides or other weed management options, such as tillage or other mechanical means of weed control.

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ANNEX I: SUMMARY OF CP4 EPSPS PROTEIN EXPRESSION DATA

The tables that follow present summary data from peer-reviewed publications and regulatory submissions. Additional information on collection and sampling methodologies can be found in the referenced sources.

Note: Expression values are represented in ug/g fresh weight unless noted otherwise. NA = Not Available

Table I.1. CP4 EPSPS protein expression data from *Beta vulgaris* events.

Event	Reference Source	Early Leaf [1]		Top[2]		Brei[3]	
		Mean	Range	Mean	Range	Mean	Range
H7-1	USDA APHIS 2003	NA	NA	161	112-201	181	145-202
	CFIA 2005	NA	NA	122	92-143	104	91-124
GTSB77	USDA 1998a, FSANZ 2005	145	130-179	285	249-370	54	46-64
	USDA 1998a, FSANZ 2005	NA	NA	190	134-273	63	50-76
	FSANZ 2005	NA	NA	172	126-193	47	32-60

1 Early Leaf = the youngest fully developed leaf was sampled at the 6-12 leaf stage.
2 Top = sampling of the leaf (immediately prior to harvest for GTSB 77).
3 Brei = A preparation of the root using a sugarbeet saw.

Table I.2. CP4 EPSPS protein expression data from *Brassica napus* events.

Event	Reference Source	Leaf		Seed	
		Mean	Range	Mean	Range
GT73	ANZFA 2000a	34	28-37	49	44-51
	ANZFA 2000a	NA	NA	18	16-22
	USDA APHIS 1998c ANZFA 2000a	NA	NA	28	18-47
	USDA APHIS 1998c	25	20-30	21	14-29
	USDA APHIS 1998c	27	16-70	28	17-37
GT200	USDA 2001[1]	NA	NA	34	26-42
	USDA 2001[1]	31	22-37	51	48-56

1 For this event data was collected for plants that were heterozygous for the transformation event and plants that were homozygous for the transformation event.

Table I.3. CP4 EPSPS protein expression data from *Brassica rapa* events.

Event	Reference Source	Seed: Range of Means
ZSR500	CFIA 1998	32-53
ZSR502	CFIA 1998	14-53
ZSR503	CFIA 1998	25-43

Table I.4. CP4 EPSPS protein expression data from *Glycine max* event GTS 40-3-2.

Reference Source	Leaf (one month)		Leaf (second month)		Seed	
	Mean	Range	Mean	Range	Mean	Range
USDA APHIS 1993	443	251-789	264	46-480	288	186-395
	495	474-526	657	523-798	239	179-303

Table I.5. CP4 EPSPS protein expression data from *Glycine max* event MON89788.

Reference Source	OSL1[1]		OSL2		OSL3		OSL4		Grain		Root		Forage	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
USDA 2007a	54	40-66	60	42-80	58	40-79	75	60-110	140	98-170	22	13-38	59	41-94

1 OSL = Over Season Leaves collected at the following developmental stages: OSL1 = V3-V4 growth stage; OSL2= V6-V8; OSL3= V10-V12; OSL4 = V14-V16.

Table I.6. CP4 EPSPS protein expression data from *Medicago sativa* events.

Reference Source	J101[1]		J163[1]		J101 x J163[1]	
	Mean	Range	Mean	Range	Mean	Range
USDA APHIS 2004c	276	220-340	317	270-380	312	260-390
	238	160-340	223	140-340	192	120-310

1 Data from forage tissue.

ANNEX II: SUMMARY OF COMPOSITIONAL ANALYSES OF GE PLANTS EXPRESSING CP4 EPSPS.

The tables that follow present summary data from peer reviewed publications and regulatory submissions. Additional information can be found in the referenced sources.

Table II.1. Proximate analysis of top tissue (aboveground tissue) from *Beta vulgaris* event GTSB77 (USDA APHIS 1998b). [1]

Analysis	Control Sample		GTSB77		Literature Range[3]
	Mean[2]	Range[2]	Mean[2]	Range[2]	
Crude Ash[4]	21.69	14.10-25.78	20.56	15.82-25.87	11.5-34.4
Crude Fibre[5]	10.52	9.59-11.70	10.64	9.03-12.40	5.9-15.9
Crude Protein[6]	15.56	12.88-16.88	16.13	13.69-17.81	8.4-23.2
Crude Fat[7]	2.22	1.47-3.17	2.19	1.43-3.07	0-4.7
Dry Matter[8]	14.37	12.95-16.43	13.99	12.76-16.50	16.0-20.0
Soluble Carbohydrates[9]	49.98	45.03-61.41	50.52	46.06-57.94	38.3-64.5

1 Data from Europe 1995 field trials.
2 n=6, all analyses conducted in triplicate and all values given on a dry matter basis (percent of dry weight) except dry matter.
3 For a description of how these values were obtained, see the original reference.
4 Crude Ash was determined using an oven method.
5 Crude fibre was determined using the Weende method.
6 Crude protein was determined using a total nitrogen value using a Kjeldahl method.
7 Crude fat was determined using a soxhlet method.
8 Dry matter was determined using an oven method.
9 Carbohydrate calculation was based on Plantedirecktoratet bek. #19 13/1-92.

Table II.2. Proximate analysis of top tissue from *B. Vulgaris* event GTSB77 (USDA APHIS 1998b). [1]

Analysis	Control Sample		GTSB77		Literature Range[3]
	Mean[2]	Range[2]	Mean[2]	Range[2]	
Crude Ash[4]	21.99	18.70-24.79	20.56	15.82-25.87	11.5-34.4
Crude Fibre[5]	9.18	8.46-9.84	10.64	9.03-12.40	5.9-15.9
Crude Protein[6]	13.00	9.45-16.24	16.13	13.69-17.81	8.4-23.2
Crude Fat[7]	2.56	2.06-3.26	2.19	1.43-3.07	0-4.7
Dry Matter[8]	14.79	11.93-17.41	13.99	12.76-16.50	16.0-20.0
Soluble Carbohydrates[9]	53.27	49.78-55.13	50.52	46.06-57.94	38.3-64.5

1 Data from Europe 1996 field trials.
2 n=6, all analyses conducted in triplicate and all values given on a dry matter basis (percent of dry weight) except dry matter.
3 For a description of how these values were obtained, see the original reference.
4 Crude Ash was determined using an oven method.
5 Crude fibre was determined using the Weende method.
6 Crude protein was determined using a total nitrogen value using a Kjeldahl method.
7 Crude fat was determined using a soxhlet method.
8 Dry matter was determined using an oven method.
9 Carbohydrate calculation was based on Plantedirecktoratet bek. #19 13/1-92.

Table II.3. Proximate analysis of top tissue from *B. Vulgaris* event GTSB77 (USDA APHIS 1998b). [1]

Analysis	Control Sample		GTSB77		Literature Range[3]
	Mean[2]	Range[2]	Mean[2]	Range[2]	
Crude Ash[4]	20.6	18.3-24.3	21.6	16.2-28.2	11.5-34.4
Crude Fibre[5]	8.46	6.11-10.4	8.76	6.56-10.7	5.9-15.9
Crude Protein[6]	16.1	10.5-18.4	14.7	10.0-18.3	8.4-23.2
Crude Fat[7]	0.79	0.73-1.03	0.92	0.76-2.16	0-4.7
Dry Matter[8]	15.3	13.9-16.5	16.3	14.9-19.6	16.0-20.0
Soluble Carbohydrates[9]	54	47.0-62.3	53.1	45.0-61.4	38.3-64.5

1 Data from USA 1996 field trials.
2 n=5, except for crude ash conducted in duplicate (n=10) all values given on a dry matter basis (percent of dry weight)except dry matter.
3 For a description of how these values were obtained, see the original reference.
4 Crude Ash was determined using an oven method.
5 Crude fibre was determined using the Weende method.
6 Crude protein was determined using a total nitrogen value using a Kjeldahl method.
7 Crude fat was determined using a soxhlet method.
8 Dry matter was determined using an oven method.
9 Carbohydrate calculation was based on Plantedirecktoratet bek. #19 13/1-92.

Table II.4. Proximate analysis of root tissue from *B. Vulgaris* event GTSB77 (USDA APHIS 1998b). [1]

Analysis	Control Sample		GTSB77		Literature Range[3]
	Mean[2]	Range[2]	Mean[2]	Range[2]	
Crude Ash[4]	5.47	4.58-6.26	6.62	4.76-9.02	3.3-17.7
Crude Fibre[5]	4.10	2.76-5.01	3.96	3.28-4.72	3.4-7.4
Crude Protein[6]	6.28	3.41-9.54	5.60	2.43-8.04	1.2-12.4
Dry Matter[7]	19.40	17.8-22.6	21.10	19.4-22.6	23.00
Soluble Carbohydrates[8]	84.1	80.3-87.2	84.1	79.0-88.1	67.3-90.9

1 Data from USA 1996 field trials.
2 n=5, except for crude ash conducted in duplicate at 2 of 5 sites (n=7). All values given on a dry matter basis (percent of dry weight) except dry matter.
3 For a description of how these values were obtained, see the original reference.
4 Crude Ash was determined using an oven method.
5 Crude fibre was determined using the Weende method.
6 Crude protein was determined using a total nitrogen value using a Kjeldahl method.
7 Dry matter was determined using an oven method.
8 Carbohydrate calculation was based on Plantedirecktoratet bek. #19 13/1-92.

Table II.5. Proximate analysis of root tissue from *B. Vulgaris* event GTSB77 (USDA APHIS 1998b). [1]

Analysis	Control Sample		GTSB77		Literature Range[3]
	Mean[2]	Range[2]	Mean[2]	Range[2]	
Crude Ash[4]	3.42	2.71-4.94	3.40	2.66-5.08	3.3-17.7
Crude Fibre[5]	4.10	3.47-5.22	3.97	3.09-5.33	3.4-7.4
Crude Protein[6]	6.25	4.81-8.19	6.25	4.94-7.88	1.2-12.4
Dry Matter[7]	20.46	14.05-23.48	20.45	13.57-23.12	23.00
Soluble Carbohydrates[8]	86.25	81.65-88.89	86.34	81.69-88.72	67.3-90.9

1 Data from Europe 1995 field trials.
2 n=6, all analyses conducted in triplicate and all values given on a dry matter basis (percent of dry weight) except dry matter.
3 For a description of how these values were obtained, see the original reference.
4 Crude Ash was determined using an oven method.
5 Crude fibre was determined using the Weende method.
6 Crude protein was determined using a total nitrogen value using a Kjeldahl method.
7 Dry matter was determined using an oven method.
8 Carbohydrate calculation was based on Plantedirecktoratet bek. #19 13/1-92.

Table II.6. Proximate analysis of root tissue from *B. Vulgaris* event GTSB77 (USDA APHIS 1998b). [1]

Analysis	Control Sample		GTSB77		Literature Range[3]
	Mean[2]	Range[2]	Mean[2]	Range[2]	
Crude Ash[4]	2.53	1.95-3.22	2.51	2.09-3.35	3.3-17.7
Crude Fibre[5]	4.19	3.87-4.60	4.15	3.88-4.62	3.4-7.4
Crude Protein[6]	4.26	3.02-5.44	4.30	3.02-5.18	1.2-12.4
Dry Matter[7]	23.88	19.18-26.37	23.93	19.53-26.22	23.00
Soluble Carbohydrates[8]	89.01	87.12-91.06	89.03	87.59-90.87	67.3-90.9

1 Data from Europe 1996 field trials.
2 n=6, all analyses conducted in triplicate and all values given on a dry matter basis (percent of dry weight) except dry matter.
3 For a description of how these values were obtained, see the original reference.
4 Crude Ash was determined using an oven method.
5 Crude fibre was determined using the Weende method.
6 Crude protein was determined using a total nitrogen value using a Kjeldahl method.
7 Dry matter was determined using an oven method.
8 Carbohydrate calculation was based on Plantedirecktoratet bek. #19 13/1-92.

Table II.7. Proximate analysis of root tissue from *B. Vulgaris* event GTSB77 (ANZFA 2001). [1]

Roots/Brei	Control		GTSB77 (untreated)		Literature Range
	Mean	Range	Mean	Range	
Crude Ash					
1995 Europe	3.4	2.7-4.9	3.4	2.7-5.1	1.1-17.7
1996 Europe	2.5	2.0-3.2	2.5	2.1-3.4	
1996 USA	5.5	4.6-6.3	6.6	4.8-9.0	
1997 Europe	2.7	2.0-3.8	2.7	2.0-4.0	
Crude Fibre					
1995 Europe	4.1	3.5-5.2	4.0	3.1-5.3	2.9-7.4
1996 Europe	4.2	3.9-4.6	4.2	3.9-4.6	
1996 USA	4.1	2.8-5.0	4.0	3.3-4.7	
1997 Europe	4.2	3.7-4.7	4.2	3.3-5.1	
Invert Sugar					
1995 Europe	1.7	0.3-3.7	1.8	0.4-4.24	0.3-2.7
1996 Europe	0.4	0.3-0.5	0.4	0.3-0.5	
1996 USA	n/d	n/d	n/d	n/d	
1997 Europe	0.6	0.3-1.7	0.7	0.3-2.6	
Amino Nitrogen					
1995 Europe	2.8	2.0-4.0	2.9	2.0-3.9	0.9-5.1
1996 Europe	1.6	0.7-2.8	1.6	0.8-2.5	
1996 USA	5.6	2.7-7.6	5.7	3.4-7.2	
1997 Europe	2.6	1.0-4.3	2.5	0.8-3.8	
Crude Protein					
1995 Europe	6.2	48-8.2	6.3	4.9-7.9	1.2-12.4
1996 Europe	4.3	3.0-5.4	4.3	3.0-5.2	
1996 USA	6.3	3.4-9.5	5.6	2.4-8.0	
1997 Europe	5.0	3.1-6.9	4.9	3.0-6.6	
Dry Matter					
1995 Europe	20.5	14.1-23.5	20.5	13.6-23.1	19.8-23.0
1996 Europe	23.9	19.2-26.4	23.9	19.5-26.2	
1996 USA	19.4	17.8-22.6	21.1	19.4-22.6	
1997 Europe	22.7	20.9-24.9	22.4	20.2-24.4	
Carbohydrate					
1995 Europe	86.3	81.7-88.9	86.3	81.7-88.7	67.3-91.0
1996 Europe	89.0	87.1-91.1	89.0	87.6-90.9	
1996 USA	84.1	80.3-87.2	84.1	79.0-88.1	
1997 Europe	88.1	84.9-91.0	88.2	85.1-91.1	
Polarisation					
1995 Europe	14.4	8.4-17.4	14.5	7.9-17.2	10.8-20.7
1996 Europe	17.3	13.8-19.4	17.3	14.1-19.4	
1996 USA	14.8	12.9-17.1	14.6	12.7-16.2	
1997 Europe	16.6	14.7-18.9	16.2	14.3-18.5	
Sodium					
1995 Europe	1.7	0.5-3.1	1.8	0.4-3.5	0.4-5.5
1996 Europe	0.5	0.3-0.8	0.5	0.2-0.8	
1996 USA	1.5	1.0-2.3	1.5	1.3-1.9	
1997 Europe	0.7	0.3-1.6	0.9	0.4-2.2	
Potassium					
1995 Europe	5.3	4.6-5.9	5.3	4.2-6.0	4.2-10.2
1996 Europe	4.9	4.1-6.0	5.0	4.0-6.4	
1996 USA	8.2	6.8-11.7	8.0	6.7-11.5	
1997 Europe	4.6	3.8-6.2	4.7	3.9-6.3	

1 All values given in g/100g dry weight except dry matter and polarization (g/100g fresh weight). Sodium, Potassium, invert sugar and Amino Nitrogen expressed as mmol/100g fresh weight.

Table II.8. Protein Content of *Brassica napus* event GT73 (Monsanto 2002). [1]

Sample Year	GT77		Westar (control)	
	Mean	Range	Mean	Range
1992	42.0	38.5-44.9	41.1	38.4-42.9
1993	41.2	38.3-45.0	41.2	38.3-45.0

1 values are % of defatted meal, ≤ 3% moisture basis.

Table II.9. Proximate Values of seed from *Brassica napus* event GT73 (Monsanto 2002). [1]

Sample Year	GT77		Westar (control)	
	Mean	Range	Mean	Range
1992	45.2	43.2-48.8	44.8	41.9-47.7
1993	45.8	43.7-47.1	45.1	42.4-47.3

1 Values are % of whole seed, ≤ 3% moisture basis.

Table II.10. Protein Content of *Brassica napus* event GT73 (Monsanto 2002). [1]

Sample		GT77		Westar (control)	
		Mean	Range	Mean	Range
% Fiber	1992	7.83	7.08-8.79	8.21	7.16-9.90
	1993	8.36	7.98-8.77	8.62	8.07-9.59
% Ash	1992	3.78	3.50-4.16	3.68	3.44-3.91
	1993	4.00	3.72-4.47	4.07	3.58-4.26
% Moisture[2]	1992	4.39	4.00-4.77	4.39	3.69-4.86
	1993	9.22	8.49-9.99	10.4	8.4-11.6
% Carbohydrate (calculated)	1992	24.6	23.0-26.9	26.4	23.6-28.0
	1993	26.1	24.4-27.1	26.4	25.8-27.9

1 All results are reported on a dry weight basis except moisture. Data are from field trials in 1992 and 1993.
2 Seed were pre-dried in 1992. In 1993 moisture analysis was performed on seed as received from the field.

Table II.11. Proximate Values of seeds from *Glycine max* event GTS 40-3-2 (Taylor *et al.* 1999). [1]

Characteristic		A5403 (control)		GTS 40-3-2	
		Mean	Range	Mean	Range
Protein	1992	41.01	37.46-44.90	40.35	36.42-44.71
	1993	41.4	40.39-42.32	41.43	39.35-44.14
Ash	1992	5.18	4.61-5.52	5.34	4.73-5.91
	1993	5.31	5.01-5.94	5.35	5.04-5.81
Moisture(g/100g fresh weight)	1992	12.68	11.10-14.30	10.56	7.67-22.65
	1993	5.73	5.18-6.19	5.74	5.32-6.20
Oil	1992	19.8	17.40-21.84	20.41	18.19-22.19
	1993	19.89	18.67-20.57	20.53	19.01-22.17
Fiber	1992	6.35	5.86-6.52	6.44	6.13-7.11
	1993	7.36	6.63-8.10	6.86	5.59-7.66
Carbohydrates	1992	34.01	32.36-35.26	33.86	32.11-35.73
	1993	33.38	31.57-35.08	32.67	27.86-35.32

1 All values are reported as percent (%) of dry weight except Moisture.

Table II.12. Composition of Soybean seeds from *Glycine max* event GTS 40-3-2 (Padgette *et al.* 1996). [1]

Characteristic		A5403 (control)		GTS 40-3-2	
		Mean	Range	Mean	Range
Protein	1992	41.6	37.5-44.6	41.4	37.0-45.0
	1993	41.5	39.7-43.35	41.4	39.6-43.2
Ash	1992	5.041	4.29-5.34	5.242	4.75-5.57
	1993	5.36	4.99-5.88	5.43	5.21-5.87
Moisture (g/100g fresh weight)	1992	8.12	7.55-8.73	8.12	7.74-8. 85
	1993	6.12	5.30-6.49	6.34	6.10-6.59
Fat	1992	15.521	14.10-18.63	16.282	14.04-19.53
	1993	20.11	18.46-21.42	20.42	18.37-23.31
Fiber	1992	7.13	5.91-7.89	6.87	5.50-7.43
	1993	6.71	5.74-7.37	6.63	5345-7.37
Carbohydrates	1992	38.11	33.9-41.3	37.12	32.1-40.0
	1993	33.0	29.3-34.8	32.7	27.6-35.0

1 All values are reported as percent (%) of dry weight except for moisture.
2 Indicates a statistically significant difference.

Table II.13. Composition of cottonseed from *Gossypium hirsutum* event MON 1445 (Nida *et al.* 1996). [1]

Characteristic		C312 (control)		MON 1445	
		Mean	Range	Mean	Range
Protein %	1993	27.8	24.6-28.9	29.6[2]	25.6-31.3
	1994	28.8	27.0-30.6	30.6[2]	28.2-31.9
Fat %	1993	23.3	20.5-24.8	23.8	19.5-26.1
	1994	24.4	23.8-25.5	25.3[2]	24.6-26.7
Ash %	1993	4.5	4.1-4.9	4.7	4.2-5.2
	1994	4.4	3.7-4.9	4.51	3.8-5.0
Carbohydrates %	1993	44.4	41.9-46.2	41.9[2]	39.2-44.0
	1994	42.4	41.0-44.4	39.6[2]	38.0-42.0
Moisture Fiber	1993	11.6	9.1-14.1	11.1	9.0-13.0
	1994	6.7	5.5-7.4	7.5	5.8-13.5

1 All values reported as percent (%) of dry weight except moisture.
2 Statistically significant difference from control.

Table II.14. Composition of forage from *Medicago sativa* events J101/J163 (USDA APHIS 2004c).

Analyte (%DW)[1]	Line	Mean	Range	Commercial Reference Range
Acid detergent fiber	Control	25.79	18.81-33.47	23.12-33.39
	J101	26.83	21.65-32.38	
	J163	28.31	20.00-39.67	
	J101 x J163	27.01	22.09-33.91	
Lignin	Control	5.07	1.64-8.10	3.86-9.65
	J101	5.78	3.86-9.11	
	J163	6.01	3.94-8.13	
	J101 x J163	5.31	3.48-8.16	
Neutral detergent fiber	Control	28.09	22.25-32.07	26.53-35.72
	J101	29.49	25.22-34.05	
	J163	30.94	24.49-43.57	
	J101 x J163	30.64	NA	
Ash	Control	11.31	8.44-15.04	8.58-15.25
	J101	13.48	8.55-28.59	
	J163	13.23	8.87-26.13	
	J101 x J163	14.41	8.26-32.50	
Carbohydrates	Control	65.08	55.44-73.53	58.03-74.38
	J101	63.32	50.30-73.64	
	J163	63.29	51.37-73.39	
	J101 x J163	63.10	48.03-74.71	
Moisture (% FW)	Control	76.77	70.70-84.20	70.90-82.10
	J101	77.11	71.10-82.40	
	J163	77.01	71.00-83.30	
	J101 x J163	75.78	70.70-83.10	
Protein	Control	21.35	16.02-28.20	15.29-25.81
	J101	21.01	15.44-24.89	
	J163	21.21	15.80-26.32	
	J101 x J163	20.49	15.53-27.11	
Total Fat	Control	2.26	1.45-3.58	1.33-3.15
	J101	2.19	1.27-4.01	
	J163	2.27	1.21-3.68	
	J101 x J163	2.12	1.5-3.13	

1 All values are reported as percent (%) of dry weight except for moisture.

Table II.15. Composition of grain from *Zea mays* event NK603 (Ridley *et al.* 2002). [1]

Component	NK603 (1998)		Control (1998)		NK603 (1999)		Controls (1999)		Commercial Hybrids
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	
Protein	12.20	10.30-14.77	12.60	11.02-14.84	12.07	10.23-13.92	11.34	10.13-13.05	7.77-12.99
Total Fat	3.61	2.92-3.94	3.67	2.88-4.13	4.16	3.87-4.48	3.60	3.24-3.84	2.57-4.95
Ash	1.45	1.28-1.62	1.49	1.32-1.75	1.38	1.23-1.65	1.34	1.25-1.50	1.02-1.94
ADF[2]	3.72	3.14-5.17	3.60	2.79-4.28	3.21	2.63-3.87	3.03	2.30-3.68	2.46-6.33
NDF[3]	10.06	7.89-12.53	10.00	8.25-15.42	10.08	8.5-12.00	10.57	9.35-11.63	8.45-14.75
Carbohydrates	82.76	80.71-84.33	82.29	80.23-83.70	82.39	80.49-84.57	83.73	81.93-84.92	82.18-88.14
Moistures %FW	11.13	9.01-13.30	11.78	8.56-14.80	7.62	7.34-7.82	7.81	7.55-8.28	7.43-9.94

1 All values reported as % dry weight except for moisture.
2 ADF=Acid Detergent Fiber.
3 NDF=Neutral Detergent Fiber.

Table II.16. Composition of forage from *Zea mays* event NK603 (Ridley *et al*. 2002). [1]

Component	NK603 (1998)		Control (1998)		NK603 (1999)		Controls (1999)		Commercial Hybrids
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	
Protein	7.14	5.57-8.98	6.8	5.49-8.69	8.71	6.37-10.79	8.86	7.03-10.96	4.98-11.56
Ash	3.81	2.36-6.80	4.02	2.46-6.28	4.38	2.82-6.44	4.44	3.35-5.80	2.43-9.64
ADF[2]	25.72	17.01-33.52	24.84	19.53-31.83	23.53	19.27-26.13	22.07	19.39-26.90	17.54-38.31
NDF[3]	42.09	36.39-49.03	42.45	35.44-53.24	37.34	31.77-44.35	37.75	34.85-41.86	27.93-54.75
Total Fat	2.36	0.69-3.64	2.17	0.61-3.42	3.24	2.06-4.49	3.05	2.09-4.02	1.42-4.57
Carbohydrates	86.71	82.68-90.32	87.11	83.71-90.03	83.67	80.43-87.53	83.65	80.64-85.52	76.50-87.29
Moistures %FW	67.02	60.30-75.00	66.24	61.00-73.70	67.53	61.60-75.20	66.30	60.40-72.60	56.50-80.40

1 All values reported as % dry weight except for moisture.
2 ADF=Acid Detergent Fiber.
3 NDF=Neutral Detergent Fiber.

Table II.17. Composition of grain from *Zea mays* event NK603 (Ridley *et al*. 2002). [1]

Component	NK603		Control		Commercial Range
	Mean	Range	Mean	Range	
Ash	1.44	1.28-1.75	1.49	1.32-1.75	0.8-1.8
Carbohydrates	82.59	80.71-84.33	82.26	80.23-83.70	83.1-89.6
ADF[2]	3.79	3.14-5.17	3.70	2.79-4.28	2.3-5.7
NDF[3]	10.38	7.89-12.53	10.32	8.25-15.42	8.2-16.1
Moisture (%FW)	11.08	9.01-13.30	11.76	8.56-14.8	6.1-15.6
Total Fat	3.54	2.92-3.94	3.59	2.88-4.13	1.7-4.3
Protein	12.43	10.30-14.77	12.66	11.02-14.84	6.7-13.4

1 All values reported as % dry weight except for moisture.
2 ADF=Acid Detergent Fiber.
3 NDF=Neutral Detergent Fiber.