A Review of the Environmental Safety of the PAT 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 phosphinothricin-N-acetyl transferase (PAT) produced in genetically engineered (GE) plants by genes isolated from *Streptomyces viridochromogenes* (*pat* gene) or *Streptomyces hygroscopicus* (*bar* gene) and presents a summary statement about the environmental safety of this protein. All sources of information reviewed herein were publicly available and include: dossiers presented to regulatory authorities; decision summaries prepared by regulatory authorities; peer-reviewed literature; and product summaries prepared by product developers. Many GE plants contain the *pat* gene for use as a selectable marker during development. In those cases, there are one or more additional transgenes contained in the plant and the final product is not necessarily glufosinate tolerant. Although this document will not address these additional genes and phenotypes, their presence should be noted when looking at data on the GE plants that express PAT.

Environmental risk assessments related to the introduction of 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, OECD 2006*). 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 cause relevant adverse effects can subsequently be evaluated for likelihood and consequence.

To date, regulatory authorities in 11 different countries have issued approvals for the environmental release of GE plants expressing the PAT protein, either by itself or in combination with other GE traits. This represents approximately 38 transformation events and includes 8 species of plant: *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), *Cichorium intybus* (chicory), *Glycine max* L. (soybean), *Gossypium hirsutum* L. (cotton), *Oryza sativa* L. (rice) and *Zea mays* L. (maize). These regulatory analyses have generally considered three categories of potential harm: (1) the PAT protein may have an adverse impact on non-target organisms; (2) transformation of the host plant and subsequent expression of the PAT protein may alter the characteristics of the plant, resulting in adverse environmental impacts (e.g. increased weediness); and (3) introgression of the gene encoding the PAT protein into a sexually compatible plant species may alter that species resulting in adverse environmental impacts (e.g. establishment of new weedy populations) (*CFIA 1995a, 1995b, 1996a, 1996b, 1996c, 1996d, 1996e, 1996f, 1998a, 1998b, 1998c, 1999, 2002a, 2002b, 2005, 2006, EC 1996, 1997, 1998, 2001, Japan BCH 1996a, 1996b, 1996c, 1997a, 1997b, 1997c, 1997d, 1997e, 1998, 1999a, 1999b, 1999c, 1999d, 2002, 2005, 2006a, 2006b, 2006c, 2006d, 2006e, 2007a, 2007b, 2008, 2009, 2010, OGTR 2002, 2003, 2006, Philippines 2005, USDA APHIS 1995a, 1995c, 1995f, 1996b, 1996b, 1996c, 1996e, 1997c, 1997f, 1998a, 1998c, 1998e, 1998g, 1998i, 1998j, 1999a, 1999b, 1999c, 2001b, 2001c, 2002b, 2003c, 2004a, 2004c, 2005, 2006b, USEPA 2001, 2005, 2009a, 2009b*).

Note that environmental effects that may be associated with the use of the herbicide glufosinate in association with GE plants producing PAT are outside the purview of this review.

Key words

PAT, *bar,* phosphinothricin, glufosinate, glufosinate ammonium, herbicide tolerant, genetically engineered, environmental risk assessment

Table 1. Regulatory approvals for the environmental release of GE plants containing PAT protein.

Species	Events/Crosses	Alternate Designations	Source of PAT Gene	United States	Canada	Japan	Australia	European Union	Brazil	Argentina	Colombia	Philippines	South Africa	Uruguay
Beta Vulgaris (sugarbeet)	ACS-BVØØ1-3	T120-7	*Streptomyces viridochromogenes*	X	X									
Brassica napus (oilseed rape/canola)	HCN1Ø		*Streptomyces viridochromogenes*	X	X	X								
	ACS-BNØØ7-1	HCN92	*Streptomyces viridochromogenes*	X	X	X	X							
	ACS-BNØØ4-7 x ACS-BNØØ1-4	MS1, RF1 ; PGS1	*Streptomyces hygroscopicus*	X	X	X	X							
	ACS-BNØØ4-7 x ACS-BNØØ2-5	MS1, RF2 ; PGS2	*Streptomyces hygroscopicus*	X	X	X	X							
	ACS-BNØØ5-8 x ACS-BNØØ3-6	MS8 x RF3	*Streptomyces hygroscopicus*	X	X	X	X							
	PHY14, PHY35		*Streptomyces hygroscopicus*			X								
	PHY36		*Streptomyces hygroscopicus*			X								
	ACS-BNØØ8-2	T45, HCN28	*Streptomyces viridochromogenes*	X	X	X	X							
Brassica rapa (bird rape/canola)	HCR-1		*Streptomyces viridochromogenes*		X									
Cichorium intybus (chicory)	RM3-3, RM3-4, RM3-6		*Streptomyces hygroscopicus*	X				X						
Glycine max (soybean)	ACS-GMØØ5-3	A2704-12, A2704-21, A5547-35	*Streptomyces viridochromogenes*	X	X	X			X					
	ACS-GMØØ6-4	A5547-127	*Streptomyces viridochromogenes*	X	X	X			X					
	ACS-GMØØ3-1	GU262	*Streptomyces viridochromogenes*	X										
	ACS-GMØØ1-8, ACS-GMØØ2-9	W62, W98	*Streptomyces hygroscopicus*	X										
Gossypium hirsutum (cotton)	DAS-24236-5	281-24-236	*Streptomyces viridochromogenes*	X										
	DAS 21Ø23-5	3006-210-23	*Streptomyces viridochromogenes*	X										
	DAS 21Ø23-5 x DAS-24236-5		*Streptomyces viridochromogenes*	X					X					
	DAS 21Ø23-5 x DAS-24236-5 x MON-Ø1445-2		*Streptomyces viridochromogenes*	*										
	DAS 21Ø23-5 x DAS-24236-5 x MON-88913-8		*Streptomyces viridochromogenes*	*										
	ACS-GHØØ1-3	LLCotton25	*Streptomyces hygroscopicus*	X			X		X					
	ACS-GHØØ1-3 x MON-15985-7	LLCotton25 x MON15985	*Streptomyces hygroscopicus*	*		X								
Oryza sativa (rice)	ACS-OSØØ1-4, ACS-OSØØ2-5	LLRice06, LLRice62	*Streptomyces hygroscopicus*	X										
	BCS-OSØØ3-7	LLRice601	*Streptomyces hygroscopicus*	X										
Zea mays (maize/corn)	SYN-EV176-9	176	*Streptomyces hygroscopicus*	X	X	X		X		X				
	PH-ØØØ676-7, PH-ØØØ678-9, PH-ØØØ68Ø-2	676, 678, 680	*Streptomyces viridochromogenes*	X										
	DKB-8979Ø-5	B16, DLL25	*Streptomyces hygroscopicus*	X	X	X								
	SYN-BTØ11-1	BT11 (X4334CBR, X4734CBR)	*Streptomyces viridochromogenes*	X	X	X			X	X	X	X	X	X
	SYN-BTØ11-1 x MON-ØØØ21-9	BT11 x GA21	*Streptomyces viridochromogenes*	*	X	X			X					
	SYN-BTØ11-1 x SYN-IR162-4	BT11 x MIR162	*Streptomyces viridochromogenes*	X										
	SYN-BTØ11-1 x SYN-IR162-4 x SYN-IR6Ø4-5	BT11 x MIR162 x MIR604	*Streptomyces viridochromogenes*	X										
	SYN-BTØ11-1 x SYN-IR6Ø4-5	BT11 x MIR604	*Streptomyces viridochromogenes*	*	X									
	SYN-BTØ11-1 x SYN-IR6Ø4-5 x MONØØØ21-9	BT11 x MIR604 x GA21	*Streptomyces viridochromogenes*	*										
	ACS-ZMØØ4-3	CBH-351	*Streptomyces hygroscopicus*	X										
	DAS-Ø6275-8		*Streptomyces hygroscopicus*	X	X	X								
	DAS-59122-7		*Streptomyces viridochromogenes*	X	X	X								
	DAS-59122-7, MON-ØØ6Ø3-6	DAS-59122-7 x NK603	*Streptomyces viridochromogenes*	*	X	X								

Table 1 (continued on next page).

Table 1 (cont'd). Regulatory approvals for the environmental release of GE plants containing PAT protein.

Species	Events/Crosses	Alternate Designations	Source of PAT Gene	United States	Canada	Japan	Australia	European Union	Brazil	Argentina	Colombia	Philippines	South Africa	Uruguay
	DAS-59122-7 x DAS-Ø15Ø7-1 x MON-ØØ6Ø3-6	DAS-59122-7 x TC1507 x NK603	*Streptomyces viridochromogenes*	*	X	X								
	DKB-89614-9	DBT418	*Streptomyces hygroscopicus*	X	X	X				X				
	MON-89Ø34-3 x DAS- Ø15Ø7-1 x MON-88Ø17-3 x DAS-59122-7	MON89034 x TC1507 x MON88017 x DAS-59122-7	*Streptomyces viridochromogenes*	X	X	X								
	ACS-ZMØØ1-9	MS3	*Streptomyces hygroscopicus*	X	X									
	ACS-ZMØØ5-4	MS6	*Streptomyces hygroscopicus*	X										
	MON-ØØ6Ø3-6 x ACS-ZMØØ3-2	NK603 x T25	*Streptomyces viridochromogenes*	*		X								
	ACS-ZMØØ2-1, ACS-ZMØØ3-2	T14, T25	*Streptomyces viridochromogenes*	X	X	X		X	X	X				
	ACS-ZMØØ3-2, MON-ØØ81Ø-6	T25 x MON810	*Streptomyces viridochromogenes*	*		X								
	DAS-Ø15Ø7-1	TC1507	*Streptomyces viridochromogenes*	X	X	X			X	X				
	DAS-Ø15Ø7-1, DAS-59122-7	TC1507 x DAS-59122-7	*Streptomyces viridochromogenes*	*	X	X								
	DAS-Ø15Ø7-1 x MON-ØØ6Ø3-6	TC1507 x NK603	*Streptomyces viridochromogenes*	*	X	X			X	X				

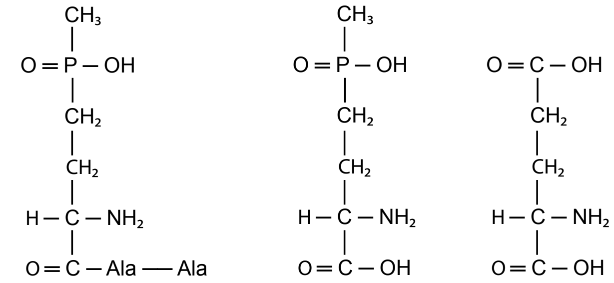
X = Approved for environmental (commercial) release.
* These are stacked events that may be considered approved in the country indicated

ORIGIN AND FUNCTION OF PAT

Phosphinothricin, bialaphos, and glufosinate ammonium

In the early 1970's a previously unknown amino acid was isolated independently from two species of *Streptomyces* by laboratories working in Germany (from *Streptomyces viridochromogenes*) and Japan (from *Streptomyces hygroscopicus*) (*Bayer* et al. *1972, Kondo* et al. *1973, OECD 1999*). Originally seen in a tripeptide with two alanine residues (see Fig. 1), the new amino acid (*L*-2-amino-4-[hydroxyl(methyl)phosphinyl] butyric acid) was given the name phosphinothricin (PT) and the tripeptide called phosphinothricin tripeptide (PTT) or bialaphos[1] (*Bayer* et al. *1972, Hoerlein 1994, Kondo* et al. *1973, OECD 1999*). In Germany, racemic mixtures were produced (*D,L*-phosphinothricin or *D,L*-PPT) and determined to have herbicidal activity. *D,L*-PPT-ammonium, referred to by the common name glufosinate ammonium (GLA) is the active ingredient in herbicide formulations marketed worldwide. In Japan, the bialaphos tripeptide was observed to have herbicidal activity and this has been commercialized as well (*Hoerlein 1994*).

Figure 1. The structure of phosphinothricin, PTT and glutamic acid.



phosphinothricin tripeptide (PTT) | phosphinothricin (PT) | glutamic acid

2

Phosphinothricin inhibits the activity of the glutamine synthetase enzyme (GS) by competitively binding in place of the normal substrate, glutamate (glutamic acid). This prevents the synthesis of *L*-glutamine, which is not only an important chemical precursor for the synthesis of nucleic acids and proteins, but serves as the mechanism of ammonia (NH_3) incorporation for plants (*Hoerlein 1994, OECD 1999, OECD 2002*). Treatment with phosphinothricin causes accumulation of ammonia and cessation of photosynthesis, probably due to the lack of glutamine (*Hoerlein 1994, OECD 1999, OECD 2002*).

ISOLATION AND FUNCTION OF PHOSPHINOTHRICIN ACETYL TRANSFERASE (PAT)

The identification of the GS inhibitor phosphinothricin from *Streptomyces* suggested that these bacteria employ a biochemical mechanism to preserve endogenous GS activity. In the late 1980s,

1 Also sometimes "bilanafos" or "bilanaphos".

2 Figure adapted from Schwartz *et al*. 2004.

two genes were identified independently based on their ability to confer resistance to phosphinothricin inhibition of GS, both of which encode a phosphinothricin acetyl transferase protein (PAT). The bialaphos resistance gene, *bar*, was isolated from *S. hygroscopicus* while the homologous gene from *S. viridochromogenes* was termed *pat* after the function of the enzyme (*OECD 1999a, Thompson* et al. *1987, Wohlleben* et al. *1998*). Both proteins have been used extensively in genetic engineering of crop plants. They both consist of 183 amino acids, with a sequence identity of 85% (*OECD 1999a, Wehrmann* et al. *1996, Wohlleben* et al. *1998*). Importantly, both proteins acetylate phosphinothricin but show no activity with glutamate, which is structurally similar, or with any other amino acids tested, indicating a high specificity (*OECD 1999a, Thompson* et al. *1987, Wehrmann* et al. *1996*). The only recorded differences in activity between the two proteins are minor differences in the optimal pH, and a significantly different affinity for acetyl-coA (a co-substrate); these differences are not expected to be meaningful *in planta* (*OECD 1999a, Wehrmann* et al. *1996*). Because the PAT proteins encoded by *bar* and *pat* are structurally and functionally equivalent, with similar molecular weights, immuno-cross-reactivity, substrate affinity and specificity, they are considered together in this document and will both be referred to as PAT protein.

The PAT enzyme acetylates phosphinothricin at the N-terminus. N-acetyl phosphinothricin has no herbicidal activity, and resistance is therefore conferred through modification of the herbicide rather than the target of its activity (*OECD 1999a, Thompson* et al. *1987, Wehrmann* et al. *1996, Wohlleben* et al. *1998*).

EXPRESSION OF PAT IN PHOSPHINOTHRICIN-TOLERANT GE PLANTS

Data for the level of expression of PAT in phosphinothricin-tolerant GE plants that have obtained regulatory approvals are available in publicly accessible regulatory documents (*ANZFA 2000, 2001a, 2001b, 2002, CFIA 1995a, 1995b, 1996a, 1996b, 1996c, 1996d, 1996e, 1996f, 1998a, 1998b, 1998c, 1999, 2002a, 2002b, 2004, 2005, 2006a, 2006b, EC 1996, 1997, 1998, 2001, EFSA 2005, 2006, 2008a, 2008b, 2009a, 2009b, FSANZ 2003, 2004a, 2004b, 2005a, 2005b, 2008, Japan BCH 1996a, 1996b, 1996c, Health Canada 2006a, 2006b, Japan BCH 1997a, 1997b, 1997c, 1997d, 1997e, 1999a, 1999b, 1999c, 1999d, 2002, 2003, 2005, 2006a, 2006b, 2006c, 2006d, 2006e, 2006f, 2007a, 2007b, 2008, 2009, 2010, OGTR 2003, 2006, Philippines 2005, USDA APHIS 1994a, b, 1995b, 1995d, 1995e, 1996a, 1996d, 1997a, 1997b, 1997d, 1997e, 1998b, 1998d, 1998f, 1998h, 1998k, 1998l, 2000, 2001a, 2002a, 2002c, 2003a, 2003b, 2004b, 2004d, 2006a, USEPA 2001, 2005, 2009a, 2009b*). Tissue types tested and sampling methodologies vary greatly. The most common method uses enzyme-linked immunosorbent assay (ELISA) to quantify the amount of protein present in a given sample, but other methods include an assay for enzymatic activity and the use of Northern blots to quantify mRNA. Normally, one or more samples are collected from plants in field trials or greenhouse experiments and the amount of protein is given as a mean accompanied by either a standard deviation or a range of observed values to show variability. The result is often quantified as a ratio to the dry weight of the sample (e.g. µg PAT/g dry weight), but some reports calculate the ratio to the fresh weight of the sample or to the total extractable protein from the sample (e.g. µg PAT/g total protein).

Variations in methodology for both sample collection and subsequent analysis make direct statistical comparisons of the data inappropriate. However, the weight of evidence suggests PAT protein is expressed at low levels (see Annex I and associated references). The highest reported levels of expression observed in each species using ELISA are reported in Table 2.

Table 2. Highest reported expression level of PAT protein using ELISA.[1]

Species	Event GE Plant	Expression	Tissue	Reference
Beta vulgaris	T120-7	966 ng/g	Top[2]	USDA APHIS 1998b
Brassica napus	Topas19/2 x T45	944 ng/g	Leaf	USDA APHIS 2002a
Glycine max	A5547-127	20202 ± 359[3] ng/g	Seed	Japan BCH 2005f
Gossypium hirsutum	LLCOTTON25	127000 ± 18000[3] ng/g[4]	Cleaned Seed	USDA APHIS 2002c
Oryza sativa	LLRICE62	84700 ng/g[4]	Leaf	CFIA 2006b
Zea mays	DAS-06275-8	935000 ng/g[4, 5]	Leaf	USDA APHIS 2004b

1 These values are not cross-comparable due to differences in sample collection and preparation methodology.
2 Top refers to all above-ground tissue (i.e. leaves and stems).
3 Reported as mean ± standard deviation.
4 Reported as ng/g fresh weight.
5 Represents the highest value in a reported range.

ESTABLISHMENT AND PERSISTENCE OF PAT-EXPRESSING PLANTS IN THE ENVIRONMENT

Familiarity with the biology of the non-transformed or host plant species in the receiving environment is typically the starting point for a comparative environmental risk assessment of a GE plant (*CBD 2000b, Codex 2003a, b, EFSA 2006a, NRC 1989, OECD 1992, 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., PAT) 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

In order to determine if GE plants expressing PAT are phenotypically different than their non-transformed counterparts, a variety of data have been collected and are presented with varying degree of detail in regulatory submissions related to the environmental release of these plants (*CFIA 1995a, 1995b, 1996a, 1996b, 1996c, 1996d, 1996e, 1996f, 1998a, 1998b, 1998c, 1999, 2002a, 2002b, 2004, 2005, 2006a, 2006b, EC 1996, 1997, 1998, 2001, Japan BCH 1996a, 1996b, 1996c, Health Canada 2006a, 2006b, Japan BCH 1997a, 1997b, 1997c, 1997d, 1997e, 1999a, 1999b, 1999c, 1999d, 2002, 2003, 2005, 2006a, 2006b, 2006c, 2006d, 2006e, 2006f, 2007a, 2007b, 2008, 2009, 2010, OGTR 2003, 2006, Philippines 2005, USDA APHIS 1994a, b, 1995b, 1995d, 1995e, 1996a, 1996d, 1997a, 1997b, 1997d, 1997e, 1998b, 1998d, 1998f, 1998h, 1998k, 1998l, 2000, 2001a, 2002a, 2002c, 2003a, 2003b, 2004b, 2004d, 2006a, USEPA 2001, 2005, 2009a, 2009b*). The data that are reported are dependent on the species of plant, but in general include information on gross morphology (e.g. height, number of leaves, number of branches or nodes, etc.), reproductive characteristics including seed production, survival and germination, as well as seedling vigor, overwintering ability, susceptibility to disease and pest pressure, and frequently the potential to volunteer following harvest. Phenotypic analyses may also include agronomic characteristics such as yield and performance in the field (*CFIA 1995a, 1995b, 1996a, 1996b, 1996c, 1996d, 1996e, 1996f, 1998a, 1998b, 1998c, 1999, 2002a, 2002b, 2004, 2005, 2006a, 2006b, EC 1996, 1997, 1998, 2001, Japan BCH 1996a, 1996b, 1996c, Health Canada 2006a, 2006b, Japan BCH 1997a, 1997b, 1997c, 1997d, 1997e, 1999a, 1999b, 1999c, 1999d, 2002, 2003, 2005, 2006a, 2006b, 2006c, 2006d, 2006e, 2006f, 2007a, 2007b, 2008, 2009, 2010, OGTR 2003, 2006, Philippines 2005, USDA APHIS 1994a, b, 1995b, 1995d, 1995e, 1996a, 1996d, 1997a, 1997b, 1997d, 1997e, 1998b, 1998d, 1998f, 1998h, 1998k, 1998l, 2000, 2001a, 2002a, 2002c, 2003a, 2003b, 2004b, 2004d, 2006a, USEPA 2001, 2005, 2009a, 2009b*). Frequently, statistically significant differences in a handful of phenotypic characteristics are reported between GE plants and controls in a given experiment (*CFIA 1995a, 1995b, 1996a, 1996b, 1996c, 1996d, 1996e, 1996f,1998a, 1998b, 1998c, 1999, 2002a, 2002b, 2004, 2005, 2006a, 2006b, EC 1996, 1997, 1998, 2001, Japan BCH 1996a, 1996b, 1996c, Health Canada 2006a, 2006b, Philippines 2005, USDA APHIS 1994a, b, 1995b, 1995d, 1995e, 1996a, 1996d, 1997a, 1997b, 1997d, 1997e, 1998b, 1998d, 1998f, 1998h, 1998k, 1998l, 2000, 2001a, 2002a, 2002c, 2003a, 2003b, 2004b, 2004d, 2006a, USEPA 2001, 2005, 2009a, 2009b*). However, these differences usually are not repeated in multiple experiments and regulatory decisions have concluded that any such differences are likely not due to the expression of the PAT protein and do not represent meaningful differences with respect to the potential for adverse impact to the environment (*CFIA 1995a, 1995b, 1996a, 1996b, 1996c, 1996d, 1996e, 1996f, 1998a, 1998b, 1998c, 1999, 2002a, 2002b, 2005, 2006a, EC 1996, 1997, 1998, 2001, Japan BCH 1996a, 1996b, 1996c, 1997a, 1997b, 1997c, 1997d, 1997e, 1999a, 1999b, 1999c, 1999d, 2002, 2005, 2006a, 2006b, 2006c, 2006d, 2006e, 2006f, 2007a, 2007b, 2008, 2009, 2010, OGTR 2002, 2003, 2006, Philippines 2005, USDA APHIS 1995a, 1995c, 1995f, 1996b, 1996b, 1996c, 1996e, 1997c, 1997f, 1998a, 1998c, 1998e, 1998g, 1998i, 1998j, 1999a, 1999b, 1999c, 2001b, 2001c, 2002b, 2003c, 2004a, 2004c, 2005, 2006b, USEPA 2001, 2005, 2009a, 2009b.*)

WEEDINESS IN AGRICULTURAL ENVIRONMENTS

All of the plant species that have been engineered to express PAT 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*). 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 PAT protein expression and any increased survival or overwintering capacity that would alter the prevalence of volunteer plants in the subsequent growing season (*CFIA 1995a, 1995b, 1996a, 1996b, 1996c, 1996d, 1996e, 1996f, 1998a, 1998b, 1998c, 1999, 2002a, 2002b, 2005, 2006, EC 1996, 1997, 1998, 2001, Japan BCH 1996a, 1996b, 1996c, 1997a, 1997b, 1997c, 1997d, 1997e, 1998, 1999a, 1999b, 1999c, 1999d, 2002, 2005, 2006a, 2006b, 2006c, 2006d, 2006e, 2007a, 2007b, 2008, 2009, 2010, OGTR 2002, 2003, 2006, Philippines 2005, USDA APHIS 1995a, 1995c, 1995f, 1996b, 1996b, 1996c, 1996e, 1997c, 1997f, 1998a, 1998c, 1998e, 1998g, 1998i, 1998j, 1999a, 1999b, 1999c, 2001b, 2001c, 2002b, 2003c, 2004a, 2004c, 2005, 2006b, USEPA 2001, 2005, 2009a, 2009b*). Following-season volunteers expressing PAT may complicate volunteer management programs, particularly if different crop species expressing glufosinate tolerance are planted in consecutive rotations. Alternative options are available for managing glufosinate-tolerant volunteers, including the use of other herbicides and mechanical weed control (*Beckie* et al. *2004, OECD 1997, OECD 2000, OECD 2001, OECD 2003a, OECD 2008, OGTR 2008*).

WEEDINESS IN NON-AGRICULTURAL ENVIRONMENTS

The primary mechanisms by which PAT 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 naturalized (or feral) population of the same crop species or other sexually compatible relatives (*Mallory-Smith and Zapiola, 2008*). Risk assessments for GE plants expressing PAT have considered the potential impacts associated with both types of introduction (*CFIA 1995a, 1995b, 1996a, 1996b, 1996c, 1996d, 1996e, 1996f, 1998a, 1998b, 1998c, 1999, 2002a, 2002b, 2005, 2006, EC 1996, 1997, 1998, 2001, Japan BCH 1996a, 1996b, 1996c, 1997a, 1997b, 1997c, 1997d, 1997e, 1998, 1999a, 1999b, 1999c, 1999d, 2002, 2005, 2006a, 2006b, 2006c, 2006d, 2006e, 2007a, 2007b, 2008, 2009, 2010, OGTR 2002, 2003, 2006, Philippines 2005, USDA APHIS 1995a, 1995c, 1995f, 1996b, 1996b, 1996c, 1996e, 1997c, 1997f, 1998a, 1998c, 1998e, 1998g, 1998i, 1998j, 1999a, 1999b, 1999c, 2001b, 2001c, 2002b, 2003c, 2004a, 2004c, 2005, 2006b, USEPA 2001, 2005, 2009a, 2009b*).

While all plants can be considered weeds in certain contexts, none of the crops for which glufosinate-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 PAT does not have a significant impact on agronomic or compositional traits (including those that are related to weediness), the evidence to date shows that expression of the PAT protein has not resulted in any altered potential for weediness for those GE plant events subjected to environmental risk assessment. PAT expression only affects the ability of the plant to survive if treated with glufosinate. Just as in agricultural environments, other management options to control glufosinate-tolerant plants in non-agricultural environments are available (*Beckie* et al. *2004, OECD 1997, OECD 2000, OECD 2001, OECD 2003a, OECD 2008, OGTR 2008*).

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. The evidence shows that expression of the PAT protein in a range of plant species has not resulted in any alteration to anticipated gene flow. However introgression of glufosinate tolerance into sexually compatible, weedy populations in agricultural or peri-agricultural ecosystems is possible and has the potential to raise management issues (*Mallory-Smith and Zapiola 2008, Warwick* et al. *2007*). In at least one instance, a regulatory decision has geographically limited the release of a herbicide-tolerant GE plant: the environmental approval of *B. rapa* event ZSR500/502 (glyphosate resistance) 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 1998d*). However, no such decisions have been made for plants expressing PAT that are glufosinate-resistant, and all of the publicly available regulatory decisions conclude that the movement of the *pat* gene to wild relatives is not a substantial risk for any of the GE plants that have been considered (C*FIA 1995a, 1995b, 1996a, 1996b, 1996c, 1996d, 1996e, 1996f, 1998a, 1998b, 1998c, 1999, 2002a, 2002b, 2005, 2006a, EC 1996, 1997, 1998, 2001, Japan BCH 1996a, 1996b, 1996c, 1997a, 1997b, 1997c, 1997d, 1997e, 1999a, 1999b, 1999c, 1999d, 2002, 2005, 2006a, 2006b, 2006c, 2006d, 2006e, 2006f, 2007a, 2007b, 2008, 2009, 2010, OGTR 2002, 2003, 2006, Philippines 2005, USDA APHIS 1995a, 1995c, 1995f, 1996b, 1996b, 1996c, 1996e, 1997c, 1997f, 1998a, 1998c, 1998e, 1998g, 1998i, 1998j, 1999a, 1999b, 1999c, 2001b, 2001c, 2002b, 2003c, 2004a, 2004c, 2005, 2006b, USEPA 2001, 2005, 2009a, 2009b*).

ADVERSE IMPACTS ON OTHER ORGANISMS IN THE RECEIVING ENVIRONMENT

The potential for PAT protein expression in GE plants to have an adverse impact on organisms has been considered in regulatory risk assessments using a weight-of-evidence approach (*CFIA 1995a, 1995b, 1996a, 1996b, 1996c, 1996d, 1996e, 1996f, 1998a, 1998b, 1998c, 1999, 2002a, 2002b, 2005, 2006a, EC 1996, 1997, 1998, 2001, Japan BCH 1996a, 1996b, 1996c, 1997a, 1997b, 1997c, 1997d, 1997e, 1999a, 1999b, 1999c, 1999d, 2002, 2005, 2006a, 2006b, 2006c, 2006d, 2006e, 2006f, 2007a, 2007b, 2008, 2009, 2010, OGTR 2002, 2003, 2006, Philippines 2005, USDA APHIS 1995a, 1995c, 1995f, 1996b, 1996b, 1996c, 1996e, 1997c, 1997f, 1998a, 1998c, 1998e, 1998g, 1998i, 1998j, 1999a, 1999b, 1999c, 2001b, 2001c, 2002b, 2003c, 2004a, 2004c, 2005, 2006b, USEPA 2001, 2005, 2009a, 2009b*). These risk assessments have generally considered the potential for the novel protein to be toxic to other organisms, as well as the history of prior environmental exposure to the protein. Toxic proteins are known to act acutely (*Sjoblad* et al. *1992*). Acute, intravenous toxicity experiments in mice show the PAT protein has no toxicity even at doses much higher than would be encountered due to environmental exposure to GE plants expressing the PAT protein (*Herouet* et al. *2005*). In addition, the PAT protein shows no homology to known toxins or allergens and is rapidly digested in experiments simulating gastric environment (*Herouet* et al. *2005*). The *Streptomyces* bacteria which are the source of PAT proteins are widespread in environments around the world, and additional species of *Streptomyces* are known to possess similar enzymatic activity, indicating that PAT protein homologs are likely ubiquitous in the environment and regulatory decisions have concluded that exposure to PAT proteins from GE plants does not represent a potential for adverse impacts on other organisms (*Herouet* et al. *2005, CFIA 1995a, 1995b, 1996a, 1996b, 1996c, 1996d, 1996e, 1996f, 1998a, 1998b, 1998c, 1999, 2002a, 2002b, 2005, 2006a, EC 1996, 1997, 1998, 2001, Japan BCH 1996a, 1996b, 1996c, 1997a, 1997b, 1997c, 1997d, 1997e, 1999a, 1999b, 1999c, 1999d, 2002, 2005, 2006a, 2006b, 2006c, 2006d, 2006e, 2006f, 2007a, 2007b, 2008, 2009, 2010, OGTR 2002, 2003, 2006, Philippines 2005, USDA APHIS 1995a, 1995c, 1995f, 1996b, 1996b, 1996c, 1996e, 1997c, 1997f, 1998a, 1998c, 1998e, 1998g, 1998i, 1998j, 1999a, 1999b, 1999c, 2001b, 2001c, 2002b, 2003c, 2004a, 2004c, 2005, 2006b, USEPA 2001, 2005, 2009a, 2009b*).

Risk assessors have also considered whether the introduction of PAT proteins into GE plants might lead to changes in the plant that would adversely impact other organisms. Phenotypic characterization (see above) as well as compositional analyses (see below) and nutritional analyses show that the introduction of PAT proteins has not had any unanticipated effects on characteristics of GE plants that might impact other organisms (*ANZFA 2000, 2001a, 2001b, 2002, CFIA 1995a, 1995b, 1996a, 1996b, 1996c, 1996d, 1996e, 1996f, 1998a, 1998b, 1998c, 1999, 2002a, 2002b, 2004, 2005, 2006a, 2006b, EC 1996, 1997, 1998, 2001, EFSA 2005, 2006, 2008a, 2008b, 2009a, 2009b, FSANZ 2003, 2004a, 2004b, 2005a, 2005b, 2008, Japan BCH 1996a, 1996b, 1996c, Health Canada 2006a, 2006b, Japan BCH 1997a, 1997b, 1997c, 1997d, 1997e, 1999a, 1999b, 1999c, 1999d, 2002, 2003, 2005, 2006a,* *2006b, 2006c, 2006d, 2006e, 2006f, 2007a, 2007b, 2008, 2009, 2010, OGTR 2003, 2006, Philippines 2005, USDA APHIS 1994a, b, 1995b, 1995d, 1995e, 1996a, 1996d, 1997a, 1997b, 1997d, 1997e, 1998b, 1998d, 1998f, 1998h, 1998k, 1998l, 2000, 2001a, 2002a, 2002c, 2003a, 2003b, 2004b, 2004d, 2006a, USEPA 2001, 2005, 2009a, 2009b*). Based on experimental evidence that PAT proteins are not toxic and the observation that exposure to PAT is widespread in the environment, regulatory authorities have concluded that the expression of PAT in GE plants does not have any meaningful potential to adversely impact other organisms (*CFIA 1995a, 1995b, 1996a, 1996b, 1996c, 1996d, 1996e, 1996f, 1998a, 1998b, 1998c, 1999, 2002a, 2002b, 2005, 2006a, EC 1996, 1997, 1998, 2001, Japan BCH 1996a, 1996b, 1996c, 1997a, 1997b, 1997c, 1997d, 1997e, 1999a, 1999b, 1999c, 1999d, 2002, 2005, 2006a, 2006b, 2006c, 2006d, 2006e, 2006f, 2007a, 2007b, 2008, 2009, 2010, OGTR 2002, 2003, 2006, Philippines 2005, USDA APHIS 1995a, 1995c, 1995f, 1996b, 1996b, 1996c, 1996e, 1997c, 1997f, 1998a, 1998c, 1998e, 1998g, 1998i, 1998j, 1999a, 1999b, 1999c, 2001b, 2001c, 2002b, 2003c, 2004a, 2004c, 2005, 2006b, USEPA 2001, 2005, 2009a, 2009b*).

COMPOSITIONAL ANALYSIS OF PAT-EXPRESSING 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. Although compositional analysis is not typically required for environmental risk assessments, it is often considered in the context of demonstrating whether or not there have been unanticipated changes to the GE plant (*CFIA 1995a, 1995b, 1996a, 1996b, 1996c, 1996d, 1996e, 1996f, 1998a, 1998b, 1998c, 1999, 2002a, 2002b, 2005, 2006a, EC 1996, 1997, 1998, 2001, Japan BCH 1996a, 1996b, 1996c, 1997a, 1997b, 1997c, 1997d, 1997e, 1999a, 1999b, 1999c, 1999d, 2002, 2005, 2006a, 2006b, 2006c, 2006d, 2006e, 2006f, 2007a, 2007b, 2008, 2009, 2010, OGTR 2002, 2003, 2006, Philippines 2005, USDA APHIS 1995a, 1995c, 1995f, 1996b, 1996b, 1996c, 1996e, 1997c, 1997f, 1998a, 1998c, 1998e, 1998g, 1998i, 1998j, 1999a, 1999b, 1999c, 2001b, 2001c, 2002b, 2003c, 2004a, 2004c, 2005, 2006b, USEPA 2001, 2005, 2009a, 2009b*). GE plants expressing PAT have undergone a variety of compositional analyses, including for proximate (protein, fat, amino acid, fiber, ash) as well as for nutritional components and known toxicants or antinutrients (such as gossypol in cotton or glucosinolates in canola) (*ANZFA 2000, 2001a, 2001b, 2002, CFIA 1995a, 1995b, 1996a, 1996b, 1996c, 1996d, 1996e, 1996f, 1998a, 1998b, 1998c, 1999, 2002a, 2002b, 2004, 2005, 2006a, 2006b, EC 1996, 1997, 1998, 2001, EFSA 2005, 2006, 2008a, 2008b, 2009a, 2009b, FSANZ 2003, 2004a, 2004b, 2005a, 2005b, 2008, OGTR 2003, 2006, Philippines 2005, USDA APHIS 1994a, 1994b, 1995b, 1995d, 1995e, 1996a, 1996d, 1997a, 1997b, 1997d, 1997e, 1998b, 1998d, 1998f, 1998h, 1998k, 1998l, 2000, 2001a, 2002a, 2002c, 2003a, 2003b, 2004b, 2004d, 2006a, USEPA 2001, 2005, 2009a, 2009b*). Although statistically significant differences between the composition of GE plants and their non-transformed counterparts have been reported, these differences have not been attributed to expression of the PAT protein, and subsequent regulatory decisions have concluded that the composition of GE plants expressing PAT is not meaningfully different with respect to potential impact on the environment (*CFIA 1995a, 1995b, 1996a, 1996b, 1996c, 1996d, 1996e, 1996f, 1998a, 1998b, 1998c, 1999, 2002a, 2002b, 2005, 2006a, EC 1996, 1997, 1998, 2001, Japan BCH 1996a, 1996b, 1996c, 1997a, 1997b, 1997c, 1997d, 1997e, 1999a, 1999b, 1999c, 1999d, 2002, 2005, 2006a, 2006b, 2006c, 2006d, 2006e, 2006f, 2007a, 2007b, 2008, 2009, 2010, OGTR 2002, 2003, 2006, Philippines 2005, USDA APHIS 1995a, 1995c, 1995f, 1996b, 1996b, 1996c, 1996e, 1997c, 1997f, 1998a, 1998c, 1998e, 1998g, 1998i, 1998j, 1999a, 1999b, 1999c, 2001b, 2001c, 2002b, 2003c, 2004a, 2004c, 2005, 2006b, USEPA 2001, 2005, 2009a, 2009b*).

CONCLUSION

The PAT protein expressed in GE plants is encoded by one of the homologous genes pat or bar, isolated from the related bacteria *Streptomyces viridochromogenes* or *Streptomyces hygroscopicus*, respectively. Environmental release approvals have been granted for 8 species of plants expressing PAT proteins in 11 different countries including at least 38 separate transformation events. Data from regulatory submissions and peer-reviewed literature show that the PAT protein expressed in GE plants has negligible impact on the phenotype of those plants, beyond conferring tolerance to the herbicide glufosinate. Risk assessments associated with regulatory review of these plants for use in the environment show that expression of PAT does not alter the potential for persistence or spread of GE plants in the environment, does not alter the reproductive biology or potential for gene flow, and does not increase the risks for adverse effects to other organisms. Although the introduction of PAT to GE plants has the potential to complicate the management of herbicide-tolerant volunteers or weedy relatives in agriculture, the evidence does not indicate that expression of PAT has impacted the effectiveness or availability of alternative control measures such as other herbicides or mechanical weed control. Taken together, these regulatory analyses support the conclusion that, for the species and environments that have been considered to date, the expression of the PAT protein in GE plants does not present any meaningful risk to the environment.

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ANNEX I: SUMMARY OF PAT 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.

Table I.1. Quantities of PAT in *Beta vulgaris* event T120-7 as detected by ELISA (USDA APHIS 1998b).

Plant Matrix[1]	% Protein[2]	ng PAT/g protein[3,4]
Roots	6.8	137
Tops (above ground)	15.0	966
Pulp (dried)	9.7	N.D.
Molasses	9.9	N.D.

1 Values reported are mean values from all sites
2 Literature values (see USDA APHIS 1998b for citation).
3 Two extracts from each sample (18 tops; 18 roots from 6 field sites) were analyzed in triplicate.
4 Limit of Detection = 2 ng/g root; 1.6 ng/g sugar, pulp; 0.4 ng/g molasses .
5 N.D. = Not Detected.

Table I.2. PAT contents in seed samples for *Brassica napus* event Topas 19/2 (HCN10 and HCN92) as detected by ELISA (USDA APHIS 2002a).

Sample ID	PAT / Sample (ng/g)
Excel 1996 (control)	N.D. [1]
HCN92	295
HCN92	295
HCN10	189
HCN10	202

1 N.D. < Limit of Quantification (0.40 ng/mL).

Table I.3. PAT expression in seed and leaf samples from *Brassica napus* lines Topas 19/2, T45, and Topas 19/2 x T45 as detected by ELISA (USDA APHIS 2002a).

Sample ID	Line/Treatment	Event	PAT/ Sample (ng/g)	Total Protein (mg/g)	PAT/ Protein (%)
Control		Topas 19/2	ND	9.51	
Plot 2	SW9782179	Topas 19/2	248	2.14	0.012
Plot 7	SW9782179	Topas 19/2	263	3.04	0.009
Plot 8	SW9782179	Topas 19/2	309	2.24	0.014
Plot 10	SW9782179	Topas 19/2	379	2.61	0.015
Plot 3	SW9782180	T45	555	2.68	0.021
Plot 5	SW9782180	T45	743	2.41	0.031
Plot 6	SW9782180	T45	717	2.17	0.033
Plot 1	SW9782213	Topas 19/2//T45	754	2.01	0.038
Plot 4	SW9782213	Topas 19/2//T45	906	2.00	0.045
Plot 9	SW9782213	Topas 19/2//T45	932	3.37	0.028
Plot 11	SW9782213	Topas 19/2//T45	944	3.12	0.030
Seed – UN	Untreated	Topas 19/2//T45	563	54.3	0.00104
Seed-TR	Treated[1]	Topas 19/2//T45	669	59.4	0.00113
Tmeal-UN	Untreated	Topas 19/2//T45	ND	85.9	ND
Tmeal-TR	Treated[1]	Topas 19/2//T45	ND	76.1	ND
RBD oil - UN	Untreated	Topas 19/2//T45	ND	ND	ND
RBD oil - TR	Treated[1]	Topas 19/2//T45	ND	ND	ND

1 Treated with glufosinate.

Table I.4. PAT expression in seeds and leaves of *Brassica napus* lines RF3 and MS8 determined by enzyme activity (OGTR 2003).

GM Canola Line	Seed µg PAT/mg Total Protein	Leaf µg PAT/mg Total Protein
RF3	0.10	1.33
MS8	0.04	0.51
RF1	Not tested	1.45
RF2	Not tested	0.7
MS1	Not tested	0.9

Table I.5. PAT expression in seeds of GM *Brassica napus* lines determined by ELISA (OGTR 2003).

GM Canola Line	µg PAT/g Seed	µg PAT/mg Total Protein
RF3	0.69	0.012
MS8	0.07	0.002
RF3xMS8	0.34	0.013
RF1	0.50	0.015
RF2	0.42	0.012
MS1	0.07	0.002
MS1xRF1	0.20	0.006
MS1xRF2	0.35	0.007

Table I.6. PAT expression in seeds of GM *Brassica napus* lines determined by ELISA (OGTR 2003).

GM Canola Line	Seed µg PAT/g Total Seed	Leaf µg PAT/mg Fresh Weight
T45	0.561	0.348
Topas	0.47	0.0843

Table I.7. Summary of mRNA expression analysis for *bar* in *Brassica napus* RF2.

Total RNA	pg *bar* mRNA/µg Total RNA (Range of Detected Values)
Leaves	0.8-1.6
Flower Buds	0.1-0.2
Seed	ND[1]
Pollen	ND

1 N.D. = Not Detected (<2pg/ µg total RNA).

Table I.8. Summary of mRNA expression analysis for *bar* in *Brassica napus* MS8 (USDA APHIS 1998l)[1].

Total RNA	Transgene Expression (pg/µg Total RNA)[2]
Leaf A	0.03
Leaf B	0.22
Flower buds 2mm A	0.14
Flower buds 2mm B	0.11
Flower buds 3mm A	0.19
Flower buds 3mm B	0.03
Dry seed	ND[3]

1 Data shown for two plants (A and B) with single samples of each tissue.
2 pGembar/SP6 plasmid used for preparation of RNA probe.
3 ND = Not Detected. Limit of Detection = 0.1pg/g total RNA.

Table I.9. Summary of mRNA expression analysis for *bar* in *Brassica napus* RF3 (USDA APHIS 1998l).

Total RNA	Transgene Expression (pg/µg Total RNA)[1]
Leaf A	1.1
Leaf B	0.2
Flower buds 2mm A	0.46
Flower buds 2mm B	0.52
Flower buds 3mm A	0.38
Flower buds 3mm B	0.34
Dry seed	ND[2]
Pollen	ND[2]

1 Data shown for two plants (A and B) with single samples of each tissue.
2 ND = Not Detected. Limit of Detection = 0.05pg/g total RNA.

Table I.10. Summary of PAT expression in seed of *Brassica napus* lines T45 (HCN28) and HCN 92 (method not reported) (CFIA 1996b).

Sample	µg/mg Sample (Reported Range)
T45 Seed	95-245
HCN 92 Seed	150-223

Table I.11. Summary of PAT expression in *Brassica napus* line T45 (HCN28) using Northern blot and ELISA (EFSA 2008).

Sample	Northern Blot[1]	ELISA
Leaves	+	NA[2]
Stems	+	NA
Roots	+	NA
Seeds	-	930 ng/g dry weight

1 Results are reported as presence (+) or absence (-) of detectable mRNA.
2 NA = Not Available.

Table I.12. Summary of PAT content of seed from *Brassica rapa* line HCR-1 (method not reported) (CFIA 1998c).

Tissue	Mean (ng/g)	Range (ng/g)
Seed	107	84-132

Table I.13. Summary of PAT protein in seeds of *Glyxine max* lines AS2704-12 and A5547-127 as detected by ELISA (FSANZ 2004a).

Sample	Year	Treatment[1]	PAT/Sample (ng/g)	Crude Protein (%)	PAT Protein as % of Crude Protein
AS2704-12	1997	NA[2]	573	37-45	0.00016
A5547-127	1998	NA	10800	37-45	0.00292
AS2704-12	1999	sprayed	879 (264)[3]	NA	0.000227
AS2704-12	1999	unsprayed	862 (272)	NA	0.000227
A5547-127	1999	sprayed	10100 (816)	NA	0.00285
A5547-127	1999	unsprayed	9971 (940)	NA	0.00283
AS2704-12	NA	sprayed	2183	38.9	0.00050
AS2704-12	NA	unsprayed	1948	38.5	0.00056
A5547-127	NA	sprayed	17471	36.5	0.0048
A5547-127	NA	unsprayed	20202	35.8	0.0056

1 sprayed = treated with glufosinate; unsprayed = not treated with glufosinate.
2 NA = Not Available.
3 Mean (Standard Deviation).

Table I.14. Summary of PAT protein detected in *Glycine max* line A2704-12 using ELISA (Japan BCH 1999b).

Tissue	Mean PAT (µg/g Fresh Weight) ± Standard Deviation	Crude Protein (% of Fresh Weight)	PAT Protein (% of Crude Protein)
Root	2.23 ± 1.29	1.95	0.011
Stem	7.63 ± 2.20	3.58	0.021
Leaf	14.5 ± 2.4	5.96	0.024

Table I.15. Summary of PAT protein detected in seeds of *Glycine max* line A2704-12 using ELISA (Japan BCH 1999b).

Sample	PAT (ng/g Sample) Mean ± Standard Deviation	Crude Protein Content (%)	PAT/Crude Protein (%)
1	1057	NA[1]	NA
2	573	NA	NA
3	862 ± 268	38.03	0.000227
4	2138 ± 33	43.5	0.00049

1 NA = Not Available.

Table I.16. Summary of PAT protein detected in *Glycine max* line A5547-127 using ELISA (Japan BCH 2006f).

Tissue	Mean PAT (µg/g Fresh Weight) ± Standard Deviation	Crude Protein (% of Fresh Weight)	PAT Protein (% of Crude Protein)
Root	3.73. ± 0.98	2.15	0.017
Stem	11.5 ± 1.8	3.62	0.032
Leaf	19.0 ± 5.0	6.70	0.028

Table I.17. Summary of PAT protein detected in seeds of *Glycine max* line A5547-127 using ELISA (Japan BCH 2006f).

Sample	PAT (ng/g Sample) Mean ± Standard Deviation	Crude Protein Content (%)	PAT/Crude Protein (%)
1	6341	NA[1]	NA
2	10800 ± 1210	NA	NA
3	9971 ± 846	35.26	0.00282
4	20202 ± 359	40.4	0.0050

1 NA = Not Available.

Table I.18. Summary of PAT content in leaves of *Glycine max* line A5547-127 as detected by ELISA (USDA APHIS 1998d)[1].

Sample	mg TEP[2]/ g Sample	µg PAT/ g Sample	% PAT/ TEP	% PAT/ Fresh Weight g/g
A5547-127	4.6	1.72	0.037	1.72×10^{-4}

1 Values are the average from two replicate extractions from two samples of 10 day old seedling leaves.
2 TEP = Total Extractable Protein.

Table I.19. Summary of PAT content in leaves of *Glycine max* line GU262 as detected by ELISA (USDA APHIS 1998f)[1].

Sample	mg TEP[2]/ g Sample	µg PAT/ g Sample	% PAT/ TEP	% PAT/ Fresh Weight g/g
GU262	4.7	3.03	0.064	3.03×10^{-4}

1 Values are the average from two replicate extractions from two samples of 10 day old seedling leaves.
2 TEP = Total Extractable Protein.

Table I.20. Summary of PAT protein in Glycine max lines W62 and W98 as detected by enzymatic activity (USDA APHIS 1996a)[1].

Tissue	Site and Year	Plant	µg PAT/g Sample[2]
Fodder (whole plant)	Arkansas 1993	W62	10.8 (6.3-15.3)
	Iowa 1993	W98	0.75 (0.65-1.0)
	Illinois 1993	W98	10.9 (9.1-12.7)
Seed	Arkansas 1993	W62	217.0 (147.1-267.3)
	Iowa 1993	W98	27.1 (15.0-39.2)
	Illinois 1993	W98	38.3 (23.5-60.9)

1 Values are the average from two replicate extractions from two samples of 10 day old seedling leaves.
2 Mean (range of observed values).

Table I.21. Summary of PAT protein expression in *Gossypium hirsutum* event 281-24-236 as determined by ELISA (USDA 2003a).

Tissue	PAT (ng/mg Dry Weight)		
	Mean[1]	Standard Deviation	Minimum-Maximum Range
Young Leaf (3-6 week)	0.43	0.12	0.18-0.67
Terminal Leaf	0.21	0.12	ND[4]-0.38
Flower	0.29	0.11	0.07[2]-0.44
Square	0.51	0.15	0.06[2]-0.79
Boll (early)	0.22	0.09	0.08[2]-0.48
Whole Plant (seedling)	0.31	0.07	0.21-0.46
Whole Plant (pollination)	0.23	0.07	0.09[2]-0.33
Whole Plant (defoliation)	0.19	0.13	ND-0.46
Root (seedling)	0.07[2]	0.05	ND-0.12
Root (pollination)	ND	NA[3]	ND-0.11
Root (defoliation)	ND	NA	ND-0.11
Pollen[5]	0.09[2]	0.15	ND-0.45
Nectar[5]	ND	NA	ND-ND
Seed[5]	0.47	0.17	0.23[2]-1.02

1 Calculated from samples at six locations.
2 Value below the limit of quantification of the method.
3 NA = Not Applicable.
4 ND = absorbance of the sample was lower than the absorbance of the lowest standard.
5 Results are given relative to fresh weight.

Table I.22. Summary of PAT protein expression in *Gossypium hirsutum* even 3006-210-23 as determined by ELISA (USDA 2004a).

Tissue	PAT (ng/mg Dry Weight)		
	Mean[1]	Standard Deviation	Minimum-Maximum Range
Young Leaf (3-6 week)	ND[4]	NA[3]	0.18-0.67
Terminal Leaf	ND	NA	ND-0.12
Flower	ND	NA	ND-ND
Square	ND	NA	ND-0.08
Boll (early)	ND	NA	ND-0.08
Whole Plant (seedling)	ND	NA	ND-0.09
Whole Plant (pollination)	ND	NA	ND-0.14
Whole Plant (defoliation)	0.11	0.05	ND-0.20
Root (seedling)	ND	NA	ND-0.07
Root (pollination)	ND	NA	ND-ND
Root (defoliation)	ND	NA	ND-ND
Pollen[5]	ND	NA	ND-ND
Nectar[5]	ND	NA	ND
Seed[5]	0.06[2]	0.06	ND-0.23[2]

1 Calculated from samples at six locations.
2 Value below the limit of quantification of the method.
3 NA = Not Applicable.
4 ND = absorbance of the sample was lower than the absorbance of the lowest standard.
5 Results are given relative to fresh weight.

Table I.23. Summary of PAT protein expression in *Gossypium hirsutum* even 3006-210-23 as determined by ELISA (USDA 20036).

Tissue	Mean Protein Expression (ng/mg dry weight)
Young Leaf (3-6 week)	0.43
Terminal Leaf	0.23
Flower	0.35
Square	0.52
Boll (early)	0.27
Whole Plant (seedling)	0.35
Whole Plant (pollination)	0.30
Whole Plant (defoliation)	0.34
Root (seedling)	0.06[2]
Root (pollination)	ND
Root (defoliation)	0.05[2]
Pollen[1]	0.05[2]
Nectar[1]	ND
Seed[1]	0.54

1 Results reported as ng/mg fresh weight.
2 Calculated concentration is less than the limit of quantification of the method.

Table I.24. Summary of PAT expression in *Gossypium hirsutum* line LLCotton25 measured by ELISA (CFIA 2004).

Tissue	PAT Protein (µg/g Fresh Weight)
Root	7.97
Leaf	52.9
Stem	36.8
Fuzzy Seed	69.9
Cleaned Seed	127
Pollen	19.2

Table I.25. Summary of PAT protein content in *Gossypium hirsutum* line LLCotton25 as measured by ELISA (OGTR 2006).

Tissue	PAT (µg/g Fresh Weight)		PAT as % of Crude Protein	Average TEP[2] (mg/g Fresh Weight ± SD)	Average PAT Content as % of TEP
	Range	Average			
Roots	5.63-10.1	7.97 ± 1.86	0.08	2.26 ± 0.22	0.35
Stems	34.3-45.5	36.8 ± 6.7	0.23	4.99 ± 0.92	0.74
Leaves	45.1-57.3	52.9 ± 6.0	0.19	7.13 ± 0.79	0.74
Pollen (frozen)	4.44-13.0	8.23 ± 3.20	NA[1]	146 ± 8	0.006
Pollen (fresh)	0.11-170	19.3 ± 39.2	NA	107 ± 21	0.018

1 Not Applicable.
2 TEP = Total Extractable Protein.

Table I.26. Summary of PAT protein content in leaves of *Gossypium hirsutum* line LLCotton25 as measured by ELISA (OGTR 2006).

Sample	PAT Protein (µg/g Fresh Weight ± SD)			
	2-4 leaf	4-5 leaf	Early Bloom	Full Bloom
Non GM Control	ND[1]	ND	ND	ND
Sprayed Once[2]	NA	85.0 ± 15.6	98.3 ± 16.8	92.6 ± 15.1
Sprayed Twice	NA	NA	NA	92.6 ± 20.3
Unsprayed	57.7 ± 5.3	74.0 ± 12.3	90.2± 14.4	75.1 ± 25.6

1 ND = not detected; NA = not applicable.
2 Sprayed with Liberty Link.

Table I.27. Summary of PAT protein content RACs of *Gossypium hirsutum* line LLCotton25 as measured by ELISA (OGTR 2006).

Sample	Average PAT Protein (µg/mg Fresh Weight) ± SD		Average Protein Content (as % of Crude Protein)	
	LL Sprayed	Unsprayed	LL Sprayed	Unsprayed
Cleaned Seed	127 ± 18	113 ± 24	NA[1]	NA
Lint Coat	1.5 ± 0.45	0.92 ± 0.50	NA	NA
Fuzzy Seed	69.9 ± 6.0	63.0 ± 10.3	0.030	0.027
Lint	0.78 ± 0.63	0.50 ± 0.42	0.003	0.003

1 NA = Not Applicable.

Table I.28. Summary of PAT protein in *Gossypium hirsutum* line LLCotton25 as measured by ELISA (USDA APHIS 2002c).

Sample	PAT Protein (µg/g Fresh Weight) ± Standard Deviation		PAT Content (% of Crude Protein)	
	Liberty Herbicide	Conventional Herbicide	Liberty Herbicide	Conventional Herbicide
Cleaned Seed	127 ± 18	113 ± 24	NA	NA
Lint Coat	1.15 ± 0.45	0.92 ± 0.50	NA	NA
Fuzzy Seed	69.9 ± 6.0	63.0 ± 10.3	0.030	0.027
Lint	0.78 ± 0.63	0.50 ± 0.42	0.003	0.003

Table I.29. Summary of PAT protein content in leaves of *Gossypium hirsutum* line LLCotton25x15985 as measured by ELISA (Japan BCH 2006a).

Sample	Protein Content (Mean ± Standard Deviation) (µg/g of Leaf)
LLCotton25 x 15985	60.9 ± 8.7[1]
LLCotton25	65.9 ± 10.6[1]

1 The difference in expression between the stacked line and the single event is not significant.

Table I.30. Summary of PAT protein content in *Oryza sativa* line LLRice62 as measured by ELISA (CFIA 2006b).

Tissue	Mean Protein Content µg/g Fresh Weight
Grain	12.1
Straw	75.3
Rice Hulls	1.56
Roots	12.7
Stems	30.9
Leaves	84.7

Table I.31. Summary of PAT protein content in *Oryza sativa* line LLRICE62 as measured by ELISA (FSANZ 2008).

Tissue	Average PAT Content (µg/g ± SD)	Crude Protein (% w/w)	PAT Protein (% of Crude Protein)
Grain (Year 1)	12.1 ± 0.6	7.19	0.017
Straw (Year 1)	75.3 ± 4.4	2.38	0.316
Grain (Year 2)	10.6 ± 1.3	7.41	0.014

Table I.32. Summary of PAT protein content in grain of *Oryza sativa* lines LLRICE06 and LLRICE62 as detected by ELISA (USDA APHIS 1998h).

Plant	mg TEP[1]/g Sample	µgPAT/g Sample	% PAT/TEP	% PAT/Fresh Weight (g/g)
LLRICE06	1.89 ± 0.49	0.419 ± 0.04	0.02	0.00005
LLRICE62	2.54 ± 0.09	12.4 ± 2.4	0.63	0.00124

1 TEP = Total Extractable Protein

Table I.33. Summary of PAT protein content in seed of *Oryza sativa* line LLRICE601 (method not reported) (USDA APHIS 2006a).

Plant	Protein Content (ng/g Fresh Weight)	% of Crude Rice Protein
LLRICE601	120	0.00034

Table I.34. PAT protein expression in *Zea mays* line Bt-176 and hybrid plants as determined by ELISA (USDA APHIS 1995a)[1].

		PAT Expression ng/g Fresh Weight			
		Seedling	Anthesis	Seed Maturity	Senescence
Leaves	Bt176	<200[4]	<200	<200	ND
	176 x 554[2]	<200	ND	ND	ND
	176 x 564[3]	<200	ND	<200	<200
Whole Plant	Bt176	ND	<200	<200	<200
	176 x 554	<200	ND	<200	<200
	176 x 564	<200	ND	ND	<200
Kernels	Bt176			ND	ND
	176 x 554			ND	ND
	176 x 564			ND	ND
Pollen	Bt176		ND		
	176 x 554		NA		
	176 x 564		ND		
Roots	Bt176	ND[4]	<100	<100	NA
	176 x 554	ND	<100	<100	NA
	176 x 564	ND	ND	<100	NA
Pith	Bt176	NA[5]	<200	<200	NA
	176 x 554	NA	ND	<200	NA
	176 x 564	NA	NA	ND	NA

1 Blank cell indicate no developmental relevance.
2 176 x 554 = hybrid progeny of CG00526-176 and untransformed CG00554 and is hemizygous for the introduced genes.
3 176 x 564 = hybrid progeny of CG00526-176 and untransformed CG00564 and is hemizygous for the introduced genes.
4 When trace amounts were detectable but not quantifiable, results are shown as < lower limit of quantification.
5 ND = Not Detected.
6 NA = Not Analyzed.

Table I.35. Expression of PAT protein in leaf tissue of male sterile *Zea mays* lines 676, 678, and 680 as determined with ELISA (USDA APHIS 1997d).

Male Sterile Corn Line	PAT Concentration µg/g Total Protein
676	601-617
678	204-278
680	<20[1]

1 Below the limit of quantification (20µg/g).

Table I.36. Expression of PAT protein in *Zea mays* line B16 (method not reported) (CFIA 1996d).

Tissue	PAT Protein Detected
Leaves	1.0-4.6 mg/g protein
Roots	+[1]
Stalk	+
Tassel	+
Cob	+
Husk	+
Kernels	-[2]
Silk	-
Pollen	-

1 + = Protein detected but quantity not reported.
2 - = Protein not detected.

Table I.37. Expression of PAT protein in *Zea mays* line B16 determined by Western blot (USDA APHIS 1995b).

Tissue	PAT Concentration (ng/µg Total Protein)	PAT Concentration (ng/mg Fresh Weight)
Coleoptile (6 days)	1.8	13.8
Leaf (24 days)	1.0	55.6
Leaf (44 days)	2.8 ± 0.1	166.0 ± 24.2
Leaf (93 days)	2.1	106.1
F¬$_2$ ovule (0 days pp)	0.8	5.5
Immature F¬$_2$¬ seed (16 days pp)	0.3	3.4
Immature F¬$_2$ seed (45 days pp)	0.3	5.7
Hybrid seed (F_1)	0.2	1.9
Root (24 days)	0.06	4.2
Root (44days)	1.3	8.1
Prop root (49 days)	1.9 ±0.3	19.8 ± 2.4
Cob (56 days)	2.2	67.2
Husk (56 days)	1.1	2.5
Silk	ND[1]	4.2
Stalk (24 days)	2.0	
Stalk (77 days)	4.6 ± 0.4	15.7
Immature tassel (49 days)	2.0	11.2 ± 2.7
Pollen	ND	30.8
Silage	ND	

1 ND = Not detected. <0.05 ng/mg in silk; <0.08 ng/mg in pollen and silage.

Table I.38. Mean level of expression of the PAT protein in *Zea mays* line Bt11 (X4334-CBR and X4743) using ELISA (ANZFA 2001a).

	Mean (µg/g Fresh Weight)			
	Leaf	Kernel	Husk	Stalk
X4334-CBR	0.0386 ± 0.0029	lod[1]	lod	lod
X4734-CBR	0.0494 ± 0.005	lod	nd[2]	nd
Control (NK4242)	lod	lod	lod	lod

1 lod (limit of detection) for the procedure is 1ng PAT/mL extract. These values are considered not above background.
2 nd = No data.

Table I.39. Mean level of expression of the PAT protein in *Zea mays* line Bt11 (method not reported) (CFIA 1996c).

	Mean (µg/g fresh weight)					
	Leaf	Tassel	Silk	Roots	Kernel	Pollen
BT11	0.049	0.027	0.005	ND[1]	ND	ND

1 ND = Not Detected.

Table I.40. Mean expression of PAT protein in *Zea mays* line CBH-351 using ELISA (USDA APHIS 1997e)[1].

Tissue	Stage 1		Stage 2		Stage 3		Stage 4	
	Total Protein (mg/g)	PAT Protein (µg/g)	Total Protein (mg/g)	PAT Protein µg/g	Total Protein (mg/g)	PAT Protein µg/g	Total Protein (mg/g)	PAT Protein µg/g
Whole Plant	12.5 ± 4.6	189.7 ± 23.5	7.8 ± 1.9	105.7 ± 16.7	5.7 ± 1.4	44.4 ± 5.4	2.9 ± 1.6	6.8 ± 1.6
Leaf	3.1 ± 1.3	45.4 ± 9.7	0.6 ± 0.1	14.0 ± 0.1	1.5 ± 0.1	2.3 ± 0.2	1.8 ± 0.2	0.0 ± 0.0
Root	0.7 ± 0.1	39.1 ± 2.7	25.8 ± 19.2	4.4 ± 0.8	0.3 ± 0.2	0.6 ± 0.5	0.6	2.2 ± 2.8
Stalk	NA[2]	NA	2.8 ± 1.2	0.5 ± 0.5	0.0 ± 0.0	0.5 ± 0.2	± 0.2	0.0 → 0.1
Tassel	NA	NA	175.0 ± 100.4	4.2 ± 1.2	NA	NA	NA	NA
Kernel	NA	NA	NA	NA	NA	NA	6.7 ± 1.1	17.8 ± 5.0

1 Values are expressed as mean ± standard deviation. All values are relative to dry weight of samples.
2 NA = Not Applicable.

Table I.41. Mean level of PAT protein expression in hybrids derived from *Zea mays* line CBH-351 using ELISA (USDA APHIS 1997e)[1].

Hybrid	Stage 1		Stage 2	
	Total Protein (mg/g)	PAT Protein µg/g	Total Protein (mg/g)	PAT Protein µg/g
Hybrid A	19.1 ± 8.2	364.0 ± 85.1	4.9 ± 2.0	40.7 ± 16.7
Hybrid B	18.3 ± 1.1	291.0 ± 4.2	7.2 ± 2.8	77.1 ± 12.9
Hybrid C	11.6 ± 6.3	227.0 ± 33.4	6.8 ± 1.3	100.2 ± 24.9
Hybrid D	9.1 ± 3.3	176.3 ± 63.0	4.1 ± 1.4	88.3 ± 23.4

1 Values are expressed as mean ± standard deviation. All values are relative to dry weight of samples.

Table I.42. Mean expression of PAT protein in *Zea mays* line DAS-06275-8 at six field trial sites in the U.S. and Canada as measured by ELISA (CFIA 2006a).

	Leaf	Root	Stalk	Grain	Pollen	Forage
PAT Protein Expression (ng/mg Dry Weight)	129.2-224.21	61.1-70.0[1]	103.3	8.94	0.73	106.9

1 Range represents means measured across multiple growth stages.

Table I.43. Mean expression of PAT protein in *Zea mays* line DAS-06275-8 in grain (method not reported) (Health Canada 2006).

	U.S.	Chile
PAT Protein Expression (ng/mg Dry Weight)	5.94	23

Table I.44. Summary of mean expression of BAR (PAT)protein in a *Zea mays* hybrid derived from DAS-06275-8 measured by ELISA (USDA APHIS 2004b).

Growth Stage	Tissue	Mean ± Standard Deviation (ng/mg Dry Weight)	Minimum-Maximum Range (ng/mg Dry Weight)
V9	Leaf	323 ± 91.0	0-538
	Root	112 ± 35.3	0-170
	Whole plant	5 ± 3.50	1-11
R1	Leaf	674 ± 98.1	539-935
	Root	253 ± 162	61-673
	Whole plant	72 ± 32.9	35-108
R4	Pollen	0 ± 0.766	0-4.07
	Stalk	282 ± 68.5	177-475
	Leaf	682 ± 254	451-1584
Maturity	Root	223 ± 105	85-511
	Forage	7 ± 7.05	1-19
	Grain	23 ± 6.33	13-33
Senescence	Leaf	0 ± 0.461	0-1
	Root	41 ± 49.5	0-148
	Whole plant	18 ± 5.27	9-23

Table I.45. Summary of PAT protein expression in *Zea mays* line DAS-59122-7 at multiple sites in the U.S. and Canada measured by ELISA (CFIA 2005).

	Leaf	Root	Stalk	Grain	Pollen	Forage
PAT Protein Expression (ng/mg Dry Weight)	0.25-11.4[1]	0.18-0.42[1]	0.38	0.1	LOD[2]	2.4

1 Range represents means measured across multiple growth stages.
2 LOD = Below the Limit of Detection (<0.30 ng/mg dry weight).

Table I.46. Summary of expression levels of PAT protein in *Zea mays* line DAS-59122-7 as measured by ELISA (USDA APHIS 2004d).

Growth Stage	Tissue	Mean ± Standard Deviation (ng/mg Dry Weight)	Minimum-Maximum Range (ng/mg Dry Weight)
V9	Leaf	11.1 ± 3.68	5.61-19.2
	Root	0.47 ± 0.15	0.27-0.87
	Whole plant	0.18 ± 0.13	0-0.40
R1	Leaf	11.2 ± 3.49	6.36-18.2
	Root	0 ± 0	0-0
	Whole plant	0.13 ± 0.03	0.07-0.20
R4	Pollen	0.27 ± 0.12	0.11-0.62
	Stalk	0.13 ± 0.23	0-0.58
	Leaf	8.13 ± 3.02	0-14.2
Maturity	Root	0.09 ± 0.12	0-0.34
	Forage	0 ± 0	0-0
	Grain	0 ± 0	0-0
Senescence	Leaf	0.38 ± 0.46	0-1.33
	Root	0.08 ± 0.11	0-0.46
	Whole plant	0 ± 0	0-0

Table I.47. Summary of expression levels of PAT protein in grain of *Zea mays* hybrids with line 59122 as detected by ELISA (EFSA 2008b/EFSA 2009a).

	59122 x 1507 x NK603		59122 X NK603		59122	
	Mean	Range	Mean	Range	Mean	Range
PAT (ng/mg Dry Weight)	2.1	1.5-3.1	0.18	0-0.46	0.09	0-0.18

Table I.48. Summary of expression levels of PAT protein in *Zea mays* line DBT418 using Western blot (USDA 1996d).

Tissue	Genotype	Mean Protein Level (µg/g Dry Weight)							
		V6-V7		Pollen Shed		Dough		Harvest	
		Mean	n ; SE	Mean	n ; SE	Mean	n ; SE	Mean	n ; SE
Leaf	Inbred[1]	351.1	7; 52.91	522.0	6; 59.04	NA	NA	60.8[6]	6; 12.46
	Hemizygous hybrid[2]	276.3	8; 25.51	501.8	8; 34.75	NA	NA	180.5	8; 24.68
	Homozygous hybrid[3]	554.9	2; 136.03	1099.4	3; 76.29	NA	NA	213.6	4; 61.92
Stalk	Inbred	NA	NA	75.8	8; 12.24	NA	NA	95.2	6; 16.86
	Hemizygous hybrid	NA	NA	60.0	8; 11.98	NA	NA	64.4	8; 8.23
	Homozygous hybrid	NA	NA	77.0	4; 11.66	NA	NA	136.3	2; 12.74
Root Ball	Inbred	95.1	7; 16.91	54.1	8; 9.15	NA	NA	24.5	7; 3.71
	Hemizygous hybrid	59.4	8; 3.53	27.5	8; 6.25	NA	NA	21.3	8; 2.23
	Homozygous hybrid	88.1	4; 21.45	69.5	4; 23.58	NA	NA	28.8	3; 7.37
Kernel	Inbred	NA	NA	NA	NA	NA	NA	6.0	6; 1.88
	Hemizygous hybrid	NA	NA	NA	NA	NA	NA	3.1	8; 0.35
	Homozygous hybrid	NA	NA	NA	NA	NA	NA	4.9	4; 0.63
Silk	Inbred	NA	NA	128.2	8; 17.21	NA	NA	NA	NA
	Hemizygous hybrid	NA	NA	29.1	8; 2.97	NA	NA	NA	NA
	Homozygous hybrid	NA	NA	133.3	2; 60.01	NA	NA	NA	NA
Pollen	Hemizygous hybrid[4]	NA	NA	BLD[5]	8; NA	NA	NA	NA	NA
	Hemizygous hybrid	NA	NA	BLD	8; NA	NA	NA	NA	NA
	Homozygous hybrid	NA	NA	BLD	4; NA	NA	NA	NA	NA
Whole Plant	Inbred	NA	NA	111.1	8; 16.50	190.5	8; 30.76	NA	NA
	Hemizygous hybrid	NA	NA	72.8	8; 5.88	39.5	7; 7.51	NA	NA
	Homozygous hybrid	NA	NA	119.5	4; 25.63	135.2	3; 10.42	NA	NA

1 The S4 inbred line (AW/BC2/DBT418 S4) is an unfinished inbred.
2 This hybrid (AW/BC2/DBT418.BS/BC1/DBT418) contains two integrated copies of DBT418 insertion.
3 This hybrid (DK.DL (DBT418)) is a "finished hybrid" with one copy of DBT418 coming from an inbred parent line.
4 An additional group of the Hemizygous hybrid (AW/BC2/DBT418.BS/BC1/DBT418) was substituted because insufficient pollen was available from the S4 hybrid.
5 Below the Limit of Detection (12.10 µg/g dry weight).
6 2 of 8 samples were below the limit of detection and not used to calculate the mean or standard error.

Table I.49. Summary of the quantification of bar mRNA transcripts in *Zea mays* line MS3 (method not reported) (CFIA 1996e).

Line	Approximate mRNA (pg/µg total RNA)				
	Leaves	Immature Kernels	Roots	Dry Seeds[2]	Germinating Seeds[2]
MS3	0.05	0.05	ND[1]	ND	ND

1 ND = Not Detected.
2 A PAT enzyme activity assay did not detect any PAT in MS3 seeds.

Table I.50. Summary of the PAT protein content *Zea mays* line MS6 as detected by ELISA (USDA APHIS 1998k).

Tissue	Mg TEP[1]/g Sample	µg PAT/g Sample	% PAT/TEP
Grain	8.73	3.54	0.04
Forage	1.31	2.01	0.15
Fodder	1.26	2.15	0.17

1 TEP = Total Extractable Protein.

Table I.51. Summary of PAT protein levels in *Zea mays* hybrid and inbred lines derived from T25 as detected by ELISA (ANZFA 2001b).

Plant	Mean Levels ± Standard Deviation (ng/mg Protein)			
	Kernel	Silage	Forage	Fodder
Hybrid T25-1	ND[2]	14.82 ± 0.86	NA[3]	NA
Hybrid T25-2	ND	12.51 ± 1.38	NA	NA
Hybrid T25-3[1]	ND	14.81 ± 1.30	NA	NA
Inbred T25	4.02 ± 0.62	119.24 ± 13.36	62.70 ± 40.07	79.91 ± 5.23

1 Plants were treated with phosphinothricin at the V8 stage.
2 ND = Not Detected.
3 NA = Data Not Available.

Table I.52. Summary of PAT protein quantities detected by ELISA in *Zea mays* lines T14 and T25 (USDA 1994b) [1].

Matrix	% Protein	ng PAT/µg Protein	µg PAT/g Matrix	% PAT in Matrix
T14 silage	0.19	13.03	36.97	3.70
T25 silage	0.05	13.54	6.62	0.67
T14 grain	1.59	0.008	0.115	0.0115

1 Two extracts from each sampel (2 each for silage, 6 for grain) were analyzed in triplicate. Means are reported from all field sites combined.

Table I.53. Summary of PAT protein expression in *Zea mays* line T25 and hybrid crosses with MON810 as detected by ELISA.

Tissue	T25 X MON810		T25	
	Mean	Min-Max	Mean	Min-Max
Leaves	33.3	17.1-54.5	33.9	11.9-64.6

Table I.54. Summary of PAT protein expression as determined by ELISA in *Zea mays* line TC1507(CFIA 2002b, EFSA 2005) [1].

Location of Cultivation	Tissue					
	Leaf	Tassel	Silk	Roots	Kernel	Pollen
Canada	<LOD[2]	<LOD	<LOD	<LOD	<LOD	NA
Chile	<LOD	<LOD	<LOD	<LOD	<LOD	NA
EU	42 pg/µg (<LOD-136.8 pg/µg)[3]	<LOD	<LOD	<LOD	<LOD	NA
United States	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD (<LOD-38.0 pg/µg)

1 Data presented are from descriptive paragraphs describing different aspects of the same data set. These have been combined for simplicity.
2 LOD = Limit of Detection (7.5 pg/µg total protein for samples from Canada, 20 pg/µg for all other locations.
3 Mean (Range).

Table I.55. SSummary of PAT protein expression as determined by ELISA in *Zea mays* line TC1507 (USDA 2001c) [1].

Leaf	Pollen	Silk	Stalk	Whole Plant	Grain	Senescent Whole Plant
<LOD2 (<LOD-40.8)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

1 All values are pg/µg protein. Mean values are listed with observed ranges in parentheses.
2 LOD = Limit of Detection (20 pg/µg total protein).

Table I.56. Summary of PAT protein expression in plants derived from *Zea mays* line TC1507 and DAS59122 as detected by ELISA (EFSA 2009b) [1].

	1507 x5912		1507		59122	
Grain	Mean	Range	Mean	Range	Mean	Range
	0.15	0.09-0.27	<LOD[2]	<LOD	0.09	0-0.18
Forage	2.53	1.01-3.97	NA	NA	NA	NA

1 Values are ng/mg dry weight.
2 LOD = Limit of Detection.