01-324-01p

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November 9, 2001

Dr. Michael J. Firko
Assistant Director
Permitting and Risk Assessment
USDA, APHIS, PPQ, Unit 133
4700 River Road
Riverdale, MD 20737

Subject: Request for Extension of a Determination of Nonregulated Status to Glyphosate Tolerant Canola Event GT200

Dear Dr. Firko,

Monsanto Company is submitting to APHIS a request for an extension of the determination of nonregulated status for glyphosate-tolerant *Brassica napus* canola event RT73 previously granted under 98-216-01p to glyphosate-tolerant canola event GT200. The glyphosate tolerance in Roundup Ready Canola event RT73 (also referred to as event GT73) is imparted by the insertion of a glyphosate tolerant EPSPS from *Agrobacterium* sp. strain CP4 as well as a variant of the glyphosate degrading enzyme, glyphosate oxidoreductase (GOX) from *Ochrobactrum anthropi* strain LBAA, designated GOXv247, into the canola genome. In comparison, glyphosate tolerant canola event GT200 (also referred to as Roundup tolerant canola event RT200) utilizes the identical EPSPS from *Agrobacterium* sp. strain CP4 as well as the non-modified GOX protein from the same donor organism. Although the two GOX enzymes vary slightly, both GOX and GOXv247 catalyze the same oxidative degradation of glyphosate, the active ingredient in Roundup® herbicide.

Although glyphosate-tolerant canola event GT200 is not intended to be commercialized independently as a Roundup Ready canola variety, the extension of nonregulated status to this event is being requested because GT200 has the potential to be present at low, adventitious levels in commercial canola varieties.

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The data submitted supports the conclusion that glyphosate-tolerant event GT200 exhibits the same phenotype, glyphosate tolerance, and other agronomic properties as the previously deregulated event RT73. Event GT200 does not differ substantially from the antecedent organism, event RT73, in that both events are unlikely to become a plant pest, a weed, or increase the weediness potential of any cultivated or wild species; are unlikely to be plant pathogens; and are not likely to be toxic to beneficial or other non-target organisms. It is on this basis that Monsanto requests the extension for the determination of nonregulated status to glyphosate tolerant canola event GT200.

The enclosed request does not contain any confidential business information. However, to protect the names of individuals at Monsanto who prepared and contributed to this document, we respectfully request that those names be blacked out in copies that might be provided to the public. These names are bracketed in the attached request for extension.

If there are any questions with regard to this submission, please call Dr. Russ Schneider at (202) 383-2866 or call me directly at (636) 737-5532.

Sincerely,

Raymond C. Dobert.

Raymond Dobert, Ph.D.
Oilseeds Lead - Regulatory Affairs

cc: Russ Schneider
Regulatory Affairs File (01-CA-069U)

01-324-01p

Request for Extension of Determination of Nonregulated Status to Glyphosate-Tolerant Canola (*Brassica napus*) Event GT200

01-CA-069U

Submitter:

Monsanto Company
700 Chesterfield Parkway North
Chesterfield, MO 63198

November, 2001

11/20/01
2/26/02 mkp

CONTAINS NO CONFIDENTIAL INFORMATION

Request for Extension for Determination of Nonregulated Status to Glyphosate-Tolerant Canola (*Brassica napus*) Event GT200

SUMMARY

The Animal and Plant Health Inspection Service (APHIS) of the U.S. Department of Agriculture (USDA) has responsibility, under the Plant Protection Act (7 U.S.C. § 7701-7772), to prevent the introduction and dissemination into the U.S. or interstate movement of regulated articles such as plant pests and genetically engineered organisms developed using plant pests or their components. The regulations provide that an applicant may petition APHIS to evaluate submitted data to determine whether a particular regulated article does not present a plant pest risk and should no longer be regulated. If APHIS determines that the regulated article does not present a plant pest risk, the petition is granted, thereby allowing unrestricted introduction of the article. Section 340.6(e) of the regulations further provides that APHIS may extend a determination to additional regulated articles upon finding that the articles do not pose a potential for plant pest risk.

Monsanto Company is submitting this Request for Extension for Determination of Nonregulated Status to APHIS for glyphosate-tolerant canola event GT200, based upon the previous determination made on Roundup Ready® canola event RT73 (98-216-01p). After concluding that event RT73 was not a plant pest and did not pose a significant risk to the environment, APHIS granted nonregulated status for RT73 on January 27, 1999. As with the previously deregulated event RT73, glyphosate-tolerant canola event GT200 (also referred to as Roundup tolerant canola event RT200) is tolerant to glyphosate, the active ingredient in Roundup® herbicide. GT200 and RT73 are distinct transformation events which were both produced via *Agrobacterium tumefaciens* transformation of the parental canola variety Westar. Event GT200 was produced using plasmid vector PV-BNGT03 while RT73 was produced using the plasmid PV-BNGT04.

Both event GT200 and the antecedent organism, RT73, were transformed with vectors containing an identical glyphosate-tolerant EPSPS gene from *Agrobacterium sp.* strain CP4 fused to a chloroplast transit peptide from the *Arabidopsis thaliana* ribulose bisphosphate carboxylase gene. Transcription of this EPSPS sequence is directed by the modified figwort mosaic virus promoter (P-CMoVb) and its termination/polyadenlyation sequence is derived from the 3' end of the pea ribulose bisphosphate carboxylase gene. The CP4 EPSPS gene in event GT200 is essentially the same as the gene imparting Roundup tolerance

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to Roundup Ready cotton, Roundup Ready corn line NK603, Roundup Ready sugarbeet line GTSB77 and Roundup Ready soybean, which have all previously been deregulated by USDA-APHIS.

Both events also contain a gene based on the glyphosate oxidoreductase (GOX) gene from *Ochrobactrum anthropi* strain LBAA, which is fused to a chloroplast transit peptide from *Arabidopsis thaliana* EPSPS gene. Transcription of the GOX sequences is directed by the modified figwort mosaic virus promoter (P-CMoVb) and the termination/polyadenlyation sequence is derived from the 3' end of the pea ribulose bisphosphate carboxylase gene. The vector used to produce events RT73 and GT200 differ by 5 base pairs in the GOX coding region, encoding for the GOX protein in event GT200 and a variant of GOX designated GOXv247 in event RT73. At the protein level, GOX and the GOXv247 variant are >99% identical, differing by only three amino acids out of 431 amino acids. In both cases, the GOX enzymes catalyze the same oxidative degradation of glyphosate. Other than the slight difference in the GOX sequence, the vectors used to produce events GT200 and RT73 contain the same genetic elements. Additionally, the GOX gene present in event GT200 is essentially the same as the GOX gene inserted into Insect-protected/Roundup Ready corn line MON802, which has previously been deregulated by USDA-APHIS.

Both GT200 and RT73 were produced via *Agrobacterium tumefaciens* transformation of the parental canola variety Westar. Data demonstrate that a single insert was inserted at a single locus in both GT200 and RT73, and that none of the elements from the backbone of the plasmid vector were transferred into either event. Events GT200 and RT73 produce very similar levels of the enzymes, CP4 EPSPS and GOX/GOXv247, which are responsible for imparting glyphosate tolerance. The two events are also similar to one another and to their nontransgenic parental canola variety with respect to key agronomic properties such as disease and pest susceptibility, yield, days to maturity, height, germination and seed shattering. In addition, the two events show similar levels of nutrients (protein, oil, ash, fiber, carbohydrates, fatty and amino acid profile) and antinutrients (erucic acid and glucosinolates) present in the seed.

Data relevant to the food and feed safety of event GT200 and the proteins expressed in canola plants containing this event have been reviewed by regulatory agencies in Canada, Japan and the United States, including food approval from Health Canada (September 12, 1997), feed approval from Agriculture and Agri-Food Canada (October 28, 1997), food approval from the Japanese Ministry of Health, Labor and Welfare (September 14, 2001) and feed approval from Japanese Ministry of Agriculture, Forestry and Fisheries (September 28, 2001). In addition, the US EPA has granted exemptions from

the requirement of a tolerance for both GOX and CP4 EPSPS proteins present in GT200 (61 FR 40338; 62 FR 52505). The conclusion of the lack of environmental impact and plant pest potential due to unconfined release of GT200 has been confirmed by Agriculture and Agri-Food Canada in its determination of environmental safety (Decision Document: DD96-07, March 22, 1996). In the United States, Monsanto initiated a consultation with the FDA on the food safety of glyphosate-tolerant canola event GT200 on April 24, 2001.

Data and information for glyphosate-tolerant canola event GT200, transformed with the plasmid vector PV-BNGT03, are provided. These data demonstrate that this canola line and its progeny are no more likely to become weeds than the antecedent organism RT73 or traditional canola varieties and are unlikely to increase the weediness potential of any cultivated plant or native wild species. In addition, like the antecedent organism, this event does not exhibit plant pathogenic properties nor does it exhibit toxicity to non-target organisms, including those organisms beneficial to agriculture.

Therefore, under regulations in 7 CFR part 340.6, Monsanto requests an extension to the determination of nonregulated status granted by APHIS on January 27, 1999 for the antecedent organism, Roundup Ready canola event RT73, to the glyphosate-tolerant event GT200. Such extension would also include any progenies derived from crosses between glyphosate-tolerant canola event GT200 and other canola varieties, and any progeny derived from crosses of this event with transgenic canola varieties that have also received a determination of nonregulated status and are no longer considered to be regulated articles under regulations in 7 CFR part 340.

CERTIFICATION

The undersigned certifies that, to the best of his knowledge and belief, this petition includes all data, information, and views relevant to the matter, whether favorable or unfavorable to the position of the undersigned, which is the subject of the petition.

Raymond C. Dobert

[Raymond C. Dobert]
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List of Abbreviations

aad	Gene encoding adenylyltransferase conferring spectinomycin and streptomycin resistance
bp	base pair
CP4 EPSPS	5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp. strain CP4
CTP	chloroplast transit peptide
ELISA	enzyme-linked immunosorbant assay
EPSP	5-enolpyruvylshikimate-3-phosphate
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
fwt	fresh weight
gox/GOX	glyphosate oxidoreductase gene/protein from *Ochrobactrum anthropi* strain LBAA
goxv247	a variant of the *gox* gene
GOXv247	a variant of the GOX protein
GT200	glyphosate-tolerant canola line 200
LB	left border of T-DNA
OSR	oilseed rape
PEP	phosphoenolpyruvate
PCR	polymerase chain reaction
RB	right border of T-DNA
RBDO	refined, bleached, deodorized oil
RT73	Roundup-tolerant canola line 73
Rubisco	Ribulose-1,5-bisphosphate carboxylase
Spc/Str	Phenotype with resistance to spectinomycin and streptomycin conferred by the *aad* gene
S3P	shikimate-3-phosphate
SSU	small subunit

Table of Contents

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

I. Rationale for the Submission of Request for Extension

A. Basis for Request for Extension of Determination of Nonregulated Status under 7 CFR340.6(e)

The Animal and Plant Health Inspection Service (APHIS) of the U.S. Department of Agriculture (USDA) has responsibility, under the Plant Protection Act (7 U.S.C. § 7701-7772), to prevent the introduction and dissemination into the U.S. or interstate movement of regulated articles such as plant pests and genetically engineered organisms developed using plant pests or their components. The regulations provide that an applicant may petition APHIS to evaluate submitted data to determine whether a particular regulated article does not present a plant pest risk and should no longer be regulated. If APHIS determines that the regulated article does not present a plant pest risk, the petition is granted, thereby allowing unrestricted introduction of the article.

In terms of a request for extension, Section 340.6(e) of the regulations provides that APHIS may extend a determination of nonregulated status to additional regulated article(s), upon finding that the additional article(s) do/does not increase the potential for plant pest risk, and should therefore not be regulated. Such a finding would be made based on an evaluation of the similarity of the additional regulated articles to an antecedent organism. The Agency has provided the following example in its guidance as a "molecular manipulation that would yield a regulated article that APHIS believes is unlikely to pose new risk issues beyond those that would have been considered in the initial determination of nonregulated status":

- *Modifications in which the antecedent organism and the regulated article in question contain different donor genes, but the donor gene used in producing the antecedent organism and the donor gene used in producing the regulated article in question encode enzymes catalyzing the same biochemical reaction (i.e., molecules that have the same substrates and products).*

When applying this guidance it is clear that a request for an extension of determination of nonregulated status to glyphosate-tolerant canola event GT200 based upon the previous approval of Roundup Ready® Canola event RT73 (98-281-01p) is appropriate.

The glyphosate tolerance of Roundup Ready canola event RT73 is imparted by the insertion of a glyphosate-tolerant EPSPS from *Agrobacterium* sp. strain CP4 as well as a variant of the glyphosate degrading enzyme, glyphosate oxidoreductase (GOX) from *Ochrobactrum anthropi* strain LBAA, designated GOXv247, into the canola genome. In comparison, glyphosate-tolerant canola

event GT200 (also referred to as Roundup tolerant canola event RT200) utilizes the identical EPSPS from *Agrobacterium* sp. strain CP4 as well as a non-modified GOX protein from *Ochrobactrum anthropi* strain LBAA. In both cases, the GOX enzymes catalyze the same oxidative degradation of glyphosate into aminomethylphosphonic acid (AMPA) and glyoxylate (OECD, 1999). It is on this basis that Monsanto requests this extension for the determination of nonregulated status to glyphosate-tolerant canola event GT200.

The specific differences between Roundup Ready Canola event RT73, previously granted nonregulated status, and glyphosate-tolerant canola event GT200 are discussed in the appropriate sections below.

B. Glyphosate-Tolerant Canola Event GT200

Monsanto developed glyphosate-tolerant canola in the early 1990s. During this period, two transformation events, RT73 and GT200 (also referred to in other documents as GT73 and RT200, respectively), were pursued as lead events. Both events performed well in field tests over several years; however, in late 1993 the decision was made to pursue commercial development of only one event, event RT73.

The basic rationale for the development of glyphosate-tolerant canola is unchanged from that provided in the previous petition submission. In summary, canola plants that are tolerant to glyphosate enables growers to utilize Roundup herbicide for effective control of weeds during the growing season and to take advantage of this herbicide's environmental and safety characteristics. Glyphosate is highly effective against the majority of annual and perennial grasses and broad-leaved weeds. Glyphosate has excellent environmental features, such as rapid soil binding (resistance to leaching) and biodegradation (which decreases persistence), as well as extremely low toxicity to mammals, birds and fish (Malik *et al.,* 1989). In addition, glyphosate is classified by the EPA as Category E (evidence of non-carcinogenicity for humans) (57 FR 8739).

II. The Canola Family

A thorough review of the taxonomy and biology of the canola family may be found in the "Consensus Document on the biology of *Brassica napus* L. (Oilseed Rape)" in the OECD Series on the Harmonization of Regulatory Oversight in Biotechnology (OECD, 1997). Information not discussed in the OECD document concerning the distribution of species that are sexually compatible with *Brassica napus* in the U.S. can be found in Sections II.A. and VI.F of the RT73 Petition.

There are no significant changes regarding canola biology from the previous petition.

A. Characteristics of the Nontransformed Cultivar

As for the antecedent organism, event RT73, glyphosate-tolerant canola event GT200 was selected from plants of the well-known Westar variety of canola (*Brassica napus* L.) (Klassen *et al.,* 1987). Since 1982, this variety has had a history of safe use in the commercial production and breeding of canola. Its pedigree and performance data spanning a six year period has been published (Klassen *et al.,* 1987). Although Westar has been a standard, as well as a source of breeding germplasm for many other registered varieties of canola, this variety is no longer competitive with the higher performing varieties available today.

III. *AGROBACTERIUM* TRANSFORMATION METHOD

The antecedent organism, Roundup Ready canola event RT73, was produced using a disarmed *Agrobacterium tumefaciens* plant transformation system. Glyphosate-tolerant canola event GT200 was produced using the same *Agrobacterium* transformation system (White, 1989; Howard *et al.,* 1990) described in Section III of the previous petition for RT73.

IV. DONOR GENES AND REGULATORY SEQUENCES

A. Plant Transformation Vector, PV-BNGT03

The antecedent organism, Roundup Ready canola event RT73, and glyphosate-tolerant canola event GT200 are distinct transformation events. The antecedent organism, RT73, was produced using the double border plant transformation vector PV-BNGT04, while event GT200 was produced using the double border plant transformation vector PV-BNGT03.

Both vectors contain an identical glyphosate-tolerant EPSPS gene from *Agrobacterium* strain CP4, and the glyphosate oxidoreductase (GOX) gene from *Ochrobactrum anthropi* strain LBAA. The vector PV-BNGT03 used to create event GT200 differs from the vector used to create the antecedent organism RT73 by five base pairs in the GOX coding region. This difference results in the production of the GOX protein in event GT200 and a variant of GOX, designated GOXv247, in event RT73. At the protein level, GOX and the GOXv247 variant are >99% identical, differing by only three out of 431 amino

acids. Other than the difference in the GOX sequence, the vectors used to produce events GT200 and RT73 contain the same genetic elements.

As described in more complete detail below and in Table 2, the sequence between the left and right border sequences in PV-BNGT03 contains the genetic elements described in Table 1 below.

Table 1. Expression Cassettes Present in Plasmid Vector PV-BNGT03

Promoter	Transit peptide	Gene	Terminator
P-CMoVb	CTP1	*gox*	E9 3'
P-CMoVb	CTP2	CP4 EPSPS	E9 3'

The plasmid map of PV-BNGT03 is shown in Figure 2. As in the antecedent organism, all of the clonings performed to construct plasmid PV-BNGT03 were performed in non-pathogenic *E. coli* strains derived from *E. coli* K-12 (*E. coli* LE392, JM101, and MM294), commonly used in molecular biology research (Sambrook *et al.*, 1989).

The vector PV-BNGT03 contains well-characterized DNA segments required for selection and replication of the plasmid in bacteria, as well as a right border for initiating the region of DNA transferred into plant genomic DNA. Other than the already described difference in the GOX coding region, the other genetic elements in the vector are the same as described in Section IV. A of the petition for the antecedent organism. This includes the descriptions of promoters, chloroplast transit peptides, transcriptional termination sequences and elements present in the plasmid backbone (origin of replication and bacterial selectable marker gene).

The previous submission for event RT73 indicated that vector PV-BNGT04 was 11,461 bp in size. This vector in fact has a size of 11,479 bp; the earlier erroneous size calculation of PV-BNGT04 incorrectly assessed the size of the E9 3' polyadenlyation region by 9 bp. Since this sequence is present twice in the vector, it results in the corrected size of the vector being 18 bp larger. This correction does not affect any analysis or conclusions made for either RT73 or GT200.

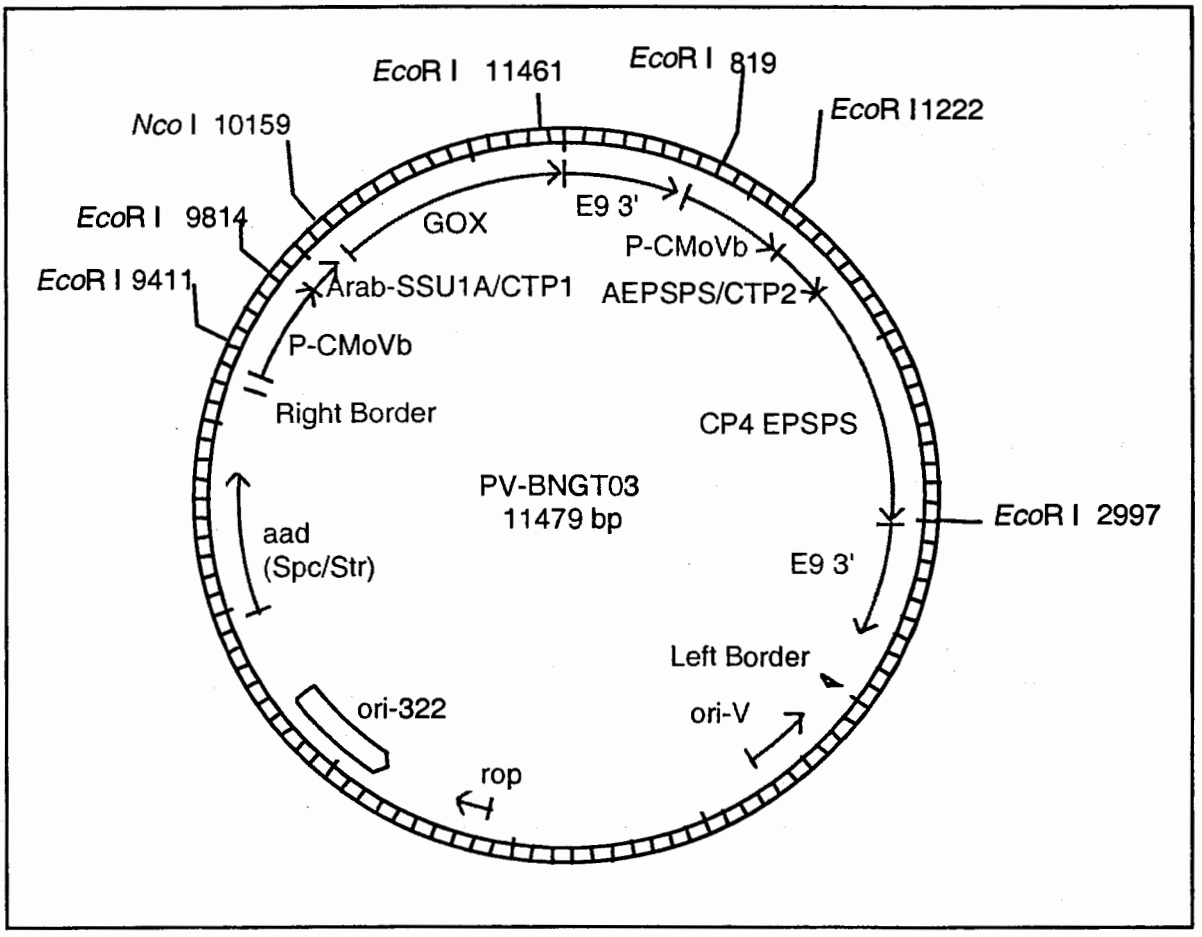

Figure 1. Plasmid Map of PV-BNGT03. The plasmid PV-BNGT03 was used to generate glyphosate-tolerant canola event GT200. The T-DNA portion of the plasmid located between the right and left borders containing the GOX and CP4 EPSPS expression cassettes was transferred to canola event GT200 using an *Agrobacterium tumefaciens* transformation system.

Table 2. Summary of the Genetic Elements in Plasmid PV-BNGT03.

Genetic Element	Function (Reference)
Right Border (RB)	A 25 nucleotide direct repeat that acts as the initial point of DNA transfer into plant cells, originally isolated from pTiT37 (Depicker *et al.*, 1982).
P-CMoVb	The 35S promoter from a modified figwort mosaic virus (Gowda *et al.*, 1989; Richins *et al.*, 1987; Sheperd *et al.*, 1987).
Arab-SSU1A/CTP1	The N-terminal of the small subunit 1A of the ribulose-1,5-bisphosphate carboxylase chloroplast transit peptide from *Arabidopsis* (Timko *et al.*, 1988).
gox	A synthetic glyphosate oxidoreductase (*gox*) gene based on the glyphosate oxidoreductase (*gox)* gene isolated from *Ochrobactrum anthropi* strain LBAA (Barry *et al.*, (1994); and, Woodward *et al.*, (1994), summarized in Appendices 2
and	3, respectively, in the RT73 petition)
E9 3'	The 3' end of the pea rbcS E9 gene, which provides the polyadenylation sites for the *gox* and *cp4 epsps* genes (Coruzzi *et al.*, 1984; Morelli *et al.*, 1985).
AEPSPS/CTP2	The N-terminal chloroplast transit peptide sequence from the *Arabidopsis* EPSPS gene (Klee *et al.*, 1987).
***cp4 epsps* syn**	The synthetic 5-enolpyruvylshikimate-3-phosphate synthase (*cp4 epsps*) gene based on the sequence from *Agrobacterium sp.* strain CP4 (Padgette *et al.*, 1996)
Left Border (LB)	Isolated from the octopine Ti plasmid, pTiA6, and containing the 25 bp direct repeat sequence that delimits the T-DNA transferred (Barker *et al.*, 1983).
ori-V	The vegetative origin of replication that permits plasmid replication in *Agrobacterium*. It was originally isolated from plasmid RK2 (Rogers *et al.*, 1987).
ori-322	A plasmid replication origin which permits propagation of DNA in bacterial hosts such as *E. coli*. (Sutcliffe, 1979).
aad (Spc/Str)	The bacterial gene encoding the Tn7 AAD 3' adenylyltransferase, conferring spectinomycin and streptomycin resistance to bacterial cells (Fling *et al.*, 1985).

B. *cp4 epsps* and *gox* Genes

As in the antecedent organism, two genes that confer tolerance to the glyphosate herbicide were introduced into glyphosate-tolerant canola event GT200: *cp4 epsps* and *gox*.

The source and characteristics of the *cp4 epsps* gene and CP4 EPSPS protein are the same as described in the petition for the antecedent organism. In addition, the same promoter and transcriptional termination element were used in the gene expression cassettes for both events.

The *gox* gene, cloned from *Ochrobactrum anthropi* strain LBAA, was also inserted into glyphosate-tolerant canola event GT200 to provide tolerance to glyphosate. Except as described below, the source and characteristics of the *gox* gene and GOX protein are the same as described in the petition for the antecedent organism. The *gox* gene encodes the glyphosate-metabolizing enzyme glyphosate oxidoreductase (GOX) (Hallas *et al.,* 1988; Barry *et al.,* 1992, OECD, 1999). As previously discussed, the GOX and the GOXv247 proteins are >99% identical, differing by only three amino acids out of more than 400. The amino acid sequence of GOX is compared to that of GOXv247 in Figure 4.

As described in the petition for the antecedent organism, the GOX variant protein, GOXv247, is modified relative to GOX by the substitution of the histidine residue at position 334 with arginine. This change effects a ten-fold lowering of the apparent K_m[2] ($appK_m$) for glyphosate in GOXv247, and thus results in an enhanced efficiency in glyphosate degradation. This modification in enzyme kinetics does not have any discernible effect on glyphosate tolerance. Although GOX is kinetically less efficient than GOXv247, the efficiency of GOX in degrading glyphosate remains sufficient to provide adequate glyphosate tolerance. Thus, both event GT200 and the antecedent organism, RT73. are tolerant to glyphosate applied at commercial rates.

[2] The Michaelis-Menton constant, K_m, is a measure of the affinity of a particular substrate for an enzyme. The lower the K_m, the higher the affinity for the enzyme.

Figure 2. Predicted Amino Acid Sequences of the GOX (lower sequence) and GOXv247 Proteins (top sequence).

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1 MAENHKKVGIAGAGIVGVCTALMLQRRGFKVTLIDPNPPGEGASFGNAGC 50
  ||||||||||||||||||||||||||||||||||||||||||||||||||||
1 MAENHKKVGIAGAGIVGVCTALMLQRRGFKVTLIDPNPPGEGASFGNAGC 50

51 FNGSSVVPMSMPGNLTSVPKWLLDPMGPLSIRFGYFPTIMPWLIRFLLAG 100
   ||||||||||||||||||||||||||||||||||||||||||||||||||
51 FNGSSVVPMSMPGNLTSVPKWLLDPMGPLSIRFSYFPTIMPWLIRFLLAG 100

101 RPNKVKEQAKALRNLIKSTVPLIKSLAEEADASHLIRHEGHLTVYRGEAD 150
    ||||||||||||||||||||||||||||||||||||||||||||||||||
101 RPNKVKEQAKALRNLIKSTVPLIKSLAEEADASHLIRHEGHLTVYRGEAD 150

151 FARDRGGWELRRLNGVRTQILSADALRDFDPNLSHAFTKGILIEENGHTI 200
    ||||||||||||||||||||||||||||||||||||||||||||||||||
151 FAKDRGGWELRRLNGVRTQILSADALRDFDPNLSHAFTKGILIEENGHTI 200

201 NPQGLVTLLFRRFIANGGEFVSARVIGFETEGRALKGITTTNGVLAVDAA 250
    ||||||||||||||||||||||||||||||||||||||||||||||||||
201 NPQGLVTLLFRRFIANGGEFVSARVIGFETEGRALKGITTTNGVLAVDAA 250

251 VVAAGAHSKSLANSLGDDIPLDTERGYHIVIANPEAAPRIPTTDASGKFI 300
    ||||||||||||||||||||||||||||||||||||||||||||||||||
251 VVAAGAHSKSLANSLGDDIPLDTERGYHIVIANPEAAPRIPTTDASGKFI 300

301 ATPMEMGLRVAGTVEFAGLTAAPNWKRAHVLYTRARKLLPALAPASSEER 350
    ||||||||||||||||||||||||||||||||||||||||||||||||||
301 ATPMEMGLRVAGTVEFAGLTAAPNWKRAHVLYTHARKLLPALAPASSEER 350

351 YSKWMGFRPSIPDSLPVIGRATRTPDVIYAFGHGHLGMTGAPMTATLVSE 400
    ||||||||||||||||||||||||||||||||||||||||||||||||||
351 YSKWMGFRPSIPDSLPVIGRATRTPDVIYAFGHGHLGMTGAPMTATLVSE 400

401 LLAGEKTSIDISPFAPNRFGIGKSKQTGPAS 431
    |||||||||||||||||||||||||||||||
401 LLAGEKTSIDISPFAPNRFGIGKSKQTGPAS 431
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C. Chloroplast Transit Peptides (CTP)

The source and characteristics of the chloroplast transit peptides used to facilitate the intercellular targeting of the GOX and CP4 EPSPS proteins to the chloroplast in glyphosate-tolerant canola event GT200 are identical to the chloroplast transit peptides previously described in the petition for the antecedent organism, event RT73 (Section IV. D).

V. Genetic Analysis and Agronomic Performance

A. Characterization of the Inserted Genetic Material Including Insert Stability

Molecular analyses were performed on glyphosate-tolerant canola event GT200 in a manner similar to that described in the petition for the antecedent organism RT73 (Section V. A).

Glyphosate-tolerant canola event GT200 was derived from an original R_0 transformant, which was obtained by *Agrobacterium* transformation of the Westar canola variety with vector PV-BNGT03. As described above, this vector (Figure 2) contains two distinct segments: the T-DNA containing the *cp4 epsps* and *gox* gene cassettes bounded by the Right and Left Borders, and the plasmid backbone containing the bacterial origins of replication and a selectable marker.

Molecular analyses were performed to characterize the DNA integrated into the genome of glyphosate-tolerant canola event GT200. Specifically, the insert number (number of integration sites within the canola genome), the copy number (the number of integrated DNA fragments within one insertion site), and the absence of backbone sequence were assessed by Southern blot analysis. Data from these analyses demonstrate that glyphosate-tolerant canola event GT200 contains a single copy of the T-DNA from PV-BNGT03 located at a single insertion site and that no detectable plasmid backbone DNA or extraneous T-DNA segments are present (see summary in Table 3). In addition, polymerase chain reaction (PCR) analyses were performed to characterize the 5' and 3' ends of the insert. All of these data support the conclusion that, as in the antecedent organism, only the full length CP4 EPSPS and GOX proteins are encoded by the insert in event GT200.

In the petition submitted for the antecedent organism (Sections V. A1 and A2), molecular characterization data was presented for glyphosate-tolerant canola event GT200, but was not discussed or summarized.

Table 3. Comparison of Genetic Elements Found in Glyphosate Tolerant Canola Events GT200 and RT73

Genetic Element	GT200[1]	RT73
CMoVb	+	+
gox	+	variant 247
cp4 epsps	+	+
E9 3'	+	+
aad (Spc/Str)	-	-
ori-V	-	-
Number of Loci	1	1

[1] + indicates the genetic element is present;
- indicates the genetic element is not present

1. Insert number and copy number

For the molecular characterization studies described below the test DNA was isolated from R_3 generation leaf or R_4 generation seed of glyphosate-tolerant canola event GT200, while the control DNA was isolated from seed of the non-transgenic parental variety Westar.

Analyses performed on DNA derived from leaves of R_3 canola GT200 plants demonstrate that only a single copy of the DNA was inserted into the canola genomic DNA at a single location in glyphosate-tolerant canola event GT200. Genomic DNA isolated from GT200 leaf tissue and Westar was digested with *SpeI*, a restriction enzyme that does not cut inside the plasmid used in transformation. Since *SpeI* does not cut within PV-BNGT03, the number of bands present in this Southern blot correspond to the number of loci where plasmid DNA has been inserted into the plant genomic DNA. The positive control on these blots was the transformation vector PV-BNGT04, which is identical to PV-BNGT03 with the exception of five bases in the GOX coding region, cut with *EcoRI*. The resulting blot was probed with ^{32}P-labelled plasmid PV-BNGT04. Figure 4 shows one very high molecular weight band in lanes 2-4, including the Westar control lane. This band represents cross-hybridizing sequences found naturally in canola. Blots using intact transformation vectors containing the plasmid backbone often show some background hybridization with plant genomic DNA. A second lower molecular weight band is seen in lane 4, which contains GT200. This single distinctive band in GT200 indicates that the T-DNA integrated at a single locus in Westar genome.

The number of copies of the T-DNA inserted into one locus was determined by digesting the genomic DNA with the restriction enzyme *Nco* I, an enzyme that cuts only once in the T-DNA of plasmid PV-BNGT03 used to generate

glyphosate-tolerant canola event GT200. The blot was probed with three 32P-labeled overlapping segment which span the entire T-DNA from PV-BNGT03. If the event contains one copy of the T-DNA, two bands should be produced, representing two border fragments containing both inserted DNA and flanking canola genomic DNA. The results are shown in Figure 5. Westar control DNA (lane 1) showed no bands, as expected for the negative control. Westar DNA mixed with PV-BNGT03 DNA (lane 2) produced the expected size band at approximately 11.5 Kb, representing the linearized plasmid. Glyphosate-tolerant canola event GT200 DNA (lane 3) produced two bands at approximately 5.4 Kb and 8.3 Kb. Since only two bands were produced in this analysis, the result establishes that glyphosate-tolerant canola event GT200 contains only one copy of the T-DNA at a single locus of integration.

2. Insert composition and structure

Only the genetic elements responsible for the expression of the glyphosate tolerance proteins were detected in glyphosate-tolerant canola event GT200. The insert (Figures 1 and 3) contains the CMoVb promoter, the *Arabidopsis* rubisco small subunit CTP, the *gox* gene, the pea E9 3' terminator, a second copy of the CMoVb promoter, the *Arabidopsis* EPSPS CTP, the *cp4 epsps* gene, and a second copy of the pea E9 3' terminator.

Genetic Elements

In order to identify the genetic elements present in glyphosate-tolerant canola event GT200, Southern blot analyses were performed. The positive control on these blots was the transformation vector PV-BNGT04. Since there are only minor sequence differences between PV-BNGT04 and PV-BNGT03 (in the *gox* coding region), the two vectors can be used interchangeably as probes and/or positive controls. The negative control was the untransformed parental line Westar. Genetic element-specific probes for the *gox* and *cp4 epsps* genes, and the *oriV/ori322/aad* backbone region were utilized as shown in Figure 3. Plasmid and genomic DNA was cut with *EcoRI* unless otherwise noted in the text or figure legends. There are six *EcoRI* sites within PV-BNGT04 and PV-BNGT03, which all occur between the left and right border sequences, as illustrated in Figure 3. In several of the blots, the antecedent event, RT73, was included in the analyses; however, this data will not be discussed here.

a. *gox* coding sequences:

Southern blot analysis was performed using genomic DNA extracted from leaf tissue from event GT200 and the parental negative control line Westar. All DNAs were cut with *EcoRI*. The blot was probed with a ^{32}P-labelled fragment containing a full-length copy of *gox* (Figure 3, Probe 1). In the previous petition for event RT73, it was stated that the probe used in this analysis was the full-

length *goxv247* sequence. The probe actually used was the full-length *gox* sequence; however, since there are only minor differences between *gox* and *goxv247*, the probe hybridizes equally well with both genes. In Figure 6, Panel A, a single band of approximately 1650 bp, the predicted size of the *gox*-containing segment is observed in lanes 1 and 5 containing PV-BNGT04 and GT200 DNA. The *gox* band in the GT200 lane migrates slightly slower than in the plasmid control lane due to matrix effects of the abundant genomic DNA in the RT73 lane. No hybridizing band is observed in the negative control lane containing Westar. This result supports the conclusion that event GT200 contains an intact *gox* coding region, with no additional detectable bands.

b. *cp4 epsps* coding sequences:
Southern blot analysis was performed in a similar manner to that described for *gox*. The blot was probed with a ^{32}P-labelled fragment containing a full-length copy of *cp4 epsps* (Figure 3, Probe 2). Figure 6, Panel B, shows a band of approximately 1775 bp, the predicted size of the *cp4 epsps*-containing segment, in event GT200 as well as in the positive control lane containing PV-BNGT04. The *cp4 epsps* band in the GT200 lane migrates slightly slower than in the plasmid control lane due to matrix effects of the abundant genomic DNA in the GT200 lane. No hybridizing band is observed in the negative control lane containing Westar. This result supports the conclusion that event GT200 contains an intact *cp4 epsps* coding region, with no additional detectable bands.

c. Ori-322, ori-V and *aad* (Str/Spc) plasmid backbone sequences:
The backbone region of the plasmid consists of the DNA between the RB and LB containing the *aad* coding region driven by a bacterial promoter, as well as *ori*-V and *ori*-322. Genomic DNA was digested with the restriction enzyme *Eco*R I. Digested control DNA was spiked with plasmid PV-BNGT03 DNA that had been previously digested with the restriction enzyme *Nco* I to linearize the plasmid. The blot was probed with two overlapping PCR-generated probes (Figure 3, Probes B1 and B2) to confirm the absence of backbone. Together, these two probes encompass the entire backbone sequence. The absence of any hybridization bands indicates the absence of detectable backbone sequence in the event. The results are shown in Figure 7. Due to the bacterial DNA contained in the molecular weight marker used in the analysis, strong hybridization occurred between the marker and the two backbone probes. Therefore, either before or after the initial exposure of the blot was obtained, the marker lane was removed from the membrane prior to generating additional exposures. However, areas of hybridization to the marker are still visible between the marker lane and lane 1 around 3.1 Kb and below 1.0 Kb. Westar control DNA (lane 1) showed no detectable hybridization bands, as expected for the negative control. Plasmid PV-BNGT03 DNA mixed with Westar control DNA (lane 2) produced one band at the expected size of ~11.5 Kb, representing the linearized plasmid. Glyphosate-tolerant canola event

GT200 (lane 3) showed no detectable hybridization bands. This result establishes that glyphosate-tolerant canola event GT200 does not contain any detectable plasmid backbone sequences including *ori*-V, *ori*-322, or the *aad* coding region.

d. Characterization of the right and left borders: There are two DNA sequences of 25 bp each within the plasmid used in the transformation of event GT200 that are defined as the "right border" and the "left border." The right border from *Agrobacterium* functions as the initiation site of the transfer of the DNA into the plant genomic DNA. The left border functions as the termination site of that transfer. As previously described (Section V. A.2.e) in the petition for the antecedent organism, PCR analysis was conducted to demonstrate that the border sequences of the inserted DNA were the endpoints of the DNA insert. As presented in the earlier petition (Section V. A.2.e, Figures 10 and 11), this analysis was also conducted with event GT200 to provide further evidence that only the T-DNA sequences are present in the DNA of event GT200. PCR primers based on the plasmid vector sequence from the region just inside the right and left border sequences were used together with primers within or beyond the border sequences (i.e., "backbone sequence") to characterize the endpoints of the DNA insert. DNA from the Westar variety served as a negative control and an appropriate plasmid vector, PV-BNGT03, served as a positive control. The results of this analysis establish that integration of the plasmid DNA in event GT200 did not proceed outside of the right or left borders.

e. Verification of Sequences at the 5' and 3' Ends of the Inserted DNA Flanking the GT200 Insert

PCR analyses were performed on genomic DNA extracted from glyphosate-tolerant event GT200 to verify the DNA sequence at the 5' and 3' ends of the insert as well as the genomic DNA flanking the 5' and 3' ends of the insert in event GT200. The positions of all primers, as well as the results of the PCR analyses, are presented in Figure 8. The control reactions containing no template DNA (lanes 3 and 7) did not generate a PCR product with either primer set, as expected. The reactions containing Westar non-transgenic canola DNA (lanes 6 and 2) did not generate a PCR product with either the 5' or 3' primer set, as expected; however, a product of 595 bp was generated with the internal control primers designed to amplify a portion of the acyl-acyl carrier protein thioesterase *Fat*A endogenous canola gene (Genbank accession X87842), indicating that the quality of the DNA was sufficient to generate PCR products. PCR performed on glyphosate-tolerant canola event GT200 DNA (lanes 8 and 4) generated the expected size products of ~440 bp, representing the 5' end of the insert and genomic DNA sequence flanking the right border, and ~1100 bp, representing the 3' end of the insert and genomic DNA sequence flanking the left border sequence. These results demonstrate that the predicted-sized PCR

products are generated from the 5' and 3' ends of the insert in glyphosate-tolerant canola event GT200.

3. Stability of glyphosate-tolerant trait and Mendelian inheritance

The stability of an insert can be determined by assessing the stability of the trait or phenotype over several generations. Genetic stability of the insert in event GT200, as expressed by tolerance to glyphosate and agronomic performance in the field was assessed under diverse environments, climatic and stress conditions across at least four generations.

The stability of the glyphosate-tolerant phenotype in event GT200 is consistent with segregation data observed in the development of this event. Selfed plants segregated at a ratio of 3 tolerant to 1 susceptible. This segregation ratio establishes that the GT200 insert behaves as a single dominant gene that is inherited in a Mendelian fashion (Table 4). The glyphosate tolerance phenotype and Mendelian transmission have been consistent over more than four generations of event GT200.

Table 4. Segregation of Glyphosate-Tolerant Trait in Event GT200.

Generation	Observed		Expected		Chi-square (X^2) value
GT200 - R1	Tolerant	50	Tolerant	54	1.185
	Susceptible	22	Susceptible	18	

Tabular value at one degree of freedom and a five percent level of significance = 3.84

4. Summary of genetic analysis

As clearly demonstrated, the only genes from PV-BNGT03 that are present in glyphosate-tolerant canola event GT200 are *cp4 epsps* and *gox*. No genetic elements from outside of the right and left borders of the T-DNA were transferred into the genomic DNA of glyphosate-tolerant canola event GT200. This conclusion was drawn from the following data: 1) the positive detection of segments containing the *cp4 epsps* and *gox* genes by Southern analysis; 2) the lack of *ori-322* and *ori-V* signals by Southern analysis; and 3) the lack of PCR amplification products using PCR primer pairs, one of which was located within the T-DNA and the other located just beyond either of the right or left border sequences. The stability of the inserted DNA has been demonstrated by the expression of the glyphosate tolerance trait over several generations and the observation of simple Mendelian inheritance of this trait.

Figure 3. Schematic Diagram of PV-BNGT03/4 Showing the Probes Used in the Molecular Characterization of Event GT200

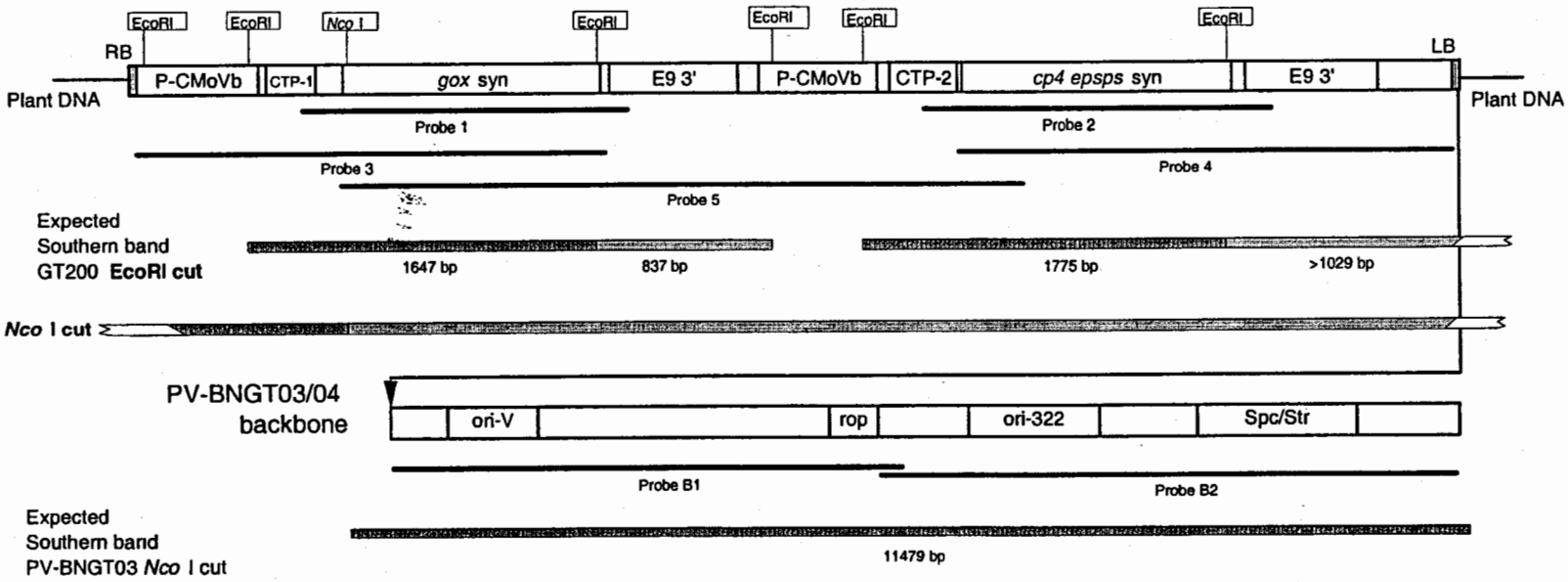
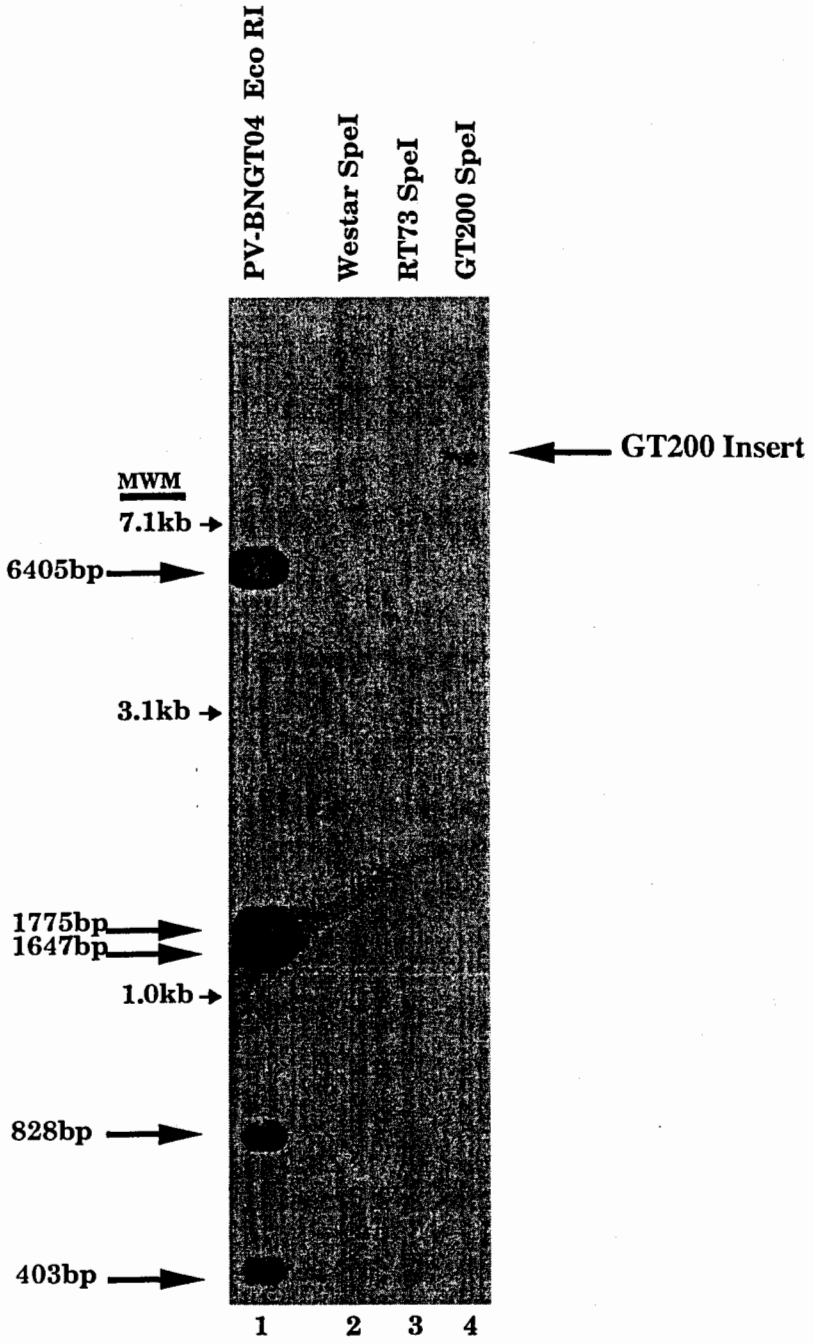
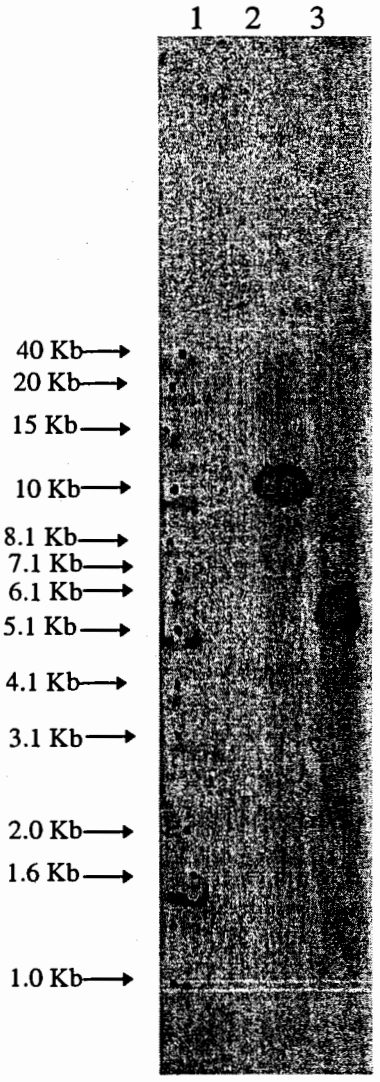


Figure 4. Southern Blot of Glyphosate-Tolerant Canola Event GT200 to Determine Insert Number



PV-BNGT04 plasmid DNA (lane 1) was digested with *EcoR*I. Westar control genomic DNA (lane 2), RT73 genomic DNA (lane 3) and GT200 genomic DNA (lane 4) were digested with *SpeI*. Each lane represents 100 pg plasmid DNA or 5 µg of genomic DNA. The digests were subjected to electrophoresis in a 0.8% agarose gel and transferred to a nylon membrane. The membrane was probed with a ^{32}P-labelled PV-BNGT04 plasmid DNA and subjected to autoradiography.

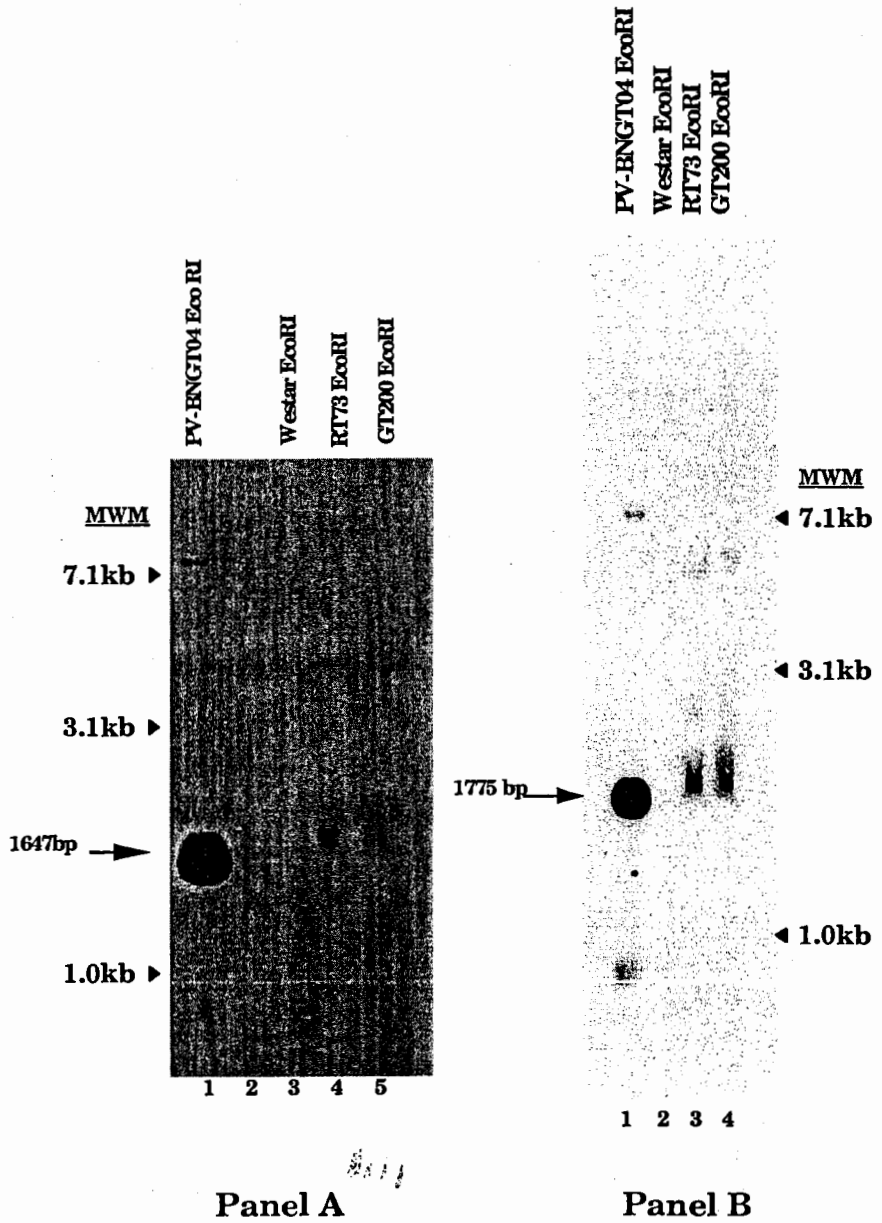
Figure 5. Southern Blot Analysis of Glyphosate-Tolerant Canola Event GT200 to Determine Copy Number



Ten micrograms of Westar non-transgenic genomic DNA extracted from seed (lane 1) and 10 µg of canola event GT200 genomic DNA extracted from seed (lane 3) were digested with *Nco* I. Approximately 76 pg of plasmid PV-BNGT03 DNA digested with *Nco* I were mixed with Westar DNA that was previously digested with *Nco* I (lane 2). The blot was probed with three ^{32}P-labeled overlapping portions of the T-DNA (probes 3, 4 and 5 in Figure 3).

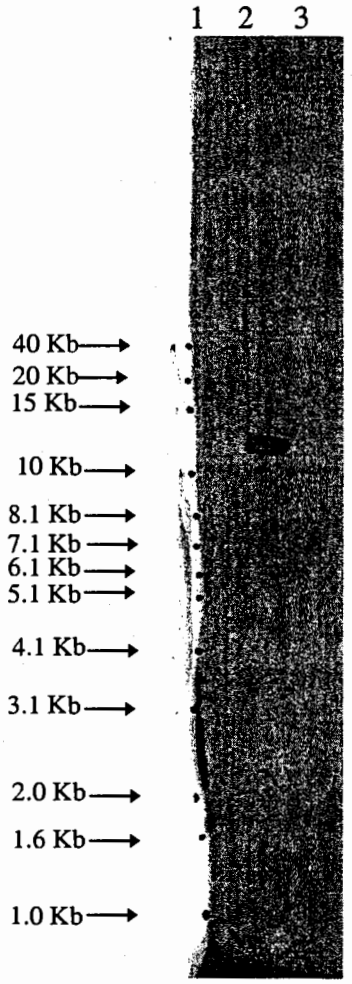
→ Symbol denotes sizes obtained from MW markers on ethidium stained gel.

Figure 6. Southern Blot Probed for *gox* and *cp4 epsps* in Glyphosate-Tolerant Canola Event GT200



PV-BNGT04 plasmid DNA (lane 1), Westar control genomic DNA (lane 2), RT73 genomic DNA (lane 3) and GT200 DNA (lane 4) were digested with *EcoRI*. Each lane represents 100 pg plasmid DNA or 5 µg of genomic DNA. The digests were subjected to electrophoresis in a 0.8% agarose gel and transferred to a nylon membrane. The membrane was probed with ^{32}P-labelled DNA from the *gox* coding region for panel A or ^{32}P-labelled DNA from the *cp4 epsps* coding region for panel B and subjected to autoradiography. The probes used are depicted in Figure 3.

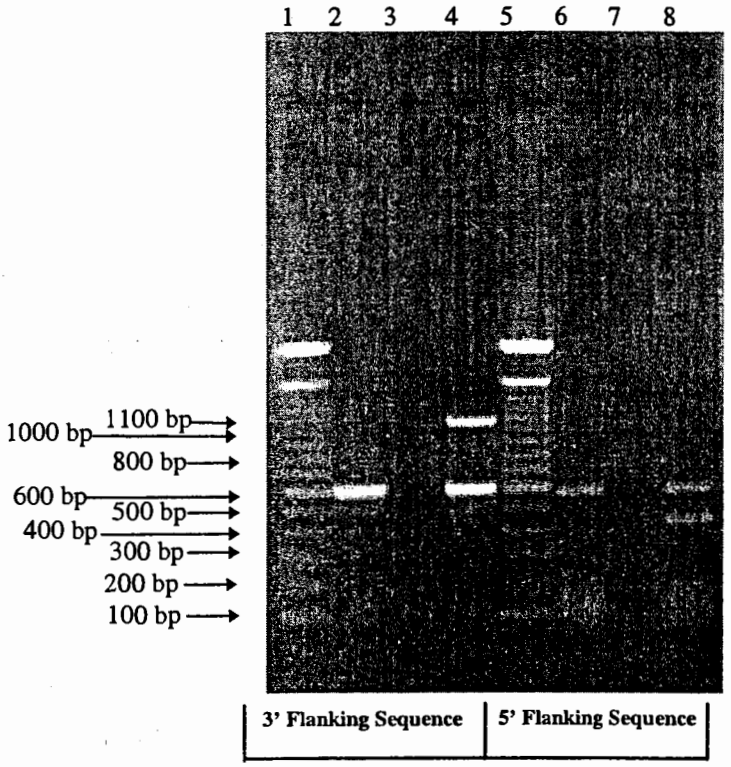
Figure 7. Southern Blot Analysis of Glyphosate Tolerant Canola Event GT200 to Assess Absence of Backbone Sequences



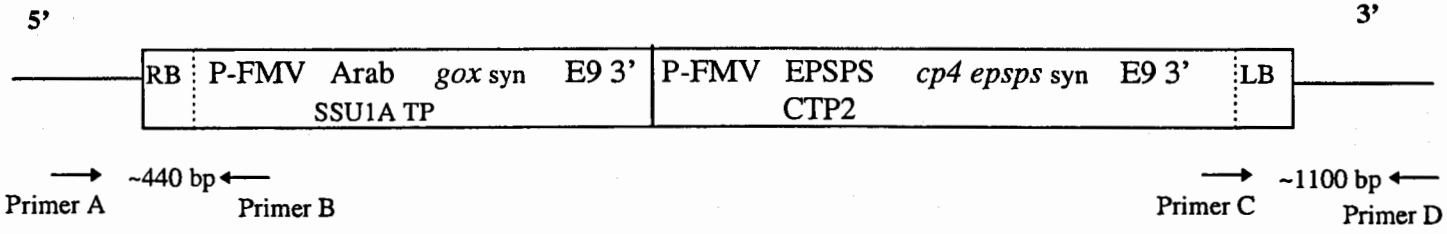
Ten micrograms of Westar non-transgenic genomic DNA extracted from seed (lane 1) and 10 µg of canola event GT200 genomic DNA extracted from seed (lane 3) were digested with *Eco*R I. Approximately 76 pg of plasmid PV-BNGT03 DNA digested with *Nco* I were mixed with Westar DNA that had been previously digested with *Eco*R I (lane 2). The blot was probed with two ^{32}P-labeled segments of DNA, encompassing the entire backbone sequence (probes B1 and B2 in Figure 3). These probes were amplified by PCR from a plasmid vector which is identical in its backbone sequence to PV-BNGT03.

⟶ Symbol denotes sizes obtained from MW markers on ethidium stained gel.

Figure 8. PCR Verification of Sequences at the 5' and 3' Ends of the Insert in Glyphosate-Tolerant Canola Event GT200



T-DNA from PV-BNGT03



PCR analyses were performed on genomic DNA extracted from GT200 or non-transgenic Westar seed using Primers A and B were used to confirm the 5' end of the insert and the 5' flanking sequence (GT200 lane 8, Westar lane 6). Primers C and D were used to confirm the 3' end of the insert, as well as the 3' flanking sequence (GT200 lane 4., Westar lane 2). An internal control primer pair based on the endogenous canola *FatA* gene (acyl acyl carrier protein thioesterase) was also added to ensure to quality of the genomic DNA. Lanes 1 and 5 contain Gibco BRL 100 bp DNA Ladder for use as a size indicator and lanes 3 and 7 were no template control PCRs. Fifteen microliters of each PCR reaction were loaded on the gel.

⟶ Symbol denotes sizes obtained from the Gibco BRL 100bp DNA Ladder.

B. Expression of the Inserted Genes

As in the antecedent organism, RT73, expression of the *cp4 epsps* and *gox* genes in event GT200 is constitutive, and both the CP4 EPSPS and GOX proteins can be detected at relatively low levels in leaves and seed. Expression throughout the plant is expected when using the *CMoVb* promoter (Sheperd *et al.*, 1987). To thoroughly characterize glyphosate-tolerant canola event GT200, the levels of CP4 EPSPS and GOX proteins were measured in leaf and seed tissue from Canadian field trials conducted in 1992. In the 1992 trials, seed used for planting event GT200 came from two separate sources. Thus, two distinct designations for seed were used: GT200H and GT200. Samples which were given the designation GT200 were identical to GT200H except that the former was heterozygous for the glyphosate tolerance genes and the latter was homozygous for these genes. Seed samples from event GT200 and the control Westar were obtained from seven locations. Due to limited availability of pure homozygous seed for planting, samples of GT200H were obtained from only three sites.

Levels of the CP4 EPSPS and GOX proteins in canola plant tissues were measured by a validated enzyme linked immunosorbent assay (ELISA) that was described in the petition for the antecedent organism, RT73.

As summarized in Table 5, analyses of CP4 EPSPS protein in seed and leaf tissue from GT200H and GT200 resulted in mean protein levels that ranged from of 0.031 μg/mg tissue (fresh weight) to 0.051 μg/mg tissue (fresh weight). Analyses of GOX in seed and leaf tissue from GT200H and GT200 gave mean levels of 0.069 to 0.142 μg/mg tissue (fresh weight). Seed from plants homozygous for the glyphosate tolerance genes appear to express slightly higher levels of the introduced proteins as expected. The absolute levels of the GOX and CP4 EPSPS proteins are low, accounting for less than 0.07% of the seed on a fresh weight basis.

The levels of CP4 EPSPS and GOX in event GT200 seed and leaf were thus comparable to levels found in those tissues of GT73 which were reported in the RT73 petition.

No CP4 EPSPS or GOX were detectable in Westar seed tissue.

Table 5. CP4 EPSPS and GOX Protein Levels in GT200 Canola Leaf and Seed

Tissue Type	CP4 EPSPS Protein (µg/mg tissue fwt)		GOX Protein (µg/mg tissue fwt)	
	GT200H	GT200	GT200H	GT200
Leaf mean: range:	0.031 0.022-0.037	n.a.[3]	0.069 0.054-0.104	n.a.[3]
Seed mean: range:	0.051 0.048-0.056	0.034 0.026-0.042	0.142 0.081-0.207	0.105 0.058-0.127
Westar[2] Leaf	ND [3]	n.a. [3]	ND [3]	n.a. [3]

[1]Values for leaf and seed samples from 1992 from three locations in Canada. CP4 EPSPS analyses were performed on single sample extracts, n=3 for leaf, n=3 for seed. GOX analyses were performed on single sample extracts, run at two loadings, n=6 for leaf, n=6 for seed.

[2] In each analysis, Westar samples were used as a negative control. Values for Westar samples were below the calculated limit of detection (LOD). The LOD was determined by computing the mean and the standard deviation for Westar control wells by ELISA. The LOD is then the mean plus three standard deviations.

[3]ND - not detected ; n.a. - not available.

C. Disease and Pest Resistance Characteristics

Glyphosate-tolerant canola event GT200 transformed with the plasmid vector, PV-BNGT03, was tested in Canadian field trials in 1992 and 1993 at over 20 locations. Growth and development characteristics, as well as disease and insect susceptibility of the transformed canola versus nontransgenic control plants were monitored regularly during the growing season.

Plots of the glyphosate-tolerant canola event GT200 and Westar control plants were visually checked for the appearance of possible disease symptoms or insect damage. In 1993, quantitative monitoring of blackleg infestation was conducted, due to the significance of this disease in canola. The overall blackleg infestation rating (on a 0-5 rating scale, with 0 showing no incidence of disease) for event GT200 was 3.95, compared to 4.11 for the control variety, Westar. This difference was not statistically significant and the value falls within the range of variability of selections from Westar (1993 Canadian Co-Op tests - Report on Co-Operative Canola/Rapeseed Test 1993).

Based on the results of the field monitoring programs and disease screening tests, no notable differences were observed in disease or pest resistance

characteristics between glyphosate-tolerant canola event GT200 and nontransgenic control or commercial lines (see also Appendix 1).

D. Compositional Analyses

Monsanto Company initiated a consultation with the FDA on the food safety of glyphosate-tolerant canola event GT200 on April 24, 2001. Studies have been carried out to compare the nutritional constituents of canola seed and toasted meal from canola RT73 with seed and toasted meal from Westar control plants that were grown, processed and analyzed under the same conditions. The constituents assessed in seed included protein, ash, moisture, fiber, carbohydrates, calories, fatty acids (including erucic acid) and amino acids, glucosinolates and sinapines. In toasted meal, a composite sample was assessed for protein, ash, moisture, fat, fiber, carbohydrate, calories and nitrogen solubility. These analyses demonstrated that event GT200 seed and a processed fraction of the seed are not materially different from, and is as safe and nutritious as its parental variety and other canola varieties on the market. These data were included as part of our consultation with the FDA on event GT200 which was initiated in April 2001, following their 1992 policy regarding "Foods Derived from New Plant Varieties." A summary of this data is included in Appendix 2.

Data relevant to the food and feed safety of event GT200 and the proteins expressed in canola plants containing this event were previously reviewed by regulatory agencies in Canada and Japan, which granted clearances including food approval from Health Canada (September 12, 1997), feed approval from Agriculture and Agri-Food Canada (October 28, 1997), food approval from the Japanese Ministry of Health, Labor and Welfare (September 14, 2001) and feed approval from Japan's Ministry of Agriculture, Forestry and Fisheries (September 28, 2001).

E. Toxicants

In addition to analyses for nutrients, glyphosate-tolerant canola event GT200 was analyzed for two antinutritional components that are normally present in canola: erucic acid and glucosinolates. The nature of these toxicants was described in the petition for the antecedent organism, RT73.

Erucic acid is a mono-unsaturated, 22-carbon fatty acid (C22:1) that is a natural constituent of canola. Fatty acid profiles, including erucic acid levels, were determined using standard methodology (AOAC, 1990). The mean erucic acid (22:1) content in GT200 and GT200H canola seed grown during the 1992 field season in Canada were 0.17% and 0.12%, respectively. These levels of erucic

acid are well below the limits allowed for human use (2% of total fatty acids) and equivalent to the Westar control.

Glucosinolates are also closely monitored due to their reported antinutritional properties (Sorensen, 1990; Downey, 1983). The total concentration of four key glucosinolates (but-3-enyl, 2-hydroxybut-3-enyl, pent-4-enyl, and 2-hydroxypent-4-enyl) must be less than 30 μmole per gram of oil-free meal for the seed to be classified as canola quality (Canola Council of Canada, 1991). GT200 and GT200H canola seed samples grown during the 1992 field season in Canada were analyzed for glucosinolates using standard methods (Daun and McGregor, 1981). The results of these analyses were compared to the commercial limits and to values for Westar from the 1992 Canadian Co-Op Trials. Means determined for the total alkyl and indolyl glucosinolates are reported in Table 6. While it is apparent that the average level of alkyl glucosinolates in event GT200 is higher than the mean value for Westar, all individual sample values are well below the 30 μmole limit and are well within established ranges and limits reported in the literature for canola. In addition, mean levels of alkyl glucosinolates in GT200 tended to be lower than those found in GT73, which has been cleared for commercial use.

To gain better insight into the variation in the level of alkyl glucosinolates in the Westar variety, Dr. Keith Downey of Ag Canada, a leading canola breeder and developer of the Westar variety, was requested to review the data for both GT200 and GT73. Dr. Downey concluded that, due to the heterogeneity inherent in the Westar variety, the variation observed would be expected for lines, such as GT200 and GT73, that were selected from the Westar genotype (see letter from Dr. Keith Downey in Appendix 6 of the petition for the antecedent organism, RT73). It is also important to note that, while this value of alkyl glucosinolates in GT200 may have been on the higher side of the range observed for Westar, it is well below the harvest survey value (17 μmol/g in 1992) for commercially produced No. 1 grade Canada canola (DeClercq *et al.*, 1992).

We conclude that there is no meaningful difference in the level of glucosinolates between event GT200 and Westar canola. Furthermore, the levels of the alkyl glucosinolates are well below the limits established for the safe use of meal derived from canola seed as an animal feed.

Table 6. Summary of Glucosinolate Analysis of GT200 Canola Seed. Values are μmoles/gram of defatted meal. Analysis performed by Ag Canada on samples collected in 1992 field trials.

Glucosinolate	Westar		GT200	
	Trial Mean[1] (range)	Co-Op Mean[2] (range)	GT200H Mean[3] (range)	GT200 Mean[1] (range)
Alkyl	8.75 (6.15-11.4)	9.66 (7.0-12.5)	12.6 (10.3-14.9)	10.7 (6.18-16.4)
Thioalkyl	0.26 (0.18-0.40)	0.36 (0.2-0.8)	0.38 (0.36-0.41)	0.33 (0.18-0.43)
Indolyl	11.4 (9.8-13.4)	11.0 (7.0-13.7)	12.1 (11.8-12.4)	11.8 (11.2-13.2)

[1] Values from seven samples, analyzed in triplicate
[2] Values from 13 samples, analyzed in triplicate
[3] Values from two samples, analyzed in triplicate

F. Agronomic Performance

Glyphosate-tolerant canola event GT200 was evaluated for agronomic performance in Canadian trials in 1992 and 1993.

Typical observations in the Canadian trials included relative emergence, vegetative growth, days to maturity, height, yield and shattering. Glyphosate-tolerant canola event GT200 was determined to be agronomically comparable to Westar and other nontransgenic commercial varieties (see summary of data in Appendix 1). Germination tests of seed of event GT200 and Westar from 1992 variety trials were conducted at the Agriculture Canada seed quality testing laboratory in Saskatoon, Saskatchewan. Germination percentages were 95% for GT200 treated with Roundup and 97% for GT200 untreated, compared with 99% for Westar, demonstrating high germination and essentially no difference between transgenic canola and nontransgenic controls. These findings, along with data collected on volunteers observed in GT200 plots the following year indicate that, as was found for the antecedent organism RT73, there is not a significant difference in dormancy between glyphosate-tolerant canola event GT200 and the parental line, Westar (Appendix 1).

VI. Environmental Consequences of Introduction

A. Overview

There are no significant changes from Section VI of the previously approved petition submission for the antecedent organism in terms of the description of the herbicide glyphosate, use of herbicides in canola, the weediness potential of glyphosate-tolerant canola, cross pollination to wild and cultivated related species, and transfer of genetic material to species to which canola cannot interbreed (e.g., "horizontal transfer"). To address the issue of the appearance of glyphosate-resistant weeds, the following section has been updated and included in this extension request. The cultivation of glyphosate-tolerant canola event GT200 would not be expected to have environmental effects different from the cultivation of event RT73, which has already been deregulated by USDA-APHIS.

B. Appearance of Glyphosate-Tolerant Weeds

Today, some 109 herbicide-resistant weed biotypes have been identified in various cropping systems in the U.S., and over half of them are resistant to the triazine familiy of herbicides (Holt and Le Baron, 1990; Le Baron, 1991; Shaner, 1995). Resistance has usually developed because of selection pressure exerted by the repeated use of herbicides with a single target site, a specific mode of action, a long residual activity of the herbicide with the capacity to control weeds year-long, and frequent applications of the same herbicide without rotation to the other herbicides or cultural control practices. Using these criteria, and based on current use data, glyphosate is considered to be a herbicide with a low risk for weed resistance (Benbrook, 1991). Nevertheless, a question has been raised about whether the introduction of crops tolerant to a specific herbicide, such as glyphosate, may lead to the occurrence of weeds resistant to that particular herbicide. This concern is based on the assumptions that the use of the herbicide will increase significantly, and possibly that it will be used repeatedly in the same location. However, other increases in glyphosate use over the previous years have been more significant than the projected increase associated with the introduction of Roundup Ready crops. A consensus document by the OECD on general information concerning glyphosate resistance points to the difficulties associated with trying to breed glyphosate-tolerant plants using traditional techniques (OECD, 1999). The desired phenotype has been elusive, because altered EPSPS derived through mutation has resulted in glyphosate-tolerant plants that have reduced biosynthesis of aromatic amino acids, which negatively impacts whole plant metabolism, agronomic fit, and plant performance.

Although it cannot be stated that evolution of resistance to glyphosate will not occur, the development of weed resistance to glyphosate is expected to be a very rare event because:

1. Weeds and crops are inherently not tolerant to glyphosate, and the long history of extensive use of glyphosate has resulted in few instances of resistant weeds (Bradshaw et al., 1997);
2. Glyphosate has many unique properties, such as its mode of action, chemical structure, limited metabolism in plants, and lack of residual activity in soil, which make the development of resistance less likely;
3. Selection for glyphosate resistance using whole plant and cell/tissue culture techniques was unsuccessful, and would, therefore, be expected to occur rarely in nature under normal field conditions.

In 1966 in Australia, it was reported that a biotype of annual rye-grass (*Lolium rigidum*) was surviving application of label recommended rates of glyphosate (Pratley *et al.*, 1996). To date, after examination of thousands of samples, only three locations have been confirmed as having the resistance population, indicating that the phenomenon is not widespread. A large body of biochemical and molecular biology experiments to determine the cause of observed weed control differences between Australian rye-grass biotypes resistant and susceptible to glyphosate indicates that the observed resistance is due to a combination of factors. Conclusions drawn to date are that the resistant biotype is easily controlled by conventional practices (tillage, other herbicides), and is caused by a complex inheritance pattern, unlikely to occur across a wide range of other species. Results of these studies have been presented (Pratley *et al.*, 1999).

Two additional reports of resistant ryegrass in northern California are being investigated. Similar to the Australian locations, these fields are small and isolated. Again, the use of mowing and other herbicides have been very effective in controlling the ryegrass. Research continues in an effort to better understand the resistance mechanism. Most recently, a population of *Elusine indica* (goosegrass) was reported to survive labelled rates of glyphosate in Malaysia; analysis found that the resistant goosegrass has a modified EPSPS that is two- to four-fold less sensitive to glyphosate than in more sensitive biotypes. Research is underway to investigate the resistance mechanism.

VII. Adverse Consequences of Introduction

Given its similarity to the previously deregulated event RT73, Monsanto knows of no unfavorable results or observations associated with glyphosate-tolerant canola event GT200 that would result in adverse consequences of introduction. In addition, we have concluded that an extension of nonregulated status to event GT200 raises no serious new issues regarding plant pest potential or potential impact on the environment relative to the previous deregulated event RT73. Therefore, Monsanto requests that glyphosate-tolerant canola event GT200 and progeny derived from traditional breeding no longer be regulated under 7 CFR part 340.6.

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APPENDIX 1. AGRONOMIC AND WEEDINESS POTENTIAL STUDIES

Appendix 1: Agronomic and Weediness Assessment of Glyphosate Tolerant Canola Event GT200

The parental variety Westar is a spring canola or oil seed rape (OSR) variety best suited to Canadian and northern U.S. environments. Comparisons between glyphosate-tolerant canola events GT200, RT73 and the parental variety Westar have been conducted in Canada, since it is the most appropriate environment for the establishment of equivalence. On the basis of observations made, as with the antecedent organism RT73, there were no notable phenotypic differences, except for tolerance to Roundup herbicide, between GT200 and the parental line Westar.

The following sections describe data and information collected from 1992 and 1993 field trials related to the biology and agronomics of event GT200, which demonstrate that GT200 is substantially equivalent to its non-modified counterpart in a series of relevant agronomic parameters. Every test was conducted such that a direct comparison was made to the parental control, Westar, grown side-by-side at each field site.

As with the antecedent organism, event GT200 was selected from a single seed of the Westar genotype, and it is certain that event GT200 inherited only a portion of the genetic variability of the parental variety (See expert letter in Appendix 6 from the petition for the antecedent organism, RT73). Therefore, additional data were collected from multiple sites in order to more accurately reflect the range of values (variability) expected for the parental genotype and allow for a reliable comparison with values.

A. Modes and/or rate of reproduction

i. Seed Production:

The yield data provided in Table A1 is from the 1993 Canadian Co-Op Test and is an average from 21 diverse test sites. The average yield was 2,000 kg/ha for Westar and 1,930 kg/ha for event GT200. The variation in performance from test site to site is a result of local weather, soil, disease and pest conditions. It is important to note that these trials are managed under "weed free" conditions, but Roundup herbicide was not applied to the plots.

As was the case for the antecedent organism RT73, the yield data for GT200 are slightly lower than yields with Westar. This was attributed to a poor seed source for GT200 from a winter nursery in Chile. Seed of GT200 and RT73 was bulked in Chile over the winter of 1992-93. To meet timelines for inclusion of this material in the Co-Op Trials, this seed was harvested earlier than considered optimal. Consequently, the slight yield reduction in the 1993 Co-Op was attributed to the slightly immature seed.

Table A1. Seed Yield for GT200 and Westar in 1993 Canadian Co-Op Trials

Location	Line or Cultivar (kg/ha)	
	GT200[1]	Westar
Lacombe	2930	3050
Loon Lake	1450	1620
Westock	3010	3160
Olds	1430	1700
High Level	1210	1210
Fort Vermilion	1930	1750
Avg. Short Season Zone	**1990**	**2090**
Scott	1890	2080
Lashburn	2180	2200
Durban	1720	2000
Melfort	2110	1660
Fort Salk.	3270	3280
Kelsey	2190	2250
Hughenden	3350	4160
Yorkton	1720	1380
Saskatoon	1860	2400
Avg. Mid Season Zone	**2250**	**2390**
Thornhill	820	750
Winnipeg	820	900
Rosebank	1670	1510
Portage	1460	1420
Brandon	1330	1330
Avg. Long Season Zone	**1220**	**1180**
Average All Zones	**1930**	**2000**

[1] - Seed of GT200 was bulked in Chile over the winter of 1992-93. To meet deadlines for Co-Op trial introduction, this seed was harvested earlier than considered optimal. Consequently, the slight yield reduction in the 1993 Co-Op may be attributed to the slightly immature seed.

ii. Pollen Production and Pollen Viability:

As a means of estimating pollen production for event GT200, yields of both GT200 and the parental variety Westar were measured in 1993 field tests. Based on these results (see Table A1), it is concluded that there is no difference expected in pollen production of event GT200 compared to Westar grown at the same locations.

iii. Time from Seeding to Maturity:

Data from the 1993 Canadian trials indicated that event GT200 was on average approximately 1-2 days later in maturation when compared to Westar. This result is consistent with the fact that GT200 is a selection from a single seed of Westar (see above in this Section). Table A2 summarizes the maturity data from a range of Canadian sites.

Table A2. Days to Maturity for Westar and GT200 from the 1993 Canadian Co-Op Tests

Location	**Line or Cultivar (days after planting)**	
	GT200	**Westar**
Lacombe	119	119
Scott	113	110
Durban	109	108
Melfort	115	113
Winnipeg	98	97
Fort Saskatchewan	120	119
Olds	129	126
Yorkton	107	105
Rosebank	101	98
High Level	121	119
Portage la Prairie	96	94
Brandon	102	102
Fort Vermilion	114	111
Saskatoon	112	110
Average	**111.1**	**109.4**

B. Dissemination Potential, Persistence and Agronomic Parameters

i. Outcrossing frequency within species:

As with the antecedent organism, there is no *a priori* scientific basis to believe that the outcrossing frequency from event GT200 to other *B. napus* varieties will be different than that of Westar. The glyphosate tolerance phenotype was transferred to other *B. napus* varieties of canola using traditional backcrossing procedures as a part of the initial breeding program using GT200. Based also on the fact that the pollen production and pollen viability are likely unchanged (as measured by yield and germination of progeny) by the genetic modification,

the outcrossing frequency within species is unlikely to be different for GT200 when compared to Westar.

ii. Volunteer Potential - Silique shattering:

The loss and local dispersal of seed by shattering of mature seed pods is a well known characteristic of canola. Agronomic practices like swathing are routinely used to limit the loss of seed before harvest; however, it is inevitable that some seed is lost. The degree of loss to shattering is largely dependent on environmental factors that influence the degree of pod maturity, the amount of moisture present, and physical disturbance of the material prior to harvest.

Brassica seeds do not have any special or specific adaptations to facilitate wide-spread dispersal (they do not blow in the wind or stick to animal fur) so the shattered seed will remain in close proximity to the original site. Since it is accepted that a high proportion of the mature shattered seed can remain viable and will germinate subsequent to harvest, the degree of shattering can be assessed by counting volunteers in plots the subsequent years. It is equally important to note that the volunteers in the subsequent field season result from not only shattering, but also spillage and other mechanisms of seed loss at harvest. Thus, counting volunteers overestimates shattering, but addresses the main issue of potential invasiveness.

Defined test sites where GT200 had been grown in 1992 were planted the following year with wheat, barley, barley/rye mixture, alfalfa and/or left fallow. As expected in the following spring, some canola volunteers were observed at some test locations. Similar to the antecedent organism, the number of GT200 volunteers was highly variable, but no reproducible differences were found upon comparison of GT200 and the Westar control. Furthermore, no differences were evident between plots of GT200 that had been treated and untreated with Roundup herbicide. The data obtained from the Canadian test sites is shown below in Table A3.

Table A3. Volunteer Canola Counts Taken in 1993 on 1992 Trial Sites (Values are plants/m^2)

Location	Westar	GT200 (untreated)[1]	GT200 (treated)[2]
Minto[3]	10[a]	11[a]	8[a]
Melfort[3]	67[a]	1[b]	3[b]
Saskatoon[3]	168[a]	170[a]	452[a]

1 Roundup herbicide was not applied to these plants.

2 Roundup herbicide was applied in 1992 at the 2 to 6 leaf stage at a rate of 0.45 kg a.i./ha.

3 Statistical significance for a listed location was determined using Duncan's Multiple Range Test p=0.05. Values followed by the same letter are not significantly different.

Although the statistical analysis of data at the Melfort site suggest that there was a significant difference in the shattering of Westar when compared with the event GT200 plots, it was noted that the Westar control plot was somewhat more mature, which may have resulted in the higher loss of shattered seed. The numbers of volunteers at Saskatoon were much larger because of adverse weather conditions during the harvest period. Although poor weather conditions increased seed loss to shattering and data variability, no significant differences were observed between the treatments.

A direct measure of seed loss to shattering was also conducted at Minto and Melfort test sites in 1993. At both locations, shattering was evaluated by placing catch pans in plots at the same time as the canola began to ripen. The results of this test are given in Table A4.

Table A4. Results of Shattering Studies Conducted in 1993 (Values are expressed as % Westar control)

Location	Westar	GT200 (untreated)[1]	GT200 (treated)[2]
Minto[3]	100[a]	83[a]	86[a]
Melfort[3]	100[a]	60[a]	70[a]

1 Roundup herbicide was not applied to these plants.

2 Roundup herbicide was applied to these plots at the 2 to 6 leaf stage at a rate of 0.45 kg a.i./ha.

3 ANOVA statistical analysis indicated no significant difference at either location. Values followed by the same letter are not significantly different from each other. Data represents the mean of four replicate plots.

Analysis of variance (ANOVA) of seed capture data did not reveal any significant differences. The results for Melfort were variable due to a heavy snowfall and high winds prior to harvest and the uneven maturity of the different test plots at this location.

On the basis of these results, it can be concluded that there are no differences between event GT200 and Westar with respect to silique shattering and potential to volunteer.

iii. Germination rate:

Germination tests of Westar and event GT200 seed harvested from 1992 trials which had been Roundup treated and untreated were conducted at the Agriculture Canada seed quality testing laboratory in Saskatoon. The results (Table A5) of these tests show that all the seed samples demonstrated high rates of germination and no differences were observed between event GT200 and Westar. These findings also support the conclusion of no differences in dormancy between GT200 and Westar (see below in this Section).

Table A5. Germination Test of Seed from 1992 Trials

Canola Cultivar or Line	Percent Germination
Westar	99 %
GT200 (untreated)[1]	97 %
GT200 (treated)[2]	95 %

1 Untreated indicates that Roundup herbicide was not applied to these plants.
2 Treated indicates that Roundup herbicide was applied to these plots at the 2 to 6 leaf stage at a rate of 0.45 kg a.i./ha.

iv. Seed dormancy:

The principle measure of seed dormancy was to determine volunteer counts in replicated GT200 and Westar plots at multiple locations. These data are reported above in the discussion related to shattering (Section B.ii.).

There is no evidence to support increased dormancy of GT200 seed as a consequence of the genetic modification or the introduced trait. The results from volunteer counts (Table A3) and germination data (Table A5), show no obvious differences between GT200 and Westar. The difference noted at the Melfort site (Table A3) can be explained by the 1-2 day maturity difference between GT200 and Westar (see Section A.iii.), and the atypical growing season in 1992 where a maturity delay was exacerbated by the excessive cold and precipitation close to harvest. Based on these results, it is concluded that event GT200 is not changed in dormancy potential relative to the parental variety Westar or other commercial canola varieties.

v. Other Agronomic Assessments:

In addition to yield assessments, data relating to plant height, lodging and percent green seed were also collected in the 1993 Canadian Co-Op trials. The mean height of event GT200 plants at twelve locations was 121.2 cm, while Westar was 115.4 cm and the average of all *B. napus* varieties in the Co-Op Test for genetically-modified plants was 119.8 cm. The mean lodging resistance score (on a 1-5 scale with 1 being good and 5 being poor) for event GT200 plants at eight locations was 2.26, while Westar was 2.58 and the average of all *B. napus* varieties in the Co-Op Test for genetically-modified plants was 2.3. The mean percent green seed for event GT200 across 21 locations was 13.0, while Westar was 10.1 and the average of all *B. napus* varieties in the Co-Op Test for genetically-modified plants was 10.7.

vi. Adaptations to stress factors:

As for the antecedent organism, introduction of the glyphosate tolerance trait into canola has not resulted in any exceptional or unexpected adaptations to stress factors which would provide event GT200 with a selective advantage.

Event GT200 has not demonstrated any observable difference in adaptation to biotic stress factors relative to Westar. Data from the 1993 Canadian Co-Op Tests rank GT200 similarly to the parental variety Westar in blackleg (*Phoma lingam*) disease susceptibility with overall disease ratings of 1.3 and 1.9 on a scale of 0-5, respectively. Like its parental variety, GT200 is susceptible to blackleg disease. In a separate experiment in a blackleg disease nursery in 1993, GT200 scored 3.81 out of a possible 5, while Westar was scored at 4.11. Analysis of variance of the ratings showed no difference between GT200 and Westar. It is concluded therefore that GT200 is unchanged in its susceptibility to blackleg disease compared to its parent variety.

Additionally, observations documented over two years of field trials indicated that there were no notable differences between event GT200 and Westar in disease and insect susceptibility, or their ability to resist abiotic factors such as drought, heat or frost. Plots of the glyphosate-tolerant canola event GT200 and Westar control plants were visually checked for the appearance of possible disease symptoms such as spotted leaves, leaf necrosis, stunted or distorted plants and wilting, which are indicative of, but not limited to, diseases such as: sclerotinia white mold *(Sclerotinia sclerotiorium),* powdery mildew *(Erysiphe communis)* and blackleg *(Phoma lingum)* (Berglund and McKay, 1998). Damage from the major insect pests of canola were monitored. This would include but not be limited to flea beetles (*Phyllotretra* spp.), aphids (*Brevicoryne brassicae* L. and *Liaphis erysimis*) and cabbage seed-pod weevils (*Ceutorhynchus assimilis*).

APPENDIX 2. COMPOSITIONAL ANALYSES STUDIES

Appendix 2: Compositional Analyses of Glyphosate-Tolerant Canola Event GT200

As a part of the program to assess the food and feed safety of glyphosate-tolerant canola event GT200, extensive compositional analyses were performed on materials obtained from field trials. Seed from all lines underwent proximate analysis (% protein, fat, ash, moisture, fiber, carbohydrate, and calculated calories). Selected seed subsamples were also analyzed for amino acid composition. Additional compositional analyses including fatty acid profile, total glucosinolates, chlorophyll, and sinapine were performed.

The compositional analyses of samples from the 1992 field trials are summarized in the sections below. Results of these analyses show that seed and a processed fraction (toasted meal) from glyphosate-tolerant canola event GT200 are not materially different from other canola in essential nutrients, and furthermore, the levels of antinutrients (glucosinolates, erucic acid, and sinapine) in GT200 seed are at or below levels currently found in commercial canola. The strategy taken for measurement of compositional parameters was to focus on the raw agricultural commodity, the canola seed. It is reasonable to infer that if the seed from a glyphosate-tolerant canola line are not materially different from parental control canola seed, then meal and oil derived from seed of that line will also not be materially different from processed fractions derived from parental control seed.

In order to provide test material for these analyses, GT200 and the parental variety, Westar were generated in field trials conducted in Canada in 1992 at up to seven locations distributed across the primary canola production areas (Manitoba, Saskatchewan, and Ontario). Seed from these trials were sent to the laboratories of Ag Canada Research Station in Saskatoon, Saskatchewan for analysis of oil, protein, chlorophyll, sinapines (total choline esters), fatty acid and glucosinolate compositions. Samples of seed were also transported to St. Louis for analysis of protein expression levels at Monsanto, and for proximate and amino acid compositional analyses at Ralston Analytical Laboratories (RAL) in St. Louis, Missouri. The latter two laboratories conducted all analyses under US EPA GLP compliance.

i. Proximate Analysis:

Proximate analyses were performed at Ralston Analytical Laboratories on canola seeds from GT200 and Westar canola from the 1992 field trials. The results of these analyses, are shown in Table B1. Components measured were protein, fat, moisture, fiber, and ash, and, with the exception of moisture, are reported on a dry weight basis. The carbohydrate content was derived by calculation. The data summarized in Table B1 establish that the levels of

these components in event GT200 are not materially different from the levels in Westar. Where literature values were available, the components analyzed from event GT200 were found to fall within these reported values.

Table B1. Summary of Proximate Analysis of Canola Seed.
Values represent means of seeds from single plots at seven field sites in 1992 trials. Analysis performed at Ralston Analytical Laboratories

Analysis	Westar[1]		GT200[1]		Literature range [4]
	Mean	Range	Mean	Range	
Protein, %DW	23.4	21.0-26.1	24.2	21.3-26.4	14.8-29.7
Ash, %DW	3.68	3.44-3.91	3.85	3.56-4.25	NA
Moisture, % [2]	4.39	3.69-4.86	4.53	4.05-4.95	NA
Fat, %DW	46.5[3]	42.3-49.9	45.5	40.7-48.5	35.6-49.0
Fiber, %DW	8.21	7.16-9.90	8.20	6.82-10.1	NA
Carbohydrates, %DW	26.4	23.6-28.0	26.4	23.8-28.9	NA
Calories, kcal/100g DW	551	536-567	546	527-559	NA

[1] n=7
[2] Samples were dried prior to analysis
[3] n=6
[4] From DeClercq, 1992

ii. Amino Acid Analysis:
When expressed on either a per dry weight of seed or per protein basis, no meaningful differences were detected in amino acid compositional analyses for event GT200. Table B2 contains the results of analysis of 18 individual amino acids calculated on a per protein basis. These data establish that there are no meaningful difference between canola seed from GT200 and Westar in terms of amino acid composition.

iii. Fatty acid Analysis:
The fatty acid composition of crude oil extracted from seeds grown in 1992 trials was measured in both event GT200 and Westar. In 1992, seed used for planting event GT200 came from two separate sources. Thus, two distinct designations for seed were used: GT200H and GT200. Line GT200 corresponded exactly to GT200H except that the former was heterozygous and the latter was homozygous for the glyphosate tolerance genes. Seed samples from event GT200 and the control Westar were obtained from seven locations. Due to limited availability of pure homozygous seed for planting, samples of GT200H were obtained from only two sites.

The means and ranges of the most abundant fatty acids are presented in Table B3. The values for all fatty acids were compared with values obtained from the Westar control as well as data obtained for Westar from the Co-Op Test in that year. Data obtained from event GT200 was in good agreement with the available literature values for the Westar variety. The data clearly establish that there are no meaningful differences between GT200 and Westar in terms of fatty acid composition.

Table B2. Summary of Amino Acid Analysis of Canola Seed.
Values reported on per protein basis (g/100 g protein dry wt.) and represent means of seeds from single plots from three field sites in 1992 trials. Analysis performed at Ralston Analytical Laboratories.

	Westar		GT200	
Amino Acid	**Mean**	**Range**	**Mean**	**Range**
Alanine	4.25	4.05-4.46	4.16	4.11-4.24
Arginine	5.62	5.30-5.85	5.64	5.40-5.88
Aspartic Acid	6.51	6.29-6.69	6.47	6.29-6.81
Cysteine	2.32	2.16-2.47	2.28	2.23-2.35
Glutamic Acid	17.0	16.2-17.4	17.4	16.4-18.1
Glycine	4.59	4.41-4.70	4.57	4.29-4.83
Histidine	2.57	2.52-2.59	2.60	2.46-2.69
Isoleucine	3.82	3.71-3.98	3.90	3.79-3.99
Leucine	6.73	6.53-6.95	6.85	6.65-7.02
Lysine	5.70	5.59-5.76	5.78	5.67-5.84
Methionine	1.85	1.78-1.94	1.80	1.76-1.85
Phenylalanine	3.82	3.71-3.94	3.87	3.75-3.95
Proline	6.24	6.09-6.36	6.48	6.29-6.81
Serine	3.89	3.76-4.01	3.87	3.75-4.03
Threonine	3.99	3.91-4.05	3.94	3.84-4.12
Tryptophan	0.98	0.97-0.99	0.99	0.97-1.01
Tyrosine	2.55	2.43-2.63	2.47	2.35-2.61
Valine	4.86	4.75-5.00	4.97	4.87-5.08

Table B3. Summary of Fatty Acid Analysis of Canola Seeds.
Values are % of fatty acid ester profile. Analysis performed by Ag Canada on samples harvested from field trials conducted in 1992

Fatty Acid	Westar		GT200		Westar Literature Reported Value [3]
	Trial Mean[1] (range)	Co-Op Range	GT200H Mean[2] (range)	GT200 Mean[1] (range)	
16:0	4.1 (3.9-4.2)	3.7-4.8	3.90 (3.8-4.0)	4.02 (3.7-4.4)	3.9
16:1	0.3 (0.3-0.4)	0.0-0.6	0.30 (0.3-0.3)	0.35 (0.3-0.4)	0.2
18:0	1.7 (1.4-2.0)	1.2-2.1	1.63 (1.5-1.8)	1.69 (1.4-2.0)	1.6
18:1	61.0 (58.8-62.5)	57.4-63.4	60.5 (59.4-61.1)	59.4 (56.6-62.0)	59.1
18:2	19.8 (18.9-20.2)	18.3-22.1	19.4 (18.5-20.0)	20.4 (19.2-22.4)	18.8
18:3	9.8 (8.1-12.1)	8.2-13.0	11.4 (10.4-12.5)	11.3 (9.6-13.4)	8.8
20:0	0.7 (0.6-0.8)	0.4-0.9	0.67 (0.6-0.7)	0.68 (0.6-0.8)	0.5
20:1	1.8 (1.7-2.0)	1.3-2.3	1.60 (1.4-2.0)	1.62 (1.4-1.8)	1.4
20:2	0.1[4]	0.1-0.2	0.13 (0.1-0.2)	0.12 (0.1-0.2)	NA
22:0	0.4 (0.3-0.4)	0.3-0.4	0.37 (0.3-0.4)	0.37 (0.3-0.4)	0.4
22:1	0.4 (0.3-0.6)	0.1-1.4	0.12 (<0.1-0.3)	0.17 (<0.1-0.3)	0.5

[1] Values from seven samples, analyzed in triplicate
[2] Values from two samples, analyzed in triplicate
[3] Data from Ackman, R.G. (1991) In Canola and Rapeseed, Production, Chemistry, Nutrition and Processing Technology. F. Shahidi, editor. Van Nostrand Reinhold, New York. 83.
[4] A single value was obtained from all seven samples

iv. *Erucic Acid:*

See discussion in Section V.E.

v. Glucosinolate Analysis:

See discussion in Section V.E.

vi. Sinapine Analysis:

Sinapine is used here as a general term for a family of choline esters naturally occurring in canola. Their impact to the poultry feed industry is significant since sinapines are known to render an off-odor to chicken eggs. These analyses were performed on defatted meal samples from canola seed produced in the1992 field trials, and the results (reported in ppm in defatted meal) are presented in Table B4.

The data establish that there is no meaningful difference in the levels of choline esters in seed between event GT200, Westar and values reported in the literature.

Table B4. Summary of Sinapine Analysis of Canola Seed.
Values are ppm in defatted meal on samples <3% moisture. Analysis performed by Ag Canada on samples collected in 1992 field trials.

	Westar		GT200	
	Trial Mean[1] (range)	**Reported Literature Range[2]**	**GT200H Mean[3] (range)**	**GT200 Mean[1] (range)**
Sinapine (ppm)	12.7 (11.6-14.3)	11.7-18.3	14.4 (12.6-16.1)	13.4 (11.4-16.0)

[1] Values from seven samples, analyzed in triplicate

[2] Values reported in Wang, X. (1992) Studies of Methodology of Sinapine Determination and Sinapine Variation in *Brassica* and *Sinapis*. University of Saskatchewan, Saskatoon, Saskatchewan

[3] Values from two samples, analyzed in triplicate

vii. Summary of Compositional Analysis

A summary of the results of the compositional analyses performed with canola seeds and toasted meal is given in Table B5. The results of all analyses show that GT200 canola seeds and toasted meal are not materially different from the control canola seeds or meal.

Table B5. Summary of Compositional Analyses Performed on Event GT200.

Component	Seed	Toasted meal
Proximate analysis	NMD	NMD
Amino acid composition	NMD	NA
Fatty acid composition	NMD	NA
Erucic Acid	NMD	NA
Glucosinolates	NMD	NA
Sinapines	NMD	NA

NMD = not materially different from the control
NA = not analyzed