Aventis CropScience

01-206-02p
Revised
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October 4, 2001

Recd.
10/04/01.
mp.

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Re: Application for an Extension of the Determination of Nonregulated Status for Glufosinate-Tolerant Canola Transformation (97-205-01p): Topas 19/2 (01-206-01p) mkp

Dear Dr. Pappu:

Enclosed please find 2 originals and 4 Confidential Business Information (CBI) and CBI-Deleted photocopies of the above referenced petition extension. This revised extension addresses the deficiencies listed in your September 17, 2001, letter. Specifically, CBI changes were addressed, as were basic presentation issues. Also included throughout the applicable sections, are better comparisons of the Topas 19/2 event with the antecedent organism T45 (petition 97-205-01p).

If you have any questions, please feel free to contact me at 919-549-2748 or Susan MacIntosh at 919-549-2780, 919-549-3929 (fax) or susan.macintosh@aventis.com.

Respectfully,

Luann Powell

Luann Powell
Registration Manager – Biotechnology

Enclosures (Contains Confidential Business Information)

Cc: Susan MacIntosh, Manager, Regulatory Affairs – Biotechnology
Rob MacDonald, Global Product Safety Management

10/9/01
mkp

01-206-02p
Revised

Application for an Extension of the Determination of Nonregulated Status for Glufosinate-Tolerant Canola Transformation (97-205-01p):

Topas 19/2

The undersigned submits this petition under 7 CFR 340.6 to request that the Director, Scientific Services, makes a determination that the article should not be regulated under 7 CFR 340.

Submitted by:

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Regulatory Affairs – Biotechnology

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October 4, 2001

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Summary

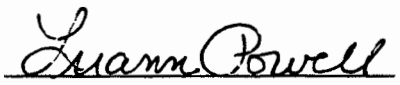
Glufosinate-Tolerant Canola line HCN92 derived from transformation event Topas 19/2, received an opinion of No Concern from the Federal Drug Administration April 20, 1995, for food and feed uses (Appendix I). On June 9, 1995, AgrEvo USA Company (now Aventis CropScience) received an opinion letter from the United States Department of Agriculture indicating that APHIS will not consider Topas 19/2 and progeny canola moved from Canada to the United States for processing as regulated articles (Appendix II). Lines derived from Topas 19/2 received approval from the Canadian Food Inspection Agency for unconfined release into the environment and use as livestock feed in March 1995 (Appendix III). Canadian food safety approvals for Topas 19/2 derived lines were granted June 1995 (Appendix IV). Prior to commercialization of various canola lines derived from Topas 19/2 in Canada, these lines were extensively field tested by Aventis Canada, formerly AgrEvo Canada, Inc. and third parties since 1991. Data collected from these fields studies, as well as greenhouse and laboratory studies, demonstrate that lines derived from Glufosinate Tolerant Canola (GTC) event Topas 19/2 1) exhibit no plant pathogenic properties; 2) is no more likely to become a weed than non-modified canola; 3) is unlikely to increase the weediness potential of any other cultivated plant or native wild species; 4) does not cause damage to processed agricultural commodities; and 5) is unlikely to harm other organisms that are beneficial to agriculture.

Since 1995, greater than 3 million acres of canola derived from the transformation event Topas 19/2 have been cultivated in Western Canada. As a component of our product stewardship efforts, Aventis has conducted a post commercialization monitoring study to assess the environmental fate of GTC volunteers. The results from these studies confirm the conclusions of the risk assessment that the GTC volunteers do not demonstrate increased weediness as compared to traditionally derived canola cultivars.

Aventis CropScience requests a determination from APHIS that Glufosinate Tolerant Canola transformation event Topas 19/2 and any progeny derived from crosses of this event with tradition canola varieties, and any progeny derived from crosses of this event with transgenic canola varieties that have also received a determination of nonregulated status, no longer be considered regulated articles under 7 CFR Part 340. Also, as was seen for the previous deregulated event T45, there were no morphological, beneficial organism, disease or pest differences between the event Topas 19/2 and the previously considered event T45.

Certification

The undersigned certifies, that to the best knowledge and belief of the undersigned, this petition includes all information and views on which to base a determination, and that it includes relevant data and information known to the petitioner which is unfavorable to the petition.



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ACRONYMS, SYNONYMS AND SCIENTIFIC TERMS

APHIS - Animal and Plant Health Inspection Service

CFIA – Canadian Food Inspection Agency

ELISA – enzyme linked immunosorbent assay

FDA – Federal Drug Administration

GA – glufosinate-ammonium

GTC – glufosinate tolerant canola

HCN05 – Topas 19/2 derived breeding line (not commercialized)

HCN10 – Canola line derived from Topas 19/2 called Independence

HCN19 – Topas 19/2 derived breeding line (not commercialized)

HCN92 – Canola line derived from Topas 19/2 called Innovator

nptII - *Neomycin phosphotransferase* II (gene)

NPTII - Neomycin phosphotransferase II (protein)

pat - - *phosphinothricin acetyltransferase* gene (origin *Streptomyces viridochromogenes*)

PAT - phosphinothricin acetyltransferase (protein)

PCR – polymerase chain reaction

POCA/Ac – construct used in the transformation of event Topas 19/2 (alias pOAC18/Ac)

T-DNA – transformed DNA

USDA - United States Department of Agriculture

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Statement of Grounds for Nonregulated Status

I. Rationale for Submission of Request for Extension

There are no changes in the rationale from the previously approved petition entitled, "Petition for Determination of Nonregulated Status: Glufosinate Tolerant Canola Transformation Event T45," petition number 97-205-01p. The specific differences between Glufosinate Tolerant Canola (GTC) Event Topas 19/2 and it's progeny and the event in the previous petition are discussed in the appropriate sections. Also, as was seen for the previous deregulated event T45, there were no morphological, beneficial organism, disease or pest differences between the event Topas 19/2 and the previously considered event T45. The new event to be considered under this extension is Topas 19/2.

Table 1 gives the molecular information for the previously approved canola transformation event T45 (97-205-01p) and the requested transformation event Topas 19/2 (01-206-02p).

Table 1. Identity and Function of DNA Elements for Canola Events T45 and Topas 19/2

Characteristic	Event T45	Event Topas 19/2
Crop	Canola	Canola
Cultivar species name	*Brassica napus* L.	*Brassica napus* L.
Parent Line	AC Excel	Topas
Transformation Method	*Agrobaterium tumefaciens* mediated transformation	*Agrobaterium tumefaciens* mediated transformation
Vector	PHOE4/Ac	POCA/Ac
Trait	Tolerance to glufosinate ammonium	Tolerance to glufosinate ammonium
Gene 1/Donor	*Phosphinothricin acetyltransferase* (*pat*) gene/*Streptomyces viridochromogenes*	*Phosphinothricin acetyltransferase* (*pat*) gene/*Streptomyces viridochromogenes*
Gene 1 Promoter/Donor	35S/Cauliflower Mosaic Virus	35S/Cauliflower Mosaic Virus
Gene 1 Terminator/Donor	35S/Cauliflower Mosaic Virus	35S/Cauliflower Mosaic Virus
Gene 2/Donor	N/A	*Neomycin phosphotransferase II* (*nptII*) gene/*Escherichia coli*
Gene 2 Promoter/Donor	N/A	*Nopaline synthase* gene/ *Agrobacterium tumefaciens*
Gene 2 Terminator/Donor	N/A	*Octopine synthase* gene/ *Agrobacterium tumefaciens*

II. The Canola Family

There are no changes from the previously approved petition submission.

III. The Transformation System, Plasmid and Parent Line Used

For transformation of plants, the vector system as described by Olszewski *et al.*, 1988, was used. The system consists of an *Agrobacterium tumefaciens* strain and two plasmid components: 1) a nononcogenic Ti-plasmid and 2) a binary cloning vector based on the plasmid pOCA18. The nononcogenic Ti-plasmid, from which the T-region has been deleted, carries the *vir* genes required for the transfer of an artificial T-DNA cloned on the second plasmid to the plant genome. On the binary vector, the gene of interest, e.g., the chimeric *pat* gene, is located between the T-DNA border sequences. The *Brassica napus* parental line used for transformation was the spring oilseed rape line Topas. Topas is a canola quality (low erucic acid low glucosinolate cultivar) that was selected for transformation based on its agronomic characteristics and its success rate for transformation with *Agrobacterium*.

The *Agrobacterium* strain

The *Agrobacterium* host strain is a rifampicin resistant derivative of C58, cured for the Ti plasmid pTiC58 (C58C1Rif^R) (Van Larebeke *et al.*, 1974). The nononcogenic Ti-plasmid, pMP90, is derived from the nopaline Ti plasmid pTiC58. The Ti region from pTiC58 has been deleted, yielding pMP90 (Koncz *et al.*, 1986). The resulting strain C58C1Rif^R (pMP90) can be used as an acceptor strain for binary vectors carrying genes of interest between the T-DNA borders, like pOCA/Ac.

A map of the vector pOCA/Ac is shown in Figure 1.

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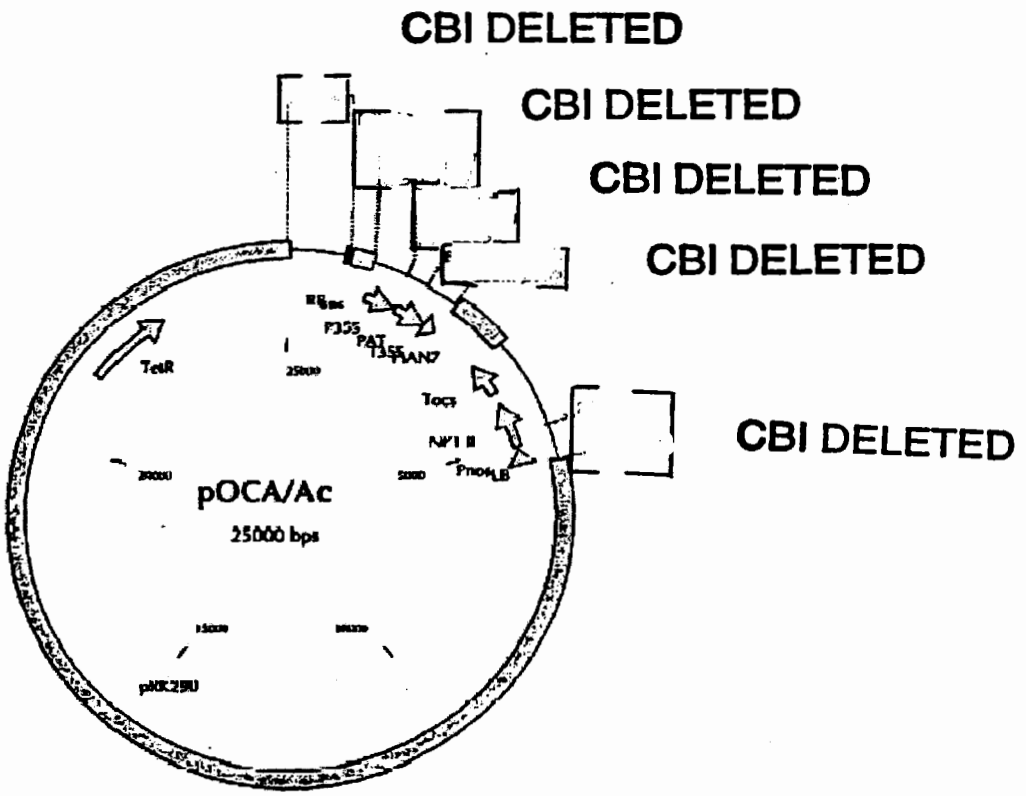
Figure 1. Map of the vector pOCA/Ac

The composition of the vector pOCA/Ac:



Genetic elements of the binary vector pOCA/Ac

Construction of the vector pOCA/Ac is described in detail in Olszewski *et al.*, (1988). The backbone of this plasmid is the broad host range vector pRK290 (Ditta *et al.*, 1980). Additionally, the vector contains the following elements:

1. A synthetic T-DNA left border from the octopine Ti-plasmid pTiAch5;
2. A chimeric Pnos/NPTII/Tocs gene;
3. The synthetic vector PiAN7 containing the ColE1 origin of replication;
4. The cos site from bacteriophage Lambda; and
5. A T-DNA Right Border fragment from the nopaline Ti-plasmid pTi37.

To obtain pOCA/AC, the synthetic *pat* gene from *Streptomyces viridochromogenes* fused to a promoter and terminator from the Cauliflower Mosaic virus was inserted as a SalI fragment.

A complete description of the DNA elements is shown in Table 2.

Table 2. Genetic Elements of the plasmid pOCA/Ac

Genetic Element	Size (Kb)	Function and Source
RB	0.9	Derived from *Agrobacterium tumefaciens* pTi37 Ti-plamid (including Right Border) (Depicker *et al.*, 1982)
Cos	0.25	cos site from bacteriophage Lambda (Feiss and Campbell, 1974)
35S	0.53	35S promoter from Cauliflower Mosaic Virus from the vector pDH51 (Pietrzak *et al.*, 1986)
pat	0.55	Synthetic *pat* gene (amino acid sequence from *Streptomyces viridochromogenes*) (Strauch *et al.*, 1993, European patent 275957 B1)
35S	0.22	35S terminator from Cauliflower Mosaic Virus from the vector pDH51 (Pietrzak *et al.*, 1986)
Ori	0.86	Derived from synthetic *E. coli* vector PiAN7 including ori ColE1 (Huang *et al.*, 1988)
Ocs	0.79	Terminator of the *octopine synthase* gene (DeGreve *et al.*, 1982 and Gielen *et al.*, 1984)
nptII	0.8	*Neomycin phosphotransferase II* gene (Beck *et al.*, 1982).
Nos	0.34	Promoter of the *nopaline synthase* gene (Depicker *et al.*, 1982)
LB	0.025	Synthetic T-DNA Left Border *from Agrobacterium tumefaciens* Ti plamid Ach5 (Gielen *et al.*, 1984)

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In summary, the event Topas 19/2 contains the same genetic elements as the deregulated event T45 (97-205-01p) with the exception of the antibiotic resistance marker gene, *nptII,* present in the Topas 19/2 event that was used solely as a transformant selection tool. As described above, the sping *Brassica napus* cultivar Topas was used for transformation. Similarly, the T45 transformation event was produced by the *Agrobacterium*-mediated transformation of another canola quality spring *Brassica napus* cultivar AC Excel.

IV. Genetic Characterization of Event Topas 19/2

A. Description, History and Mendelian Inheritance

The *pat* locus has been inherited in the Mendelian fashion, fitting the prediction for a single locus, in Topas 19/2.

F_2 generation analysis

In the F_2 generation, every plant deriving from ABCD lines was 100% tolerant to herbicide spray treatment (Table 3). Glufosinate ammonium application to 720 F_2 plants resulted in 548 survivors. Therefore, approximately 1/3 of the total population was susceptible to the herbicide. The segregation of plants, derived by selfing of the cross 19/2 x Aventis CropScience-[ ] was expected to be 3:1 for a single dominant gene with homozygous parents. CBI DELETED

Table 3. Assessment Results of Spray Treatment

123 F_1 Seeds	4 R_1 Seeds
104 seeds grown ↓ 88 fast germination ↙ ↘ 46 plants sprayed — 42 plants check non-sprayed ↓ 100% tolerance	4 plants sprayed Seed produced from here are referred to as A, B, C, D lines. 29 — 78 — 14 — 66 ↓ — ↓ — ↓ — ↓ 100% — 100% — 95% 100% tolerance

The BC_1 generation was screened for glufosinate-tolerance to the herbicide glufosinate ammonium. Of the 659 plants sprayed with glufosinate ammonium, 356 died and 305 survived which matches the 1:1 ratio (χ^2= 4.26, 1 df). Backcrossing the original Topas 19/2 transformant into any non transformed population would give a 1:1 ratio for glufosinate-tolerance. The cross of the homozygous Topas 19/2 with the non-allelic [ ] resulted in 100% heterozygous, tolerant plants. The additional cross with Excel represents a backcross and gave a 1:1 segregation versus the herbicide tolerance: Excel [ ]. CBI DELETED CBI DELETED

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BC_2 & BC_3 generation analysis

Of the 605 plants, 297 were tolerant and 308 plants non-tolerant ($\chi^2 = 0.2$ n/s, 18 df). The results were always a 1:1 segregation ratio. In Table 4, examples of BC_2 and BC_3 backcrosses show consistency of this 1:1 segregation pattern.

Table 4. BC_2 and BC_3 Backcrosses Showing Consistency of the 1:1 Segregation Pattern

PEDIGREE	COUNT	TOL	NON-TOL	RATIO
91/4.C10 [E x ([ ] x E)] x E	27	11	16	1:1
91/4.C16 [P X (19/2 X P)] x P	34	18	16	1:1
91/4.C19 [W x ([ ] x W)] x W	34	15	19	1:1
91/4.C20 [W x (W X 19/2)] x W	35	19	16	1:1
91/4.C22 (295 x 19/2) x 295	36	18	18	1:1
91/4.C23 (296 x 19/2) x 296	35	20	15	1:1
91/4.C25 [E x (19/2 x E)] x E	36	17	19	1:1
91/4.C26 [P x (P x [ ])] x P	35	17	18	1:1
91/4.C29 [P x (19/2 x P)] x P	34	16	18	1:1
91/4.C33 [A x ([ ] x A)] x A	36	19	17	1:1
91/4.C35 [W x ([ ] x W)] x W	35	19	16	1:1
91/4.C37 [W x (19/2 x W)] x W	34	15	19	1:1
91/4.C41 A x [A x (A x [ ])]	35	15	20	1:1
91/4.C56 P x [P x (19/2 x P)]	9	5	4	1:1
91/4.C56 P x [P x (19/2 x P)]	27	12	15	1:1
91/4.C62 295 x (295 x 19/2)	36	15	21	1:1
91/4.C65 296 x (296 x 19/2)	18	8	10	1:1
91/4.C66 P x (19/2 x P)	35	19	16	1:1
91/4.C67 W x [W x (W x [ ])]	34	19	15	1:1
TOTALS	605	297	308	$\chi^2 = 0.2$ n/s, 18 df

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Table 5. 3:1 Resistant/susceptible reaction for herbicide tolerance (Chi² = 95% probability)

TOPAS 19/2 *B. NAPUS* x *B. NAPUS* CROSSES			
GENOTYPE BC_3 F_1	**# SPRAYED**	**# SUSCEPTIBLE**	**CHI-SQUARE[a] 95% PROBABILITY**
Cyclone x TOPAS 19/2	72 72	27 31	0.28 n/s 1.43 n/s
N89-17 x TOPAS 19/2	73 72	31 36	1.28 n/s 4.00[b]
Elect x TOPAS 19/2	73 50	37 24	4.37[b] 2.19 n/s
N90-740 x TOPAS19/2	72 70	33 33	2.32 n/s 2.72 n/s
N90-933 x TOPAS19/2	63 72	34 33	5.23[b] 2.32 n/s
Maintainer x TOPAS19/2	65 70 66	29 35 29	1.72 n/s 3.89[b] 1.49 n/s

[a] Testing a 3:1 resistant/susceptible reaction assuming the herbicide tolerance gene is a single dominant gene

[b] Indicates the material tested deviated significantly from a 3:1 ratio

n/s Not statistically significant

B. DNA Analysis of Event Topas 19/2

1. Southern Blot Analysis for *pat* and *nptII* genes

Southern blot analysis of genomic DNA extracted from the transformed plants and digested with restriction endonucleases was used to confirm the number of insertion events and the subsequent structure of the inserted T-DNA as well as the stability of the insert over multiple generations.

Both the *pat* and *nptII* probes were used to clarify the inserted DNA structure. The results of these analyses were used to establish a restriction map of the insert.

Plant DNA was extracted from HCN19 and Excel leaf tissue using the Dellaporta DNA Miniprep method. Restriction analysis, with several different endonucleases, was performed, then the DNA separated by gel electrophoresis. Capillary transfer of the DNA onto Gene Screen Plus membrane followed, using the protocol described by Sambrook.

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] Plant genomic DNA extracted from c.v. Excel was fortified with DNA from the plasmid pOCA18/Ac (pOAC/Ac) and was used as a positive control. The positive controls were fortified at a concentration equivalent to a single copy inserted into the *Brassica* genome.

Figure 2. Southern Analysis of HCN19 [**CBI Deleted Copy** **] CBI DELETED**

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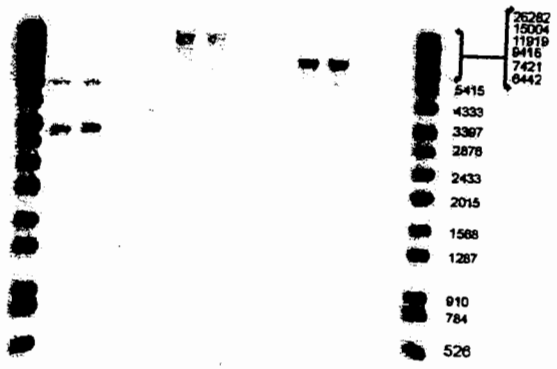

HCN 19

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Figure 3. Southern Analysis of HCN19 [

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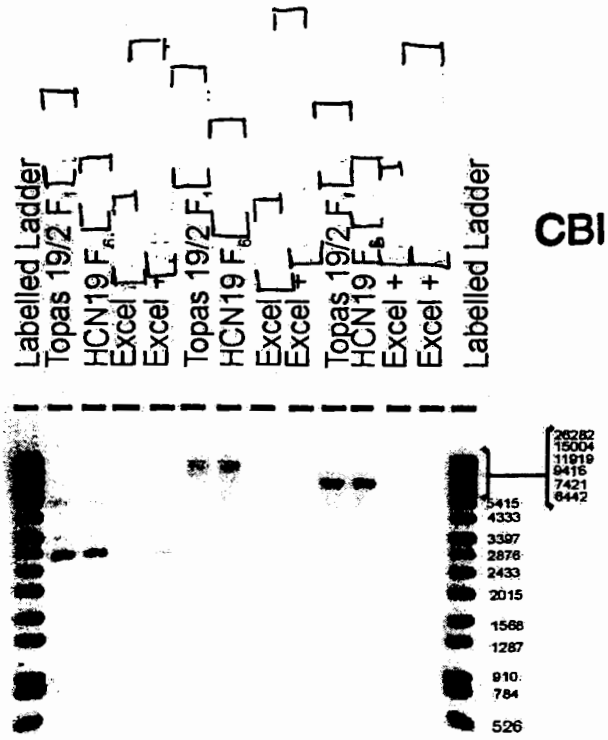

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HCN 19

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Interpretation of HCN19 Southern data

The objective was to analyses the insertion site of HCN19 and to evaluate the stability of the insertion by comparing the original transformant with the final breeding line (pedigree seed).

- The *pat* gene was used as a radioactive labeled probe (black rectangle in Fig.2). Subsequently the filter was stripped of the pat probe and rehybridized using the *npt*II gene as radioactive labeled probe (black rectangle in Fig.3)
- DNA isolated from AC Excel was digested with three different enzymes and used as a negative control. No banding pattern is detected with either *npt*II or *pat* as a probe.
- DNA isolated from AC Excel was fortified with plasmid pOCA/Ac, digested with three different restriction enzymes and used as positive control. *Pat* and *npt*II probe resulted in signals thus recognizing the DNA originating from plasmid pOCA/Ac.

Stability of Insert: Using three different restriction enzymes no changes of the insertion pattern were detected comparing a F1 and a F5 (pedigree) generation. This is true whether using *pat* or *npt*II as a probe.

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[ ] Identical banding patterns are observed between the R_1 (selfed seed of original transformant) generation and the F_5 of the cultivar HCN19. This demonstrates a stable integration of the T-DNA into the line HCN19.

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2. Border Integrity

Canola breeding lines HCN92 and HCN10 were created through *Agrobacterium*-mediated gene transfer using the vector pOCA/Ac. The aim of this study was to evaluate the possibility that regions of the vector outside the right and left border sequences have been integrated into the genome of the transgenic rape lines. (See Appendix V for the complete study.)

In order to determine if any DNA sequence outside of the two borders of the plasmid pOCA/Ac is stably integrated into the genome of the two rape line HCN92 and HCN10, a detailed Southern Analysis was performed. Nine different radioactive labeled probes were used for the hybridization. These nine probes in combination represent the whole pOCA/Ac vector region outside the right and left border.

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None of the nine probes hybridization signals could be detected with genomic DNA prepared from HCN92 and HCN10 plants, whereas clear hybridization bands appeared with plasmid spiked genomic DNA. These data strongly suggest that no region outside the two border sequences of the binary vector pOCA/Ac are integrated into the genome of the two rape lines.

3. Summary

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Results from southern analysis of genomic DNA probed [

] as well as

the stability of the insert over multiple generations.

Overlapping PCR and Southern Analyses were used to determine the presence of vector sequences located outside of the T-DNA. Data indicate that no region outside the right and left border sequences of vector pOCA/Ac was integrated into the plant genome.

C. Gene Expression of Event Topas 19/2

The content of PAT and NPTII proteins in the transformation event Topas 19/2 and the derived line HCN10, was determined in seed samples by Enzyme Linked Immunosorbent Assay (ELISA). A polyclonal antibody was used that detected both active and inactive PAT protein.

No PAT or NPTII proteins were detected in the nontransformed control (Excel –1996). Results are shown in Table 6.

Table 6. PAT and NPTII Contents in Canola Seed Samples as Detected by ELISA

Sample ID	PAT/Sample (ng/g)[1]	NPT II/Sample (pg/g)[2]
Excel-1996	ND	ND
Innovator (HCN92)	295	ND
Innovator (HCN92)	295	ND
Independence (HCN10)	189	ND
Independence (HCN10)	202	ND

[1] ND < LOQ (0.40 ng/mL)
[2] ND < LOQ (100 pg/mL)

The quantity of PAT protein in whole seed, leaf tissue and processed samples (toasted meal and refined bleached and deoderized oil) from transformation events Topas 19/2, T45 and a cross of T45 with Topas 19/2 was also determined by ELISA. Seed was collected from plants (SW9782213) derived from a conventional cross of a line produced from the glufosinate tolerant transformation event T45 with another glufosinate tolerant line derived from the transformation event Topas 19/2. In addition, leaf tissue was collected

from a T45 derived line (SW9782180) and from a Topas 19/2 line (SW9782179). The quantity of PAT protein ranged from 0.001 % of total protein in the seed to 0.04 % of total protein in leaf tissue. There was no PAT protein detected the toasted meal or in the processed oil. The seed was processed into toasted meal and processed oil fractions at the POS Pilot Plant Corp. in Saskatoon, Saskatchewan. Table 7 shows the PAT content in the leaf, meal and seed samples. There were no differences in PAT content in the leaf tissue, whole seed and processed samples (toasted meal and refined bleached and deoderized oil) between the previously deregulated event T45 and the new event being considered in the petition extension, Topas 19/2.

Table 7. PAT Content in Canola Leaf, Meal and Seed Samples as Detected by ELISA

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

In 1996, seed was collected from a field trials at Rosthern, SK, Canada, and was used for a processing study conducted at POS Pilot Plant Corp, Saskatoon, SK. The processing study generated samples of desolventized meal, toasted meal, crude oil and refined oil. Processed fractions were shipped to Xenos Laboratories for determination of PAT and NPTII protein content in the processed fractions. Protein levels were determined quantitatively by means of ELISA. Results and Limits of Quantitation are given in Tables 8 and 9, respectively.

Table 8. PAT, NPT II and Protein Contents in Canola Seed and Processed Fractions as Detected by ELISA

Cultivar	Fraction	PAT (ng/g)	Total Protein (mg/g)	NPT II (pg/g)
Excel	toasted meal	ND	46.0	ND
HCN10	toasted meal	ND	44.9	ND
HCN10	toasted meal	ND	41.9	ND
Excel	Desolventized meal	ND	80.8	ND
HCN10	Desolventized meal	ND	124	ND
HCN10	Desolventized meal	ND	116	ND
Excel	Refined oil	ND	ND	ND
HCN10	Refined oil	ND	ND	ND
HCN10	Refined oil	ND	ND	ND
Excel	crude oil	ND	ND	ND
HCN10	crude oil	ND	ND	ND
HCN10	crude oil	ND	ND	ND
HCN19	Seed	921	48.84	1777
Excel	Seed	ND	29.32	ND

Table 9. Limits of Quantitation for PAT and NPTII Assays

Matrix	PAT		NPTII	
	ng/ml	ng/g (equivalent)	pg/ml	pg/g (equivalent)
Seed	0.40	70.0	70.0	350
Toasted Meal	0.40	70.0	70.0	350
Desolventized meal	0.40	70.0	70.0	490
Crude Oil	0.40	70.0	70.0	140
DRB Oil	0.40	70.0	70.0	140

Neither PAT nor NPTII protein was detectable in the processed fractions of either the negative control material (AC Excel) or the glufosinate tolerant canola line HCN10. Both PAT and NPTII protein were detected in the raw unprocessed seed of the transgenic line HCN19 that was included as a positive control. The bench scale processing conditions employed in this study denature the target proteins to the extent that they are no longer detectable by the ELISA assays used in this study.

V. Agronomic Performance of Event Topas 19/2 and/or Progeny

As seen for the deregulated event T45, there were no differences in morphology and in disease and insect resistance between the event Topas 19/2 and its progeny compared with the non-transgenic counterpart. Also, there is no obvious increase in volunteers, increase in seed dormancy or other variation indicative of increased weediness. In addition, the

expected segregation ratios were observed for a single *pat* locus as detailed above in Section IV.

A. Field Tests of Event of Topas 19/2 and/or Progeny

Prior to receiving approval in Canada, lines derived from the transformation event Topas 19/2 were evaluated from 1991 through 1995. Topas 19/2 was cultivated on millions of acres and, as part of Aventis' stewardship program, monitored at multiple sites over multiple years. The purpose of the trials was to increase seed, advance generations, demonstrate agronomic performance, and to evaluate segregation ratios and/or collect samples for compositional, nutrient and protein analysis.

B. Agronomic, Disease and Pest Characteristics

Canadian field trials continued to be conducted after commercialization. In 1998, a field test to compare the agronomic characteristics between *B. napus* line Topas 19/2//T45 and the nontransformed line AC Excel was performed. Data was obtained from replicated field plots in the mid-growing season zone in western Canada. Agronomic characteristics, including growth habit and reproductive biology for the glufosinate tolerant *B. napus* canola line Topas 19/2//T45 were substantially equivalent to the non-glufosinate tolerant canola cultivar AC Excel. Also, assessments based on plant vigor (emergence to flowering), flowering interval, canopy height, maturity dates, resistance to lodging, pod density, pod length and width, seed number per pod and yield all fell within the range of values observed for the commercial standard evaluated in this trial. Table 10 gives the agronomic performance data.

Table 10. Comparison of Agronomic Characteristics Between the Glufosinate Resistant *B. napus* line Topas 19/2//T45 and the Commercial line AC Excel at Balcarres, SK.

	Topas 19/2//T45[1]		AC Excel[2]		
	Mean	Std. Dev.	Mean	Std. Dev.	
EMERGENT/m²	123.13	34.68	160.50	66.10	NSD[3]
ESTABLISHMENT (1-9)	4.75	1.83	5.25	2.06	--
VIGOUR (1-9)	6.75	0.71	6.75	1.26	--
FLOWER INTERVAL (days)	29.5	2.67	27.0	0.00	NSD[3]
HEIGHT (cm)	122.50	14.88	121.25	6.29	--
MATURITY (days)	99.00	5.66	105.00	9.24	NSD[3]
LODGING (1-9)	8.13	0.64	8.50	0.58	--
DENSITY (1-9)	8.25	0.46	7.75	0.50	--
POD LENGTH (cm)	5.00	0.53	5.88	1.18	--
POD WIDTH (cm)	0.20	0.00	0.20	0.00	--
SEED NO./pod	20.75	2.91	20.25	1.25	NSD[3]
YIELD (g/ m²)	193.38	52.59	182.00[a]	81.02[a]	NSD[3]

[1] 4 plots untreated, 4 plots treated with Liberty Herbicide
[2] 4 plots untreated
[3] NSD indicates not significantly different ($p<0.0500$)
[a] 3 plots only

It can be concluded that there are no substantial differences in the agronomic characteristics between glufosinate tolerant *B. napus* canola, Topas 19/2//T45 and the commercially available counterpart AC Excel. It can also be concluded that a treatment with Liberty Herbicide did not impact growth habits or reproductive biology. Growth habit and reproductive biology assessments of Topas 19/2//T45 fell within the range of values observed for the commercial standard AC Excel evaluated in this trial. Overall, the presence of the *pat* gene did not result in any secondary effects, which impacted the agronomic characteristics of the glufosinate tolerant *B. napus* Topas 19/2//T45.

In another field test, the agronomic characteristics were examined for oilseed rape line SW02631, derived from a conventional cross of a line produced from the glufosinate tolerant transformation event T45 with another glufosinate tolerant line derived from the transformation event Topas 19/2. In 1995, the agronomic characteristics of line SW02631 were evaluated at four locations across Western Canada and in 1998, the line was evaluated in a confined field trial in Sweden. Agronomic characteristics evaluated included plant height, plant stand density, days to flowering days to maturity, lodging and plot yield. The results demonstrate that SW02631 is an early maturing cultivar with equivalent height, lodging, plant stand and yield characteristics to the commercial nontransgenic cultivars AC Excel and Legend. The agronomic characteristics for the cultivar SW02631 are typical for oilseed rape. The transformation of the oilseed rape lines used in the breeding of SW02631 has not impacted on the agronomic characteristics of the cultivar. No pleitropic effects on the agronomic performance of the cultivar are indicated.

Yet another field test examined the agronomic characteristics as well as the seed composition of Innovator (Topas 19/2) and traditionally derived non-transgenic counterparts. Based on 1996 private co-op testing data published by the Western Canada Canola/Rapeseed Recommending Committee, the seed composition and agronomic characteristics of the transgenic *B. napus* canola line Innovator (HCN92) is substantially equivalent to those of and three nontransgenic canola (Cyclone, Legend, Excel) varieties evaluated. Summary data was obtained from field plots across the short, mid and long growing zones in western Canada. The crop height, black leg susceptibility, lodging potential, maturity and yield of Innovator fell within the range of values observed for the commercial standards evaluated in the trials (Tables 11 and 12). Values for seed oil, protein, fatty acids and glucosinolates for Innovator line also fell within the range observed for the non-transgenic counterparts. All transgenic and non-transgenic varieties tested, regardless of growing zone, contained erucic acid and total glucosinolate levels below the mandatory limits of 2% and 30 µmoles/gram, respectively. Overall, the presence of the gene which codes for the PAT protein or the transformation event yielding Innovator did not result in any secondary effects which might impact either the seed composition or the agronomic characteristics of the glufosinate tolerant *B. napus* line.

Table 11. Susceptibility score (0-10) of various *B. napus* varieties at two locations in Western Canada in 1996

Entry	Type	Rosthern	Winnipeg	Mean
Cyclone	Non-transgenic	1.37	0.74	1.06
Legend	Non-transgenic	1.41	0.89	1.15
Excel	Non-transgenic	1.82	1.13	1.48
Innovator	Topas 19/2 (HCN92)	1.14	0.98	1.06
Non-trans Mean		1.53	0.92	1.23

Table 12. Summary of agronomic and seed composition of various *B. napus* varieties from private coop trials across Western Canada in 1996

Entry	Type	Yield	% of Checks	Oil %	Protein %	Height (cm)	Lodge Score	Maturity days[1]
Cyclone	Non-transgenic	350.92	107.93	44.75	46.85	111.2	2.2	98
Legend	Non-transgenic	319.31	97.39	46.10	47.05	105.9	2.5	97
Excel	Non-transgenic	313.39	95.58	46.85	46.35	113.9	2.1	98
Innovator	Topas 19/2 (HCN92)	318.79	97.23	46.40	45.60	106.3	2.4	97
Non-trans Mean		327.87	100.30	45.90	46.75	110.3	2.3	98

ND = not determined.
[1] long and short growing season only

C. Monitoring

Beginning in 1996, Aventis (then AgrEvo) undertook a survey of 1995 glufosinate tolerant canola production fields, adjacent non-agricultural areas, and transportation routes to document the occurrence and fate of *B. napus* volunteers and related species (Deschamps *et al.*, 1997) (See Appendix VI for complete report). The survey was repeated on the same sites in 1997 with a few modifications. No evidence was found to suggest that the weediness of transgenic volunteers is different from the non-transgenic counterparts. No evidence was found for out-crossing to weedy relatives. Proper management practices are the key to controlling or containing volunteer canola (transgenic or otherwise) and its weedy relatives.

The approach chosen to assess the occurrence and fate of glufosinate tolerant *B. napus* canola and related weedy in species in cultivated areas was to systematically scout and

sample 1995 Innovator (HCN92) fields and adjacent fields throughout the 1997 growing season. In 1996, ten sites in East-Central Saskatchewan where glufosinate tolerant canola (Innovator HCN92) was grown in 1995 were selected. To provide a comparison, five additional sites in the same region where non-transgenic canola was produced in 1995 were surveyed in a similar manner. These same 15 sites were re-surveyed in 1997.

The specific survey methods were similar to those used to obtain the annual weed surveys published in the province of Saskatchewan (Thomas *et al.*, 1996). The surveyor walked a predefined "W" pattern in the field and took weed counts from 20 quadrats (0.5 m by 0.5 m) spaced 50 paces apart. The number of quadrats counted was increased from 10 in 1996 to 20 in 1997 to compensate for an anticipated reduction in the population of canola volunteers two years following the canola crop. Assessments were taken at the following times: 1) early spring prior to cultivation and seeding (early May); 2) following crop emergence but prior to application of any herbicide (early June); 3) following herbicide application (July), and 4) after harvest just prior to freeze-up (mid-October).

Appropriate non-agricultural areas in the vicinity of 1995 Innovator (HCN92) fields were identified during the site selection process. These areas included roadways, fence lines, sloughs, grassed areas, and building sites. Late in the growing season when *Brassica* and related species are in the reproductive stage, the non-crop areas were surveyed for their presence. The survey area was not extended more than 20 m from the margin of the field, as it is unlikely for seed to be spread a greater distance by wind or harvest operations. If any were discovered within the sample area, green tissue from the plant(s) was sampled and tested for the presence of the PAT enzyme by ELISA.

Storage areas and transportation tours were also surveyed late in the growing season. Each transportation route was surveyed over a distance of 10 km measured from each location in the survey where Innovator (HCN92) was grown or stored. A segment of 100 m out of each kilometer was walked and individual plants of *Brassica* or related weed species found along the roadside or adjacent ditch was counted. Green tissue samples were collected from each individual counted for analysis by ELISA to determine whether the plant possessed the glufosinate tolerance trait.

After two years of monitoring the occurrence and fate of glufosinate tolerant canola volunteers and weedy relatives following the 1995-growing season in Saskatchewan, the following conclusions can be made:

(1) no evidence was found to suggest that glufosinate tolerant canola behaves differently as a volunteer than does standard non-transgenic canola;
(2) spontaneous out-crossing to weedy relatives does not appear to be a factor; and
(3) proper management practices are the key to controlling or containing volunteer canola (transgenic or otherwise) and its weedy relatives.

By using weed management techniques familiar to growers such as herbicides, cultivation, and mowing, these species of the family Cruciferae are easily controlled. Our monitoring program has shown that glufosinate tolerant canola, like standard canola, is readily removed from subsequent crops by herbicides familiar to and commonly used by farmers.

The same can be said for the related weed species. The mowing of ditches along municipal roads effectively prevents volunteer canola escapes from producing seed and establishing feral populations. Wherever sound vegetation management practices are employed, the potential for volunteer canola (herbicide tolerant or traditional) to become an environmental problem is limited.

VI. Potential for Environmental Impact from Non-contained Use of Event Topas 19/2 and progeny

A. Non Target Organisms

To evaluate the potential impact on non-target organisms the behavior of honey bees foraging on Topas 19/2 derived canola (*Brassica napus*) was evaluated. Canola flowers produce an abundant supply of nectar for insect pollinators. Cross pollination of flowers often occurs when bees are searching for nectar. Because a genetic modification may elicit a biochemical change in plants, it is important to evaluate the impact of the transformation of the crop on the behavior of honey bees. Studies were therefore conducted in 1993 and 1994.

Hives were placed adjacent to HCN92 with an untranformed variety used as a negative control. Inspection of brood chambers revealed queens actively laying, with both eggs and larvae present in abundance with minimal honey stores in the brood chambers. The bees were observed to be actively foraging in the transgenic crops.

Colonies were again inspected approximately two weeks after the commencement of canola flowering. The lower honey super was approximately 75% filled, while the upper super was 25% filled. Cells were partially filled with a very light honey characteristic of canola. Bee populations in the honey supers as well as larvae in the brood chamber had increased markedly from the commencement of canola flowering. The presence of drones near the hive entrance was noted. The queen was observed to be actively laying, and both eggs and developing brood were observed.

After the canola flowering had terminated, approximately 60 kg of honey was extracted from the hives. The extracted honey was light in color, characteristic of canola, and highly viscous. Fresh supers were introduced to both colonies.

Further autumn inspections of the hives were conducted and honey production found to be normal with bee populations above average.

Bee behavior of the colony in 1993 was normal and in 1994 the behavior of the colony located near the transgenic canola field was no different from the colony located near the non-transgenic canola field. Results from these studies indicate that honey bees will actively forage on glufosinate tolerant canola and produce a light colored honey. Hive development was observed to be normal during and subsequent to flowering of both transgenic and non-transgenic canola crops. Prior to overwintering, the health of both hives was rated as above average condition.

B. Summary

There were no significant differences, apart from the intended changes, demonstrated in field tests of event Topas 19/2//T45 and progeny compared with the non-transgenic parent line with regard to emergence, flowering interval, maturity, seed number per pod and yield. No morphological, beneficial organisms in the environment, disease or pest differences between event Topas 19/2 and progeny and the previously considered GTC event were noted. There is no reason to think cultivation of event Topas 19/2 and its progeny will have environmental effects different from cultivation of event T45, the other GTC event previously considered by APHIS, or will cause damage to raw or processed agricultural commodities. No adverse consequences from the introduction of event Topas 19/2 and its progeny are expected.

VII. Statement of Grounds Unfavorable

No unfavorable information and data have been demonstrated for GTC transformation event Topas 19/2 and its progeny.

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Appendix I

United States Food and Drug Administration:

Consultation letter dated July 6, 1995, confirming no safety concerns for glufosinate canola lines HCN92, HCN10 and HCN05, derived from Event Topas 19/2

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Washington DC 20204

July 6, 1995

Sally L. Van Wert, Ph.D.
Manager, Regulatory Affairs - Biotechnology
AgrEvo USA Company
Little Falls Centre One
2711 Centerville Road
Wilmington, DE 19808

Dear Dr. Van Wert:

This is in response to your letter dated May 15, 1995, regarding your genetically modified glufosinate tolerant canola line HCN92, derived from transformation event Topas 19/2, about which you concluded consultations with the agency in March, 1995. You note that AgrEvo has produced other lines of glufosinate tolerant canola (lines HCN05 and HCN10) that represent additional crosses with event Topas 19/2; AgrEvo has identified no safety issues associated with these additional lines. You state your understanding that consultation with the agency about food and feed issues pertinent to a genetically engineered variety is undertaken for each new transformation event, and that consultation is not necessary for additional crosses with that event so long as those crosses do not raise safety concerns and the germplasm is grown in accordance with good standard agricultural practices. You ask that we confirm that this understanding is correct.

As you are aware, FDA has encouraged developers of new plant varieties to consult with the agency before marketing to ensure that all potential safety, nutritional, and regulatory issues have been addressed and resolved prior to commercial distribution. When one line derived from a transformation event has been shown to raise no such issues, we believe that it is unlikely that other lines generated from the same event would raise issues that would be the subject of a consultation with the agency. However, should a line show characteristics that would raise safety or regulatory issues, we would encourage and expect developers to consult with the agency to ensure those issues were resolved prior to marketing, regardless of the origin of the line. It is, of course, as always, the responsibility of developers of new foods to ensure that the foods they offer to consumers are safe and comply with all applicable legal and regulatory requirements.

I trust that this information adequately responds to your request. If we can provide further assistance, please contact us.

Sincerely yours,

for Laura M. Tarantino, Ph.D.
Chief
Biotechnology Policy Branch, HFS-206
Division of Product Policy
Center for Food Safety
and Applied Nutrition

Appendix II

United States Department of Agriculture

Opinion letter dated June 9, 1995, for importation of canola seed (lines HCN10, HCN92 and HCN05 derived from Event Topas 19/2) for processing into oil

United States Department of Agriculture

Animal and Plant Health Inspection Service

Biotechnology, Biologics, and Environmental Protection

JUN - 9 1995

Dr. Sally Van Wert
Manager, Regulatory Affairs - Biotechnology
AgrEvo USA Company
Little Falls Centre One
2711 Centerville Road
Wilmington, DE 19808

Dear Dr. Van Wert:

This in response to your letter of April 17, in which you requested an opinion on the regulatory authority of Biotechnology Permits, Biotechnology, Biologics, and Environmental Protection, Animal and Plant Health Inspection Service (APHIS), U.S. Department of Agriculture, with respect to your company's glufosinate resistant canola lines (transformation event Topas 19/2 and varieties derived from it). You referred to three canola varieties that have been developed from Topas 19/2, namely, HCN92 (Innovator), HCN10, and HCN05.

Based upon the information in your letter and the intended use of this plant material for processing, we believe that these lines should pose no plant pest risk. Therefore, APHIS will not consider Topas 19/2 and progeny canola seed, moved from Canada to the United States for processing, as regulated articles under our regulations (7 CFR Part 340).

We have based our decision on the factors summarized below:

1. The intended use of the canola seeds is processing the seeds to extract the oil. After processing, the remaining plant material is nonviable.

2. For years, canola seeds have been shipped from Canada for processing at facilities located in the United States. APHIS is unaware of any plant pest problems that have been associated with such seed shipments or the handling of the remaining plant material after processing of the seeds.

3. APHIS takes note of the environmental analysis on canola line conducted by Agriculture and Agri-Food Canada in which they concluded that the canola is safe for cultivation in Canada and that the AgrEvo canola is no more competitive than other canola varieties.

4. APHIS also believes that standard industry practices for the shipment of the canola to a processing plant are adequate and should not present any plant pest risk. There is no indication that the shipment of other canola varieties has ever resulted in a plant pest risk.

5. The unique trait of the above referenced AgrEvo canola lines, namely, glufosinate resistance, would confer a selective advantage only if these plants or their offspring were treated with glufosinate. APHIS believes that it is unlikely that canola will escape, germinate, grow to reproductive maturity, and pollinate wild or cultivated relatives whose offspring will be treated with glufosinate and thereby, exhibit a selective advantage. Even if such an unlikely sequence of events were to occur and result in a plant population that could not be controlled with glufosinate, alternative chemical and mechanical control practices are currently available that should be effective.

We must emphasize that our opinion regarding these canola lines is expressly limited to the conditions that you have described, namely, shipment of seed to processing plants in the United States. Under the circumstance of this intended use (i.e., shipment to a processing facility), APHIS would not regulate these canola lines under APHIS regulations found under 7 CFR Part 340. This opinion on canola lines Topas 19/2 also applies to progeny of Topas 19/2 crosses with other canola lines that are not regulated articles under 7 CFR Part 340. However, if this plant material is shipped for other uses or purposes, it may be subject to regulation under 7 CFR Part 340. The canola seed is subject to all other applicable phytosanitary standards and regulations.

If you have any further questions about this matter, please feel free to call Dr. David Heron on Area Code (301) 734-7612.

Sincerely,

John H. Payne
Acting Director

Appendix III

Agriculture and Agri-Food Canada: Decision Document DD 95-01

Determination of Environmental Safety of AgrEvo Canada Inc.'s Glufosinate Ammonium-Tolerant Canola

and

Approval letter dated March 13, 1995, from Agriculture and Agri-Food Canada to AgrEvo Canada, Inc. for release into the environment and use as livestock feed

Agriculture and Agri-Food Canada
Agriculture et Agroalimentaire Canada

Plant Products Division
Food Production and Inspection Branch
Agriculture and Agri-Food Canada

MAR 15 1995
AgrEvo OTTAWA

Decision Document DD95-01

Determination of Environmental Safety of Agrevo Canada Inc.'s Glufosinate Ammonium-Tolerant Canola

This Decision Document has been prepared to explain the regulatory decision reached under the guidelines Dir94-08 Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits and its companion document Dir94-09 The Biology of *Brassica napus* L. (Canola/Rapeseed), and the proposed guidelines Pro94-04 Guidelines for the Assessment of Plants with Novel Traits as Livestock Feed.

The Plant Biotechnology Office and the Feed Section of the Plant Products Division have evaluated information submitted by AgrEvo Canada Inc. regarding a glufosinate ammonium-tolerant and kanamycin-resistant canola line. They have determined that this plant with novel traits does not present altered environmental interactions when compared to currently commercialized canola varieties and is considered substantially equivalent to canola currently approved as livestock feed.

Unconfined release into the environment, including feed use of HCN92, and other *B. napus* lines derived from it, but without the introduction of any other novel trait, is therefore considered safe.

(publié aussi en français)

March 10, 1995

This bulletin is published by the Plant Products Division, Agriculture and Agri-Food Canada. For further information, please contact the Plant Biotechnology Office or the Feed Section at:

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Table of Contents

I. Brief Identification of The Plant With Novel Traits (PNT)

Designation(s) of the PNT:	HCN92
Applicant:	AgrEvo Canada Inc.
Plant Species:	Canola (*Brassica napus* L.)
Novel Traits:	Glufosinate ammonium (herbicide) tolerance; kanamycin (antibiotic) resistance
Trait Introduction Method:	*Agrobacterium tumefaciens*-mediated transformation
Proposed Use of PNT:	Production of *B. napus* for seed oil for human consumption and seed oil and meal for livestock feed. These materials will not be grown outside the normal production area for canola.

II. Background Information

AgrEvo has developed a *Brassica napus* canola line tolerant to glufosinate ammonium, a broad spectrum non-residual herbicide. This *B. napus* line, referred to as HCN92 in the present document, will allow the use of glufosinate ammonium as a post-emergence herbicide, thus providing an alternative for weed control in canola production, and reducing reliance on soil-incorporated herbicides.

The development of HCN92 was based on recombinant DNA technology, by the introduction of two bacterial genes into a line of *B. napus*. A gene conferring tolerance to glufosinate ammonium was inserted, coding for phosphinothricin acetyltransferase, an enzyme that inactivates glufosinate ammonium through acetylation. Another gene, conferring resistance to kanamycin, was also inserted; this gene is of no agronomic interest but was used to select modified plants from those that remained unmodified at the development stage.

HCN92 has been field tested in Canada under confined conditions in Saskatchewan (1990-94), Alberta (1991-94), Manitoba (1991-94), and Ontario (1993-94).

AgrEvo has provided data on the identity of HCN92, a detailed description of the modification method, data and information on the stability of the gene insertion, the role of the inserted genes in donor organisms and the role of regulatory sequences in donor organisms, their molecular characterization, and full nucleotide sequences. The novel proteins were identified and characterized, including their potential toxicity to livestock and non-target organisms, allergenicity, and levels of expression in the plant and feed. Numerous detailed scientific publications were also supplied.

Agronomic characteristics such as seed production, time to maturity, flowering period, and male and female fertility were compared to those of unmodified *B. napus* counterparts. Effects of HCN92 residues on growth and productivity of the following season's grain, forage, and pulse crops were assessed.

AgrEvo has also provided data on HNC92's survival adaptations: silique shattering potential, seed dormancy, seed dispersal mechanisms, vegetative vigor, reproductive characteristics, and the emergence in subsequent years of volunteer plants under mechanical or chemical fallow conditions. Stress adaptation was evaluated, including susceptibilities to various *B. napus* pests and pathogens, to abiotic stresses such as soil salinity and moisture regimes, and to herbicides other than glufosinate ammonium that are normally used on canola crops. Invasiveness studies were performed under disturbed, undisturbed, and agronomic conditions.

Data to support the efficacy of HCN92 as a livestock feed were provided. A proximate analysis to include crude protein, crude fat, crude fiber, ash and gross energy were supplied for the whole seed, processed meal and oil content.

Agriculture and Agri-Food Canada (AAFC) has reviewed the above information, in light of the assessment criteria for determining environmental safety of plants with novel traits, as described in the regulatory directive Dir94-08:

- potential of the PNT to become a weed of agriculture or to be invasive of natural habitats,
- potential for gene-flow to wild relatives whose hybrid offspring may become more weedy or more invasive,
- potential for the PNT to become a plant pest,
- potential impact of the PNT or its gene products on non-target species, including humans, and
- potential impact on biodiversity.

AAFC has also reviewed the above information in light of the assessment criteria for determining safety and efficacy of livestock feed, as described in Pro94-04:

- potential impact on livestock, and
- potential impact on livestock nutrition.

III. Description of the Novel Traits

1. Glufosinate Ammonium Tolerance:

- Phosphinothricin (PPT), the active ingredient of glufosinate ammonium, inhibits glutamine synthetase, which results in the accumulation of lethal levels of ammonium in susceptible plants within hours of application.

- The phosphinothricin tolerance gene engineered into HCN92 codes for PPT-acetyltransferase (PAT). This enzyme detoxifies phosphinothricin by acetylation into an inactive compound. It has extremely high substrate specificity; experimental data clearly showed that neither L-PPT's analog L-glutamic acid, D-PPT, nor any protein amino acid can be acetylated by the PAT enzyme.

- The PAT gene was originally isolated from *Streptomyces viridochromogenes*, an aerobic soil actinomycete. The PAT enzyme is therefore naturally occurring in the soil. More generally, acetyltransferases are ubiquitous in nature.

- The gene is linked to a constitutive promoter, and protein expression was detected in roots, leaves, buds and seeds. However, it was not detected in stem tissue, protein extracts from the pollen, or unprocessed honey. Maximum expression was 0.001% of total plant protein.

- The expressed PAT enzyme was compared to the bacterial protein: molecular weights were similar, indicating that the protein had not been glycosylated nor had it undergone post transcriptional modifications. Studies showed that the enzyme was inactivated within one minute when subjected to typical mammalian stomach conditions and was inactivated during processing of canola seed into feed ingredients.

- The gene nucleotide sequence and the enzyme amino acid sequence were provided. The nucleotide sequence showed no significant homology the toxins or allergens entered in to GENEBANK DNA database.

2. Kanamycin Resistance:

- Kanamycin is an aminoglycosidic antibiotic that binds to bacterial ribosomes thus disrupting normal protein synthesis and killing the bacterial cell.

- The kanamycin-resistance gene codes for an enzyme that prevents kanamycin from binding to ribosomes, thereby rendering the cells resistant. The exact nature of the enzyme is considered Confidential Business Information by AgrEvo. The source of the gene was described, and the full nucleotide sequence was provided.

- The gene is linked to a weak constitutive promoter; expression was consistently stronger in root tissue, but was also observed in buds, leaves, and crude seed samples. The enzyme was not detected in unprocessed honey or pollen samples and was inactivated during processing of canola seed into feed ingredients.

- The expressed enzyme was compared to the bacterial protein: molecular weights were similar, indicating that the protein had not been glycosylated nor had it undergone post-transcriptional modifications.

- The nucleotide sequence showed no significant homology with the toxins or allergens entered in the GENEBANK DNA database.

3. Development Method:

- *Brassica napus* cultivar Topas was transformed using a disarmed non-pathogenic *Agrobacterium tumefaciens* vector; the vector contained the T-DNA region of an *Agrobacterium* plasmid from which virulence and plant disease-causing genes were removed, and replaced with genes coding for glufosinate ammonium tolerance and kanamycin resistance. The T-DNA portion of the plasmid is known to insert randomly into the plant's genome and the insertion is usually stable, as was shown to be the case in HCN92.

- The transformant was crossed with *B. napus* line ACSN3, then with AC Excel; HCN92 was derived from a bulk of single F_3 plants selected from the cross.

4. Stable Integration into the Plant's Genome:

- The provided data showed that there was no incorporation of any coding region from outside the T-DNA borders and that gene integration occurred at only one insertion site.

- HCN92 is several generations removed from the original transformant. Comparisons between the original transgenic plant and the HCN92 line show no difference in the presence and expression of both genes, nor in the insertion site.

IV. Assessment Criteria for Environmental Safety

1. Potential of the PNT to become a weed of agriculture or to be invasive of natural habitats

AAFC evaluated data submitted by AgrEvo on the reproductive and survival biology of HCN92, and determined that vegetative vigor, overwintering capacity, flowering period, time to maturity, seed production, and dormancy were within the normal range of expression of characteristics in unmodified *B. napus* counterparts. HCN92 has no specific added genes for cold tolerance or winter hibernation; no overwintered plants were observed by AgrEvo in post-harvest

years of field trials, and the number of volunteers in the year following a field trial were comparable between plots of HCN92 and counterpart *B. napus*. Seed morphology and average seed weight did not change, indicating that seed dispersal potential was not altered.

Based on the submitted data, AAFC has determined that HCN92 did not show any stress adaptation other than its resistance to glufosinate ammonium. Its resistance or susceptibility to major *B. napus* pests and pathogens (e.g., blackleg, sclerotinia, flea beetles) fall within the ranges currently displayed by commercial varieties. Moisture stress had a significant negative effect on both HCN92 and its counterparts.

The biology of *B. napus*, described in Dir94-09, shows that unmodified plants of this species are not invasive of unmanaged habitats in Canada. According to the information provided by AgrEvo, HCN92 was determined not to be different from its counterparts in this respect. Invasiveness was studied in disturbed and undisturbed habitats. Data showed that HCN92 was neither more invasive nor more persistent than commercial counterparts. No competitive advantage was conferred to glufosinate ammonium-tolerant plants, other than that conferred by tolerance to glufosinate ammonium.

Glufosinate ammonium is not used in normal crop rotation cycles, and resistance is therefore not an issue of concern in weed management control. Glufosinate-resistant *B. napus* volunteer plants can easily be managed by mechanical means and other available chemicals used to control *B. napus*.

The above considerations, together with the fact that the novel traits have no intended effect on weediness or invasiveness, led AAFC to conclude that HCN92 has no altered weed or invasiveness potential compared to currently commercialized canola varieties.

NOTE: A longer term concern, if there is general adoption of several different crop and specific herbicide weed management systems, is the potential development of crop volunteers with a combination of novel resistances to different herbicides. This could result in the loss of the use of these herbicides and any of their potential benefits. Therefore, agricultural extension personnel, in both the private and public sectors, should promote careful management practices for growers who use these herbicide tolerant crops, to minimize the development of multiple resistance.

2. Potential for Gene Flow to Wild Relatives Whose Hybrid Offspring May Become More Weedy or More Invasive

Brassica napus plants are known to outcross up to 30% with other plants of the same species, and potentially with plants of the species *B. rapa, B. juncea, B. carinata, B. nigra, Diplotaxis muralis, Raphanus raphanistrum*, and

Erucastrum gallicum (Dir 94-09). Studies show that introgression of the herbicide tolerance gene is most likely to occur with *B. rapa*, the other major canola species and an occasional weed of cultivated land especially in the eastern provinces of Canada.

If glufosinate ammonium-tolerant individuals arose through interspecific or intergeneric hybridization, the novel traits would confer no competitive advantage to these plants unless challenged by glufosinate ammonium. This would only occur in managed ecosystems where glufosinate ammonium is used for broad spectrum weed control, e.g., in the cultivation of plant cultivars developed to exhibit glufosinate ammonium tolerance and in which glufosinate ammonium is used to control weeds. As with glufosinate ammonium-tolerant *B. napus*, these herbicide tolerant individuals, should they arise, would be easily controlled using mechanical and other available chemical means. Hybrids, if they developed, could potentially result in the loss of glufosinate ammonium as a tool to control these species. This, however, can be avoided by the use of sound crop management practices.

The above considerations led AAFC to conclude that gene flow from HCN92 to canola relatives is possible, but would not result in increased weediness or invasiveness of these relatives.

3. Altered Plant Pest Potential

The intended effects of both novel traits are unrelated to plant pest potential, and *Brassica napus* is not a plant pest in Canada (Dir94-09). In addition, agronomic characteristics, stress adaptation, and qualitative and quantitative composition of HCN92 were shown to be within the range of values displayed by currently commercialized *B. napus* varieties, leading to the conclusion that plant pest potential was not inadvertently altered.

AAFC has therefore determined that HCN92 did not display any altered pest potential.

4. Potential Impact on Non-Target Organisms

Data presenting the effect of plant residue from HCN92 on agronomic performance of succeeding crops were examined by AAFC for wheat, barley, lentils, peas, flax and alfalfa. No significant differences in either plant counts or grain yield between the HCN92 and counterpart canola plots were identified. This is an indirect indication that soil bacteria, involved in maintaining soil fertility, are not negatively affected by HCN92 plant residues.

PAT activity was not detected in pollen grains, neither was it detected in unprocessed honey collected from a bee colony which had foraged in the glufosinate-tolerant *B. napus* line. No negative impact on bees foraging in

Agriculture and Agri-Food Canada
Agriculture et Agro-alimentaire Canada

Food Production and Inspection Branch
Direction générale de la production et de l'inspection des aliments

Plant Industry Directorate
Plant Products Division
59 Camelot Drive
Nepean, Ontario K1A 0Y9
Tel.: (613) 952-8000
Fax.: (613) 992-5219

HCN92
HCN 10-
HCN 05

Your file / Votre référence

Our file / Notre référence

RECEIVED
AGRICULTURE DIV.
MAR 15 1995
AgrEvo OTTAWA

March 13, 1995

3625-6-10H1

Mr. Conor Dobson
AgrEvo Canada Inc.
Manager, Government Affairs
213-1600 James Naismith Drive
Gloucester, Ontario
Fax: (613) 748-5728

Dear Mr. Dobson:

We have reviewed your application for unconfined field release and for livestock feed use of the transformed HCN92 canola line (*Brassica napus*). These plants have been transformed with genes that confer tolerance to the herbicide glufosinate ammonium and a selectable marker.

On the basis of the information provided to us, the unconfined release of HCN92 should not pose any concern to environmental safety, and is therefore authorized in Canada. HCN92 and its byproducts were found to be substantially equivalent to traditional varieties, and are therefore approved for use as livestock feed ingredients in Canada. The enclosed Decision Document, dated March 10, 1995, which explains the rationale behind our decision, will be made publicly available.

The present authorization relates to HCN92, all other *Brassica napus* spring varieties resulting from the same transformation event, and all their descendants, provided no inter-specific crosses are performed, provided the intended use is similar, and provided these plants do not display any additional novel traits.

If at any time, your company becomes aware of any new information regarding risk to the environment, including risk to animal or human health, that could result from this release, you must immediately provide such information to this office.

Please note that, while determining the environmental safety of plants with novel traits is a critical step in the commercialization of these plant types, other requirements may still need to be addressed, including a food safety assessment by Health Canada, and Variety Registration by Agriculture and Agri-Food Canada. We will inform provincial agencies of this decision.

Yours sincerely,

Glenn Hansen
Director
c.c.: Provincial Contacts, EC, HC, Seed Program Officers, Variety Section, Feed Section, Director, Plant Protection

Enclosure (Decision Document)

Appendix IV

Health Canada: Health Protection Branch

Approval letter dated February 16, 1995, indicating "no objection to the sale as food of refined oil" from canola line HCN92, derived from Event Topas 19/2

and

Approval letter dated June 27, 1995, indicating "no objection to the sale as food of refined oil" from canola line HCN10, a sister line to HCN92 which was derived from Event Topas 19/2

Health Canada | Santé Canada
Health Protection Branch | Direction générale de la protection de la santé
Tunney's Pasture
Ottawa, Ontario
K1A 0L2

February 16, 1995

RECEIVED
AGRICULTURE DIV.
FEB 16 1995
AgrEvo OTTAWA

Mr. Conor Dobson
Manager, Government Affairs
Hoechst NOR-AM AgrEvo Inc.
Agriculture Division
1600 James Naismith Drive
Suite 213
Gloucester, Ontario
K1B 5N4

Dear Mr. Dobson:

This will refer to the Novel Food Submission concerning a transgenic, glufosinate ammonium-tolerant canola (*Brassica napus*) variety developed through the use of recombinant DNA techniques.

Officers of the Health Protection Branch have reviewed the information AgrEvo Inc. provided for the assessment of the glufosinate ammonium tolerant canola variety. According to the submitted data, the procedure used in developing the novel canola variety of note consisted of the following:

(i) The gene encoding for the expression of the protein phosphinothricin acetyl transferase (PAT) from *Streptomyces viridochromogenes*, was introduced into canola (*Brassica napus*) var 19/2; then

(ii) The transformant produced in (i) was backcrossed with unmodified canola lines to produce the transgenic canola variety HCN92 which is tolerant to glufosinate ammonium herbicide.

The PAT gene is linked to the kanamycin resistant marker gene (NPTII) derived from *Escherichia coli*. Consequently, the glufosinate ammonium tolerant canola contains 2 new genes and the proteins expressed by them.

We also note that neither the canola wholeseed nor the meal is expected to be used as food. On the basis of the data submitted, we have no objection to the sale as food of the refined oil from the glufosinate ammonium tolerant canola varieties developed through the use of the above-noted transgenic technique.

.../2

It should be noted that this opinion is solely with respect to the suitability for sale as food of the oil from glufosinate ammonium tolerant canola. The issue of registration of glufosinate ammonium for use on this crop is being addressed separately through the normal pesticide registration process.

Concerning the acceptability of the meal for use in animal feed, we would suggest that you continue to keep in direct contact with Agriculture and Agri-Food Canada as the evaluation of animal feed falls under the purview of that Department.

Please note that we are providing our colleagues in Agriculture and Agri-Food Canada with a copy of this letter.

Yours truly,

S.W. Gunner, Ph.D.
Director General
Food Directorate

cc: S.W. Ormrod

Health Canada / Santé Canada
Health Protection Branch / Direction générale de la protection de la santé

Sir Frederick Banting Building
Tunney's Pasture 2204A1
Ottawa, Ontario
K1A 0L2

RECEIVED
JUL - 5 1995
REGULATORY
AgrEvo

June 27, 1995

RECEIVED
AGRICULTURE DIV.
JUN 29 1995
AgrEvo OTTAWA

Mr. Connor Dobson
Manager, Government Affairs
Hoechst NOR-AM AgrEvo Inc.
Agriculture Division
1600 James Naismith Drive
Suite 213
Gloucester, Ontario
K1B 5N4

Dear Mr. Dobson:

This will refer to your letter of April 17, 1995 requesting clarification of the novel food status of HCN10, a sister canola line to HCN92. HCN92 is the glufosinate ammonium tolerant canola line which was the subject of a Food Directorate opinion indicating no objection to the use of refined oil from that line as food in Canada (February 16, 1995).

We note that HCN10, the sister line which is the subject of your recent request, is derived from the same original transformant, var 19/2, as was HCN92. Furthermore, only conventional breeding techniques have been used subsequent to the original transformation event to develop line HCN10. This conventional breeding involved crossing of progeny from 19/2 with the variety Profit to develop line HCN10 while HCN92 was developed by crossing with the variety Excel. No new characteristics or significant differences from the phenotypic characteristics of HCN92 are reported for HCN10.

Based upon the information submitted, the Food Directorate opinion of February 16, 1995 regarding HCN92 is also valid for line HCN10.

Please note that we are providing our colleagues in Agriculture and Agri-Food Canada with a copy of this letter.

Sincerely,

Paul Mayers
Head, Office of Food Biotechnology

cc: G. Hansen, AAFC

Canada

Appendix V

Report from PlanTec GmbH:

Southern Analysis Performed on the Innovator (HCN92) and Independence (HCN10) Rape Lines

PlantTec
Biotechnologie GmbH
Forschung & Entwicklung

A59464

Hermannswerder 14
14473 Potsdam · Germany
Tel. +49 331-27 567-0 · Fax +49 331-27 567-77
e-mail PlantTec@PlantTec.de

PlantTec · Hermannswerder 14 · 14473 Potsdam

Title

Southern analysis performed on the Innovator (HCN 92) and Independence (HCN 10) rape lines

Author

Dr. Ursula La Cognata

Completed on

October 1997

Performing Laboratory

PlantTec GmbH
Forschung & Entwicklung
Hermannswerder 14
14473 Potsdam Germany

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1.Objective:

Canola breeding lines HCN 92 and HCN 10 were created through *Agrobacterium* mediated gene transfer, using the vector pOCA/Ac (Figure 8). The aim of this study was to evaluate the possibility that regions of the vector outside the right and left border sequences have been integrated into the genome of this transgenic rape lines.

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Taken together, probes 1-9 in combination represent the whole pOCA/Ac vector region outside of the right and left border.

Southern analysis was performed in the following way: genomic rape DNA was prepared according to published procedures. In each case approximately 40 microgrammes of genomic DNA were digested with the appropiate restriction enzyme(s) and wildtype as well as HCN 92 or HCN 10 DNA were loaded site by site onto the same agarose gel. As a positive control for hybridization the same restriction digests were performed in parallel, adding 2 nanogrammes of pOCA/Ac plasmid DNA to the genomic wildtype DNA. After gel electrophoresis the DNA was transferred to nylon filters and hybridized with either probe 1, probe 2 and probe 3 in combination, probe 4, probe 5, probe 6 and probe 7 in combination, probe 8 and probe 9. After the first round of hybridization all seven filters were stripped and hybridized with a second probe, namely [ ] CBI Deleted

3.Results:

Hybridization of the seven filters with either probe 1 to probe 9 led to the same result: in the lanes where genomic DNA was spiked with plasmid DNA the expected hybridizing bands were obtained. In contrast no hybridizing bands became visible in those lanes where solely genomic DNA of transgenic HCN 10 or HCN 92 plants was loaded (see Fig.1 to 7).
Hybridization of the same filters with the [ ] led to the following results: in all cases CBI Deleted
where genomic DNA was spiked with plasmid DNA, hybridizing bands of the expected length were obtained. Additionally, hybridizing bands of similar intensity were obtained as well in all lanes where solely genomic DNA of HCN 10 or HCN 92 plants were loaded (see Fig.9 to 15).

4.Summary and Conclusion

To investigate the possibility if any region outside the two border sequences of the binary vector pOCA/Ac has been integrated into the genome of the Glufosinate tolerant rape lines HCN 10 and HCN 92, a detailed Southern analysis was performed. Nine different DNA fragments, in combination representing the whole pOCA/Ac region outside the two border sequences, were used for hybridization. With none of these probes hybridization signals could be detected with genomic DNA prepared from Innovator or Independence plants, whereas clear hybridizing bands appeared with plasmid spiked genomic DNA. In contrast, the hybridization signals obtained for plasmid DNA and HCN genomic DNA were of similar intensity if the PAT coding region was used as a probe. Taken together, these data strongly indicate that no region outside the two border sequences of the binary vector pOCA/Ac are integrated into the genome of the two rape lines.

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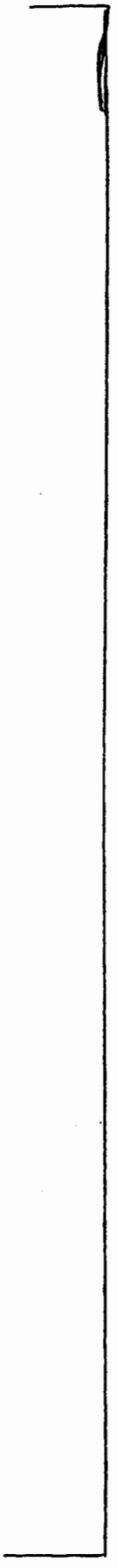
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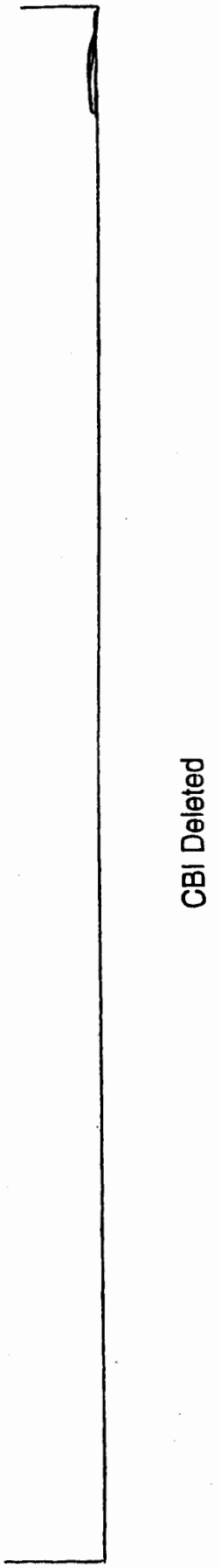
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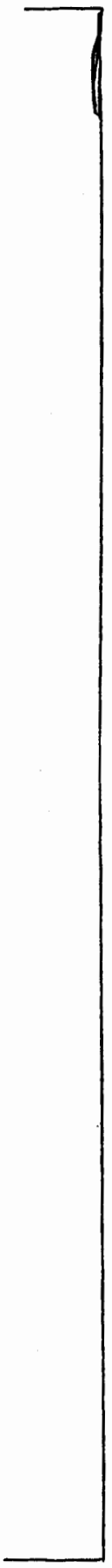
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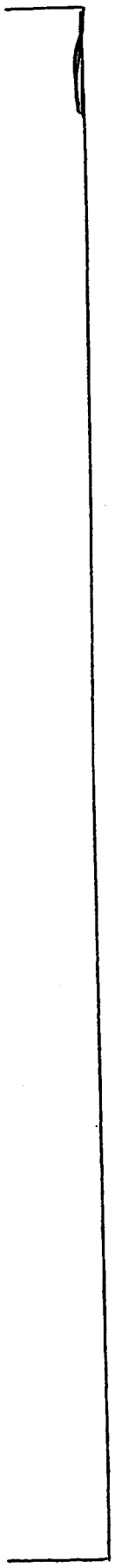

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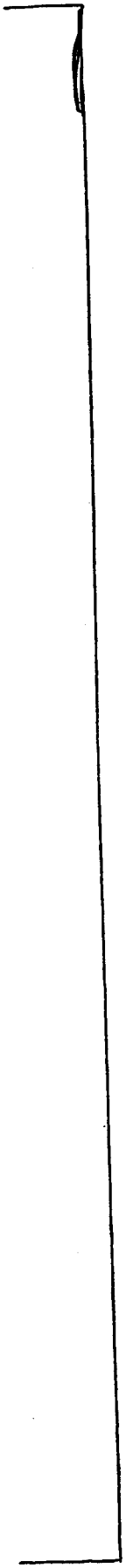
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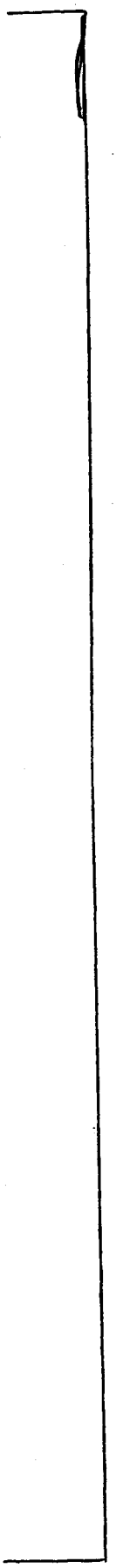

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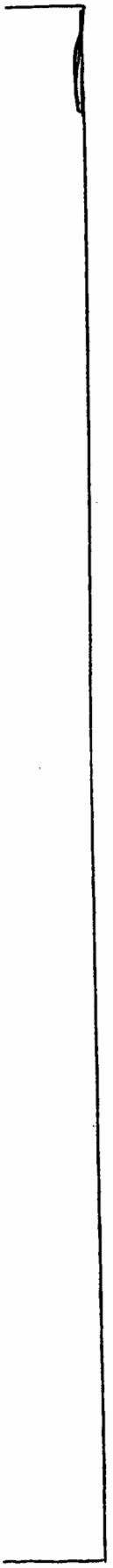

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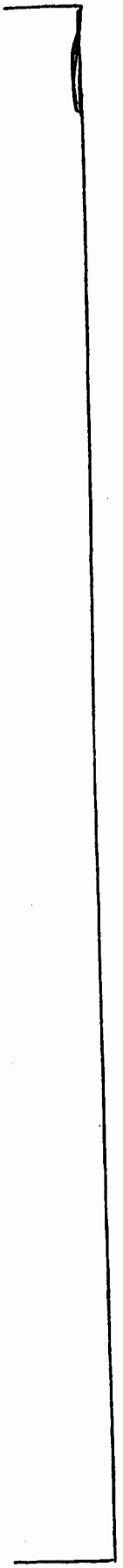
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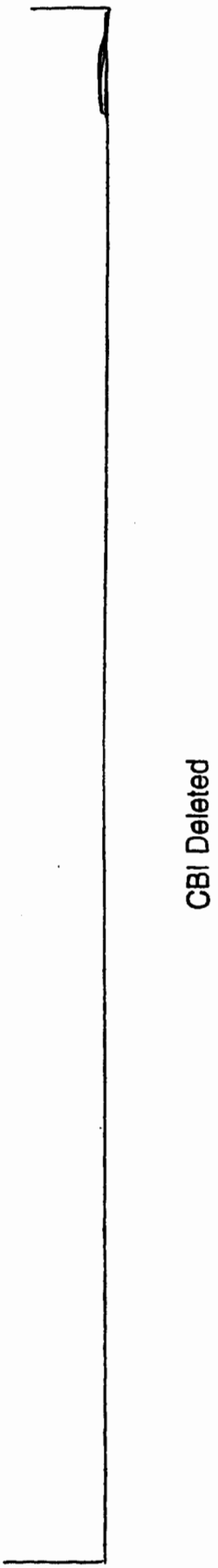
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Appendix VI

Monitoring Program to Assess the Occurrence and Fate of Glufosinate Tolerant Canola Volunteers and Weedy Relatives Following the 1995 Growing Season in Saskatchewan

by

Deschamps and Dorn

Monitoring Program to Assess the Occurrence and Fate of Glufosinate Tolerant Canola Volunteers and Weedy Relatives Following the 1995 Growing Season in Saskatchewan

Report of 1997 Findings
July 23, 1998

Authors
Wendy Dorn and Raymond Deschamps
AgrEvo Canada Inc.
295 Henderson Drive
Regina, SK
CANADA S4N 6C2

AgrEvo Canada Report Number
AC198-16
International Reference:
A57551

Protocol Number
97AC16

CONFIDENTIALITY STATEMENT

This report is confidential. No part of the report or any information contained herein may be disclosed to any third party without the written prior authorisation of AgrEvo Canada Inc..

APPROVALS PAGE

Authors

Wendy Dorn _____ _____

(signature) (date)

Dr. Raymond Deschamps _____ _____

(signature) (date)

Product Safety Manager

Robert MacDonald _____ _____

(signature) (date)

Table of Contents

ABSTRACT

In 1996, the first year of a survey to assess the occurrence and fate of glufosinate tolerant canola volunteers and weedy relatives following the 1995 growing season in Saskatchewan was conducted in the former transgenic canola fields, in neighbouring fields, an along roadsides. The survey was repeated on the same sites in 1997 with a few modifications. No evidence was found to suggest that the weediness of transgenic volunteers is different from the non-transgenic counterparts. No evidence was found for out-crossing to weedy relatives. Proper management practices are the key to controlling or containing volunteer canola (transgenic or otherwise) and its weedy relatives.

1.0 INTRODUCTION

In the province of Saskatchewan in 1995, glufosinate tolerant canola (*Brassica napus* cv. "Innovator") developed by AgrEvo Canada Inc. was grown on an estimated 8000 ha under an "Identity Preserved" program administered by the grain company marketing this canola variety.

In response to concerns regarding the potential of glufosinate tolerant canola to become a problem weed or the glufosinate tolerance trait to transfer to related weed species, AgrEvo Canada Inc. had the unique opportunity to collect data under a multi-site production scale scenario. Beginning in 1996, AgrEvo undertook a survey of 1995 glufosinate tolerant canola production fields, adjacent non-agricultural areas, and transportation routes to document the occurrence and fate of *Brassica napus* volunteers and related species (Deschamps, 1997). This survey was continued in 1997. The results of the second year of the survey are summarised in this report.

2.0. OBJECTIVES

The overall objectives for the survey were:

1. To assess the occurrence and fate of glufosinate tolerant *Brassica napus* canola volunteers.
2. To assess the occurrence and fate of weed species related to *Brassica napus* canola. Of primary interest were weedy relatives that may have received the glufosinate tolerance trait through outcrossing.

The specific objective for 1997 was to obtain a second year of data from the sites surveyed in 1996.

3.0. METHODS

The methods employed for the 1997 survey were identical to those used in 1996 except where noted.

3.1. Overview.

The project was separated into 3 specific modules focusing on:

1) cultivated areas including the fields in which Innovator canola was grown in 1995 and adjacent fields,
2) non-agricultural areas in the vicinity of 1995 Innovator fields such as shelterbelts, fence lines, sloughs, or abandoned farmyards,
3) storage areas and transportation routes.

The first module was conducted at specified intervals over the growing season (detailed below). Modules 2 and 3 were conducted later in the growing season when seed bearing plants could be identified. The rationale behind delaying the assessments for Modules 2 and 3 was that from an ecological point of view, the only individuals of concern were those that successfully reproduced resulting in the potential for re-establishment of the population in the next season.

3.2. Module 1: Cultivated areas.

The approach chosen to assess the occurrence and fate of glufosinate tolerant *Brassica napus* canola and related weedy species was to systematically scout and sample 1995 Innovator fields and adjacent fields throughout the 1997growing season.

In 1996, ten sites in East-Central Saskatchewan where glufosinate tolerant canola (Innovator) was grown in 1995 were selected (Table1). To provide a comparison, five additional sites in the same region where non-transgenic canola was produced in 1995 were surveyed in a similar manner. These same 15 sites were re-surveyed in 1997. An overview of the crops planted at each site in 1996 and 1997 is provided in Table 1.

The specific survey methods were similar to those used to obtain the annual weed surveys published in the province of Saskatchewan (Thomas et al., 1996). The surveyor walked a predefined "W" pattern in the field and took weed counts from 20 quadrats (0.5 m by 0.5 m) spaced 50 paces apart. The number of quadrats counted was increased from 10 in 1996 to 20 in 1997 to compensate for an anticipated reduction in the population of canola volunteers 2 years following the canola crop.

Assessments were taken at the following times:
1) early spring prior to cultivation and seeding (early May),
2) following crop emergence but prior to application of any herbicide (early June),
3) following herbicide application (July), and
4) after harvest just prior to freeze-up (mid-October).

Detection of plants in the test field possessing the glufosinate tolerance trait was incorporated into the first assessment. To determine whether a volunteer canola plant or weedy relative had the glufosinate tolerance trait, every second quadrant was marked with flags and treated with glufosinate ammonium herbicide at 1 kg/ha. The field was re-visited 48 hours to one week later and survivors were documented.

3.3. Module 2: Non-agricultural areas in the vicinity of 1995 Innovator fields.

Appropriate non-agricultural areas in the vicinity of 1995 Innovator fields were identified during the site selection process. These areas included roadways, fence lines, sloughs, grassed areas, and building sites (Table 1). Late in the growing season when *Brassica* and related species are in the reproductive stage, the non-crop areas were surveyed for their presence. The survey area was not extended more than 20 m from the margin of the field, as it is unlikely for seed to be spread a greater distance by wind or harvest operations. If any were discovered within the sample area, green tissue from the plant(s) was sampled and tested for the presence of the PAT enzyme by enzyme-linked immunosorbent assay using a commercially available kit similar to the assay reported by Bauer-Weston et al. (1996).

3.4. Module 3: Storage areas and transportation routes.

This survey was conducted late in the growing season for the same reasons as stated for Module 2 above. Each transportation route was surveyed over a distance of 10 km measured from each location in the survey where Innovator was grown or stored. A segment of 100 m out of each kilometre was walked and individual plants of *Brassica* or related weed species found along the roadside or adjacent ditch was counted. Green tissue samples were collected from each individual counted for analysis by enzyme-linked immunosorbent assay to determine whether the plant possessed the glufosinate tolerance trait.

4.0. RESULTS AND DISCUSSION

4.1. Module 1 Cultivated areas: 1997 findings.

As expected, the density of volunteer canola plants counted in the first survey of 1997 (Tables 2 and 3) was much lower than seen in 1996. It was somewhat surprising to find no volunteer canola in the first 1997

survey of non-transgenic sites (Table 3). Volunteers were present by the time the second survey was conducted following crop emergence. As seen in 1996, the weed control program employed by the producer was effective in controlling volunteer canola (refer to survey #3). With the exception of *Thlaspi arvense* (THLAR), levels of related weed species in the test fields were negligible (Tables 2 and 3). There appeared to be no differences in densities of volunteer canola or of related weed species between Innovator and non-transgenic sites.

The glufosinate treatment applied at 1 kg/ha to every second quadrat within the test field at the time of the first survey revealed that the majority of canola volunteers in 1997 were still glufosinate tolerant Innovator (Table 4). However, finding *Thlaspi arvense* individuals that survived the glufosinate spray treatment was unexpected. Tissue samples were not taken to test for the PAT enzyme but it is extremely unlikely that these plants were glufosinate tolerant. *Thlaspi arvense* is not listed among those species that will successfully hybridise with canola (Scheffler and Dale, 1994; Sindel, 1997). The genus *Thlaspi* is not among the 51 genera of species most closely allied to *Brassica napus* in the tribe Brassiceae (Warwick and Black, 1993). Spring-germinating *Thlaspi arvense* is normally controlled by 0.4 kg/ha glufosinate ammonium. Survival of these individuals may be attributed to a spray error or to interception of the intended dose by a shading plant.

In the fields adjacent to the test fields, low numbers of volunteer canola and weedy relatives were found in 1997 (Table 5). Two notable exceptions to that were Innovator fields 1.2 and 3.1 where canola was the crop in 1996 (Table 1). High numbers of volunteers in a field the year following a canola crop are typical. The low numbers of individuals reported for surveys #3 and #4 once again indicate that these species are easily controlled.

4.2. Module 2: Non-agricultural areas in the vicinity of Innovator fields.

Unlike cultivated areas, adjacent non-agricultural areas are often unmanaged and therefore represent an opportunity for Innovator escapes or weedy relatives possessing the glufosinate tolerance trait through outcrossing, if present, to establish and perpetuate the population. Canola seed can be disseminated to these areas by a variety of mechanisms. Canola windrows can actually be blown across or off of a field in a strong wind. During harvest, the small fraction of seed that is not collected in the grain tank but is blown out the back of the harvester with the canola straw and chaff can be spread into non-crop areas.

Canola volunteers growing in non-crop areas in the vicinity of fields where Innovator was grown in 1995 were even more rare in 1997 than in 1996 as expected (Table 6). At one site out of three (Innovator Site 8), those found were shown to be glufosinate tolerant. These individuals were growing in a narrow shallow ditch separating the test field from an adjacent field. Interestingly, canola was the crop planted in the adjacent field in both 1996 and 1997. The practice of following one canola crop with another in the next season is not recommended because of the potential for significant disease problems in the second crop. It is unclear whether the individuals counted in 1997 were progeny of the volunteers present in this same area in 1996 or new introductions (e.g. from harvest operations of the adjacent canola field in 1996). It is clear that canola (standard or transgenic) can establish in these unmanaged non-agricultural areas.

4.3. Module 3: Storage areas and transportation routes.

Additional areas where volunteer glufosinate tolerant canola or related weed species may establish include areas around storage bins and along transportation routes. Canola or weed seeds from grain transport trucks represents a mechanism for long range dispersal.

As was the case in 1996, a few canola volunteers were found growing along roadsides leading away from canola production fields in 1997 (Table 7). Once again, none were glufosinate tolerant.

In 1996, we observed that none of the volunteer canola found growing in ditches was at the reproductive stage even very late in the season. This was the case in 1997 also. The vegetation in the ditches along municipal roads in Saskatchewan are either cut for hay or simply mowed at least once each growing season. Therefore, these ditches do not represent a

habitat where volunteer canola or its weedy relatives would be expected to complete their life cycle and bear seed.

5.0. CONCLUSIONS

After two years of monitoring the occurrence and fate of glufosinate tolerant canola volunteers and weedy relatives following the 1995 growing season in Saskatchewan, the following conclusions can be made:

(1) no evidence was found to suggest that glufosinate tolerant canola behaves differently as a volunteer than does standard non-transgenic canola,
(2) spontaneous out-crossing to weedy relatives does not appear to be a factor, and
(3) proper management practices are the key to controlling or containing volunteer canola (transgenic or otherwise) and its weedy relatives.

The final conclusion warrants elaboration. By using weed management techniques familiar to Saskatchewan producers such as herbicides, cultivation, and mowing, these species of the family Cruciferae are easily controlled. Our monitoring program has shown that glufosinate tolerant canola, like standard canola, is readily removed from subsequent crops by herbicides familiar to and commonly used by farmers. The same can be said for the related weed species. The mowing of ditches along municipal roads effectively prevents volunteer canola escapes from producing seed and establishing feral populations. Wherever sound vegetation management practices are employed, the potential for volunteer canola (herbicide tolerant or traditional) to become an environmental problem is limited.

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Table 1: Survey site description. The Test Field was planted to canola (glufosinate tolerant or non-transgenic in 1995.

Site	Year	Test Field	Adjacent Fields			Non-Crop Areas
			#1	#2	#3	
Innovator 1	1996	wheat	barley	canola	flax	Roadway, Fence line, Slough
	1997	durum	flax	durum	barley	
2	1996	barley	wheat	n.a.	n.a.	Roadway, Fence line
	1997	flax	canola	n.a.	n.a.	
3	1996	wheat	canola	n.a.	n.a.	Roadway, Grassy area
	1997	wheat	peas	n.a.	n.a.	
4	1996	wheat	canola	n.a.	n.a.	Roadway, Grassy area
	1997	wheat	peas	n.a.	n.a.	
5	1996	wheat	w. wheat	n.a.	n.a.	Roadway, Fence line, Slough
	1997	oats	w. wheat	n.a.	n.a.	
6	1996	wheat	flax	n.a.	n.a.	Roadway, Fence line, Slough
	199	canola[b]	-	-	-	-

7	1996 1997	fallow flax	n.a n.a	n.a. n.a.	n.a. n.a.	Roadway, Slough
8	1996 1997	wheat wheat	canola canola	wheat barley	n.a. n.a.	Roadway
9	1996 1997	wheat wheat	n.a canola	n.a. n.a.	n.a. n.a.	Roadway, Slough
10	1996 1997	wheat fallow	n.a n.a	n.a. n.a.	n.a. n.a.	Roadway, Slough, Fence line

[a]n.a. = not applicable. Field was separated from other cropland by a major barrier such as a ravine, grid road, surface water, or woodlot.
[b]Since canola was planted in the Test Field, this site was not surveyed in 1997.

Table 1(continued): Survey site description. The test field was planted to canola (glufosinate tolerant or non-transgenic in 1995.

Site	Year	Test Field	Adjacent Fields			Non-Crop Areas
			#1	#2	#3	
Non-transgenic 1	1996 1997	barley durum	fallow flax	n.a. durum	n.a. barley	Roadway, Slough, Fence line

2	1996 1997	barley wheat	barley wheat	n.a. n.a.	n.a. n.a.	Roadway, Slough, Fence line
3	1996 1997	wheat fallow	fallow n.a.	n.a. n.a.	n.a. n.a.	Roadway, Fence line, Slough
4	1996 1997	wheat barley	oats canola	n.a. n.a.	n.a. n.a.	Roadway, Fence line, Slough
5	1996 1997	wheat barley	canola barley	n.a.	n.a.	Roadway, Fence line

Table 2: Occurrence of *Brassica napus* (BRANA), *Brassica rapa* (BRARA), *Sinapis arvensis* (SINAR), and *Thlaspi arvense* (THLAR) over the 1997 growing season in fields where glufosinate resistant canola variety "Innovator" was grown in 1995.

Species	Number of Plants / m²				Species	Number of Plants / m²			
BRANA	Survey #1[a]	Survey #2	Survey #3	Survey #4	BRARA	Survey #1	Survey #2	Survey #3	Survey #4
Site 1	11	29	1	0	Site 1	0	0	0	0
2	15	18	0	0	2	0	0	0	0
3	13	28	5	0	3	0	0	0	0
4	n.a.[b]	2	0	0	4	n.a.	0	0	0
5	n.a.	0	1	0	5	n.a.	0	0	0
7	2	2	2	0	7	0	0	0	0
8	19	34	6	1	8	0	0	0	0
9	n.a.	5	0	0	9	n.a.	0	0	0
10	6	2	0	0	10	0	0	0	0
Mean (n)	11 (6)	13 (9)	1.7 (9)	0.1 (9)	Mean	0	0	0	0
SINAR	Survey #1[a]	Survey #2	Survey #3	Survey #4	THLAR	Survey #1	Survey #2	Survey #3	Survey #4
Site 1	0	1	0	0	Site 1	2	11	0	0
2	0	0	3	0	2	10	16	0	0
3	0	0	0	0	3	1	73	2	0
4	n.a.	0	0	0	4	n.a.	39	0	15
5	n.a.	0	0	0	5	n.a.	0	0	0
7	0	0	1	0	7	30	1	0	0
8	0	8	1	1	8	18	13	0	0
9	n.a.	1	0	0	9	n.a.	14	0	0
10	0	0	0	0	10	5	0	0	0
Mean	0	1.1 (9)	0.6 (9)	0.1	Mean	11 (6)	19(9)	0.2 (9)	1.7(9)

[a]Timing of surveys was as follows: #1 - prior to spring cultivation for seedbed preparation (early May); #2 - following emergence of crop but prior to postemergent herbicide application (early June); #3 - following postemergent herbicide application (July); #4 - following combining (October).
[b]n.a. = not applicable. No information due to early seeding.

Table 3: Occurrence of *Brassica napus* (BRANA), *Brassica rapa* (BRARA), *Sinapis arvensis* (SINAR), and *Thlaspi arvense* (THLAR) over the 1997 growing season in fields where standard, non-transgenic canola varieties were grown in 1995.

Species	Number of Plants / m²			
BRANA	Survey #1[a]	Survey #2	Survey #3	Survey #4
Site 1	n.a.[b]	12	4	0
2	n.a.	n.a.	18	0
3	0	0	5	0
4	0	5	1	0
5	0	124	0	0
Mean	0	35	5.6	0

Species	Number of Plants / m²			
BRARA	Survey #1	Survey #2	Survey #3	Survey #4
Site 1	n.a.	0	0	0
2	n.a.	n.a.	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
Mean	0	0	0	0

SINAR	Survey #1	Survey #2	Survey #3	Survey #4
Site 1	n.a.	0	0	0
2	n.a.	n.a.	1	0
3	1	1	1	0
4	2	0	0	0
5	1	0	1	0
Mean	1.3	0.2	0.6	0

THLAR	Survey #1	Survey #2	Survey #3	Survey #4
Site 1	n.a.	0	0	0
2	n.a.	n.a.	0	0
3	0	3	3	0
4	3	5	0	0
5	3	0	0	0
Mean	2	2	0.6	0

[a]Timing of surveys was as follows: #1 - prior to spring cultivation for seedbed preparation (early May); #2 - following emergence of crop but prior to postemergent herbicide application (early June); #3 - following postemergent herbicide application (July); #4 - following combining (October).
[b]n.a = not applicable. No information due to early seeding.

Table 4. Number of *Brassica napus* (BRANA) and *Thlaspi arvense* (THLAR) that survived the application of glufosinate ammonium following survey #1 over the 1997 growing season in 1995 Innovator fields. Values represent the total number of individuals in 10 sprayed quadrats.

	BRANA		THLAR	
Site	Sprayed	Survivors	Sprayed	Survivors
Innovator #2	11	8	3	1
#3	5	3	1	1
#7	0	0	13	3
#10	5	3	0	0
Mean	5	3.5	4.2	1.2

Table 5. Occurrence of *Brassica napus* (BRANA), *Sinapis arvensis* (SINAR), and *Thlaspi arvense* (THLAR) over the 1997 growing season in fields adjacent to 1995 Innovator fields. Values represent total individuals counted in 20 quadrats.

	BRANA				SINAR				THLAR			
Site	#1[a]	#2	#3	#4	#1	#2	#3	#4	#1	#2	#3	#4
Innovator Sites												
1.1	7	3	1	0	1	2	3	0	42	0	0	0
1.2	1220	267	0	4	0	0	0	6	2	4	0	0
1.3	n.a.[b]	3	0	0	n.a.	0	0	0	n.a.	134	17	0
2.1	seeded to canola				0	0	0	0	0	0	0	0
3.1	n.a.	311	0	0	n.a.	3	0	0	n.a.	0	0	0
4.1	n.a.	6	0	0	n.a.	4	0	0	n.a.	1	0	0
5.1	n.a.	2	5	0	n.a.	0	0	0	n.a.	0	2	0
8.1	seeded to canola				0	0	0	0	0	0	0	0
8.2	2	19	2	0	0	0	0	0	23	10	0	0
9.1	seeded to canola				0	0	0	0	0	0	0	0
Mean	**161**	**87**	**1**	**0.5**	**0.1**	**0.9**	**0.3**	**0.6**	**11**	**15**	**1.9**	**0**
Non-transgenic Sites												
1.1	0	0	0	0	4	0	0	0	3	3	2	0
2.1	0	n.a.	1	0	0	n.a.	0	0	2	n.a.	2	0
4.1	seeded to canola				0	0	0	0	0	0	0	0
5.1	0	0	0	1	7	8	0	0	2	0	0	0
Mean	**0**	**0**	**0.3**	**0.3**	**2.7**	**2**	**0**	**0**	**1.7**	**1**	**1**	**0**

[a]Timing of surveys was as follows: #1 - prior to spring cultivation for seedbed preparation (early May); #2 - following emergence of crop but prior to postemergent herbicide application (early June); #3 - following postemergent herbicide application (July); #4 - following combining (October).
[b]n.a = not available. No information due to early seeding.

Table 6. Occurrence of canola volunteers in non-crop areas adjacent to or in the vicinity of fields where canola (Innovator or non-transgenic) was grown in 1995. Values reported represent total individuals counted in 20 quadrats. If a particular area was not present in a site, "n.a." appears in the column. Values in parentheses represent the number possessing the PATenzyme as determined by immunoassay.

Non-crop area	Fence lines	Ditches	Sloughs	Grassy areas
Innovator 1	2(0)	n.a.	n.a.	n.a.
2	0	0	n.a.	n.a.
3	n.a.	2(0)	n.a.	0
4	n.a.	0	n.a.	n.a.
5	0	n.a.	n.a.	n.a.
7	n.a.	0	0	n.a.
8	n.a.	15(15)	n.a.	n.a.
9	n.a.	1(0)	n.a.	n.a.
10	0	0	n.a.	0
Non-transgenic 1	0	0	n.a.	n.a.
2	0	0	n.a.	0
3	0	0	n.a.	0
4	0	n.a.	0	n.a.
5	0	0	n.a.	n.a.

Table 7. 1997 Survey of roadside ditches leading away from 1995 canola production fields and/or storage sites. A single 100 m segment of ditch was surveyed per kilometre for 10 kilometres. Values reported represent total individuals counted. Values in parentheses represent the number possessing the PAT enzyme as determined by immunoassay. BRANA = *Brassica napus* ; Other = *Brassica rapa, Sinapis arvensis, Thlaspi arvense.*

Site	Total BRANA Counted	Total Other Counted
Innovator 1	0	0
2	0	0
3	0	0
4	9(0)	0
5	0	0
6	0	0
7	0	0
8	0	0
9	0	0
10	0	0
Mean	0.9	0
Non-transgenic 1	0	0
2	0	0
3	0	0
4	0	0
5	0	0
Mean	0	0

Appendix VII

Confidential Business Information Justification

Aventis

CONFIDENTIAL BUSINESS INFORMATION JUSTIFICATION

The information claimed as confidential within this application may fall into two categories, namely (1) the genotype/phenotype description and (2) commercial development information. The genotype/phenotype description category includes names and information about the recipient plant, the phenotype of the regulated article, vectors, mode of transformation, gene coding regions, associated regulatory sequences and expressed traits. Commercial development information includes the names and locations of cooperators, collaborators, investigators, and contacts.

This confidential business information justification is submitted by Aventis CropScience USA LP ("Aventis USA"). Aventis USA is made up of the former AgrEvo USA Company and Rhone Poulenc Ag Company. Aventis USA is part of the worldwide Aventis CropScience Group of companies which also includes Aventis CropScience NV (the former Plant Genetics Systems N.V.) and Aventis CropScience GmbH (the former Hoechst Schering AgrEvo GmbH). All of these entities are referred to as Aventis in the statements given below.

GENOTYPE/PHENOTYPE DESCRIPTION

Central to the commercial value of Aventis' biotechnology products is the genetic information that confers the desired traits on the plant product, as well as the technical means by which the desired products have been achieved. Aventis has spent many person years in developing its expertise in the field of plant biotechnology, concurrent with the expenditure of millions of dollars on biotechnology research. In the rapidly growing and highly competitive industry of biotechnology products, Aventis has a leading edge.

Aventis has been working on the development of genetically enhanced plants, particularly those with herbicide tolerance, since the early 1980's and can document the large sums of money spent in research and testing costs. The uniqueness of Aventis' products lies in the transformation and regeneration methods and/or the combination of genetic components in the vectors transferred into the genomes of the recipient plants. The transformation and regeneration methods may be Aventis proprietary methods or available through licensing of other's proprietary methods. The genetic components in these vectors include the coding sequence for the expression of the trait(s), and regulatory sequences such as promoters, enhancers, introns, termination and polyadenylation sequences. In certain cases the recipient plant strain used is tantamount for regeneration and other desired features. Although the information on the transformation methods, recipient plant strains, or on each of these vector components may be in the public domain, the particular combination of the components put together by Aventis is unique and represents a great expenditure of time and money.

Competitors (which include Monsanto/DeKalb, Syngenta, DuPont/Pioneer, Dow Mycogen) of Aventis cannot presently duplicate Aventis' commercially valuable products without going through the painstaking process of trial and error development and testing of many different combinations of genetic information and plant strains. Access to genotype and/or phenotype description information, including the donor organisms and the recipient plant, for Aventis' products would allow competitors to create similar products that would result in a market share loss for Aventis of millions of dollars. By performing simple copy

work, these competitors would avoid the expenditure of dollars, research time and effort used by Aventis to develop its commercial products. Furthermore, the release of genotype and phenotype information would provide competitors with commercially valuable knowledge about particular products that Aventis is planning to commercialize and the likely timeframe for commercialization. Such information would be extremely useful to these companies in developing their own marketing and development strategies.

COMMERCIAL DEVELOPMENT INFORMATION

The disclosure of information about the names of cooperators, collaborators, investigators, research farm on-site personnel or contacts and the location and characteristics of the field experiments will provide Aventis' competitors with invaluable information about Aventis' marketing strategy, and could cause severe harm to Aventis' competitive standing in the industry.

In particular, release of the choice of cooperators and collaborators provides the competition with knowledge about the individuals and organizations that Aventis has found, through experience and investigation, to be most expert. Information on the location and characteristics of the field experiments will directly, or with little effort, provide the identity of the cooperators and collaborators. There is no doubt that competitors would seek to use the services of the entities found most expert by Aventis, and limit or block access of these sources. This could be accomplished by prices for services being increased, or by competitors acquiring exclusive licenses with these individuals and organizations, or by entering into contracts that would essentially tie up the time and facilities of such entities.

Maintaining the good will of the cooperators and collaborators is also a very important consideration for Aventis' success. The release of information that would directly or indirectly identify these entities could cost Aventis considerable good will and the breach of an agreement with the entity concerned. This could lead to the loss of the entity as an expert source. If Aventis is forced to use alternative cooperators and collaborators, it would take time to identify high technical performance, and it would represent a loss of the valuable expertise and understanding built-up with former entities. This, in turn, could result in a delay in bringing products to market, which would cost Aventis sums in to the millions of dollars.

Additionally, the disclosure of information about cooperators and collaborators would provide strong insights into Aventis' marketing strategy by revealing where Aventis is planning to introduce the products, and the schedule for such introduction.