

Food Safety of GM Crops in Canada: toxicity and allergenicity

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PURPOSE OF ANALYSIS

The food safety assessment process for genetically modified (GM) crops in Canada is reputed to be robust and effective. The following analysis was undertaken to test the rigor with which toxicity and allergenicity are assessed prior to the release of GM crops into commerce in Canada.

AVAILABLE GM FOOD SAFETY DATABASE

Responsibility for assessing the food safety of GM crops rests with Health Canada, which posts their Novel Food Information Decisions (hereafter called Decisions) for approved GM crops at their website (<http://www.hc-sc.gc.ca>).

Of the 42 GM crops approved for Canada, 40 Decisions are currently mounted for public appraisal (Table 1)¹.

Table 1. Current status (December 1999) of Novel Food Information Decisions (no. of crops) on the Health Canada website

	Corn	Soy	Canola	Cotton	Potato	Tomato	Squash	Flax
Mounted	15	1	10	4	4	3	2	1
Missing	0	0	1	1	0	0	0	0
TOTAL	15	1	11	5	4	3	2	1

TOXICITY ASSESSMENT

No Actual Testing. No laboratory or feeding trial measurement of toxicity is presented in 70% (28 of 40) of the available crop Decisions (Table 2). The issue of potential toxicity in all canola and cotton crops is dealt with by assuming that a) all human exposure to GM plant toxins will occur only through consumption of oil, b) toxicity risk derives solely from proteinaceous material, and because c) all proteinaceous material is removed in the process of refining the oil, therefore, d) there is no risk, and hence, no need for testing. The evidence upon which each of these assumptions is made is not presented.

¹Of those missing, 1 is in the 1994-1996 interval, and 1 is in the 1998-1999 interval. All 1997 Decisions are available.

Table 2. Health Canada-approved approaches to toxicity testing in 40 GM crops

	NO ACTUAL TESTING		TOXICITY TESTED
Approach	Assume that all potential risk is removed in the oil extraction process	Rely on heuristic ¹ (assumptions-based) reasoning (only)	Conduct <i>acute</i> oral toxicity of the refined target protein(s)(only), simulated digestion of the target protein (only), plus heuristic reasoning
GM Crops	all 9 canola and all 4 cotton	3 tomato, 1 flax, and 5 corn	1 soybean , 10 corn
% of 40	70%		30%

¹lack of amino acid and/or nucleotide homology between the target protein, e.g. CryIA(x), PAT, CP4 EPSPS, and known mammalian protein toxins; characterization of the target protein vs. that of known mammalian toxins

For crops such as canola and cotton, extraction of the oil for human consumption leaves a protein-rich byproduct which is commonly fed to livestock, which then feed into the human food chain. Health Canada takes responsibility for assessing only the directly human consumable products (e.g. the oil), and defers responsibility for assessing safety of GM crops for animal feed to either

- the *Canadian Food Inspection Agency* (CFIA) (Decisions issued through early December 1996, and from 8 July 97 onwards)² or
- to *Agriculture and Agri-Food Canada* (Decisions issued between 19 December 96 and 3 April 97, inclusive)

Safety of GM Canola and Cotton Byproducts for Livestock. Given the Health Canada assumption that any GM toxins would be confined to proteinaceous byproducts, one might expect to see more rigorous testing of toxicity concentrated on the protein-rich livestock feed fraction.

- The Decision Documents posted by the CFIA (http://www.cfia-acia.agr.ca/english/plant/pbo/home_e.html) for GM canola (11) and cotton (3) report the same

²Only 32 of the 42 GM crops approved in Canada appear to be represented by Decision Documents on the CFIA website (). They are not in the same order as those on the Health Canada website, nor are they labelled with the same names. For example,

- Health Canada’s *Novel Food Information* document FD/OFB-096-100-C “Insect Resistant Cotton Lines 531 and 757” conforms to part of
- the CFIA *Decision Document 96-14*: “Determination of Environmental Safety of Bollgard Insect Resistant Cotton”, which authorizes the release of lines 531, 757 **and 1076**.

heuristic reasoning previously demonstrated in the context of human food safety, e.g. no homology to known toxins or allergens, rate of target protein inactivation in simulated digestion etc. No actual testing of toxicity (or allergenicity) is reported.

- Under *Section V. Nutritional Assessment Criteria as Livestock Feed* of the same CFIA Decision Documents, assessments of anti-nutritional factors and nutritional composition are reported. However, with two exceptions³, no actual lab or feeding trial assessment of toxicity (or allergenicity) to livestock is referenced. Very similar approaches are found in the Decision Documents for the other crops in the “no actual testing” category (Table 2).

For the canola and cotton crops, as well as for other crops, heuristic (assumptions based) reasoning is widely used in place of actual experimentation. In virtually every case, it is reasoned that because the target proteins synthesized in response to the transgene insertions, e.g. CryIA(x) or specific enzymes as PAT, either do not share characteristics commonly found in mammalian protein toxins, or do not show either amino acid and/or nucleotide homologies with known mammalian toxins, then the crops themselves are presumed to have no toxicity risk. And thus, there is no need for testing. The assumptions and logic of this position are disputed later in the paper.

In sum, 70% of the currently available GM crops, including all of the canola and cotton crops approved for commerce in Canada, have not been subjected to any actual lab or animal toxicity testing, either as refined oils for direct human consumption or indirectly as feedstuffs for livestock. The same finding pertains to all 3 GM tomato Decisions, the only GM flax, and to five GM corn crops (Pioneer Hi-Bred Imidazolinone Tolerant Corn(34171R); BASF Sethoxydim Tolerant Corn; Zeneca Imazethapyr Tolerant Corn EXP1910IT; PGS Novel Hybridization System for Corn; and Pioneer Hi-Bred’s Imidazolinone Tolerant (IG) Corn) (Appendix Table 1).

Toxicity Tested. For 12 of the 40 crops, including the only GM soybean approved for use in Canada as well as ten of the 15 approved GM corn crops and 1 of the 4 approved GM potato crops, some actual testing is reported, as well as the usual heuristic reasoning. In each case, all testing is limited to the purified target protein(s) only. For these 12 crops, the toxicity of a purified protein is assessed by some or all of the following:

- a. Protein characterization
- b. Digestive fate in simulated gastric and intestinal fluids
- c. Acute oral toxicity
- d. Amino acid and/or nucleotide homology.

³Decision Document 96-14 (<http://www.cfia-acia.agr.ca/english/plant/pbo/dd9614e.html>) pertains to Bollgard cotton lines 531, 757, and 1076. “A four week rat feeding trial showed no difference in weight gain of animals fed diets containing 10% raw cottonseed meal from line 531 vs. C312” (the parental control line). No comment on the other two lines, or any evidence of how the trial was conducted.

Decision Document 95-05 (<http://www.cfia-acia.agr.ca/english/plant/pbo/dd9505e.html>) pertains to glyphosate tolerant soybean (GTS 40-3-2), which was subjected to feeding trials with dairy cattle (raw soybeans for 29 days) and with broilers (soybean meal for 6 weeks), with “equivalent performance” to the control variety

Features identified through these measurements were compared with those of known protein toxins, with the invariable finding of “no risk”. Details of the mouse or rat feeding trials, such as number of animals and rate and duration of exposure, are sketchy to nonexistent, and vary widely among Decisions. None of the “feeding trials” involved whole grain or processed meal, with the Bollgard cotton and glyphosate tolerant soybean exceptions noted above, for which reported parameters are “weight gain” and “performance”, respectively. No effort was reported to test risk of *chronic* exposure, reflecting the risk to humans routinely consuming GM crops over time, as through longterm feeding trials.

ALLERGENICITY ASSESSMENT

No measure of allergenicity was provided for any of the 40 available crops. All inferences of safety were based on the same type of heuristic reasoning as that used for toxicity testing.

DEFICIENCIES OF THE GM FOOD SAFETY ASSESSMENT PROCESS

The process of toxicity and allergenicity testing of GM foods in Canada may be criticized on several grounds:

1. Food safety assessment is largely an assumptions-based process. Most or all of the conclusions of food safety for individual GM crops are based on inferences and assumptions, rather than on actual testing. Evidence is needed to substantiate and validate these assumptions.
2. Many of the assumptions are, in turn, based on other assumptions which do not appear to have been validated.
 - a. Whether heuristic reasoning or actual measurement, restricting consideration to only the target protein(s) assumes that the only factor which distinguishes transgenic from conventional crops is the target protein(s) coded for by the transgenes themselves. Theoretical as well as empirical evidence suggests that this is an unsound assumption, particularly for transgenes that act at the level of secondary metabolism (e.g. glyphosate).
 - b. The logic is internally inconsistent for assessment of the potential toxicity (and allergenicity) of canola and cotton. If such toxins or allergens exist, and are in fact present in the protein-rich residue left after refining the oils, why is toxicity assessment not more rigorous prior to feeding the protein-rich meal to livestock? Where are the trials showing lack of harm to fed livestock, or that meat and milk from livestock fed on GM feedstuffs are safe?
3. Lab animal feeding trials were reported for just 30% of the approved GM crops, and were limited to acute exposures to single purified proteins. Insufficient information is given on which to assess the statistical rigor of the lab animal feeding trials, although none appear to have been published in the refereed literature.

4. Feeding trials with whole grain or processed oil or meal - the forms which would more realistically test for both the expected and unexpected toxins or allergens - are not conducted, with the partial exception of Bollgard cotton and glyphosate tolerant soybean. Insufficient information is given on which to assess the statistical rigor of the feeding trials, although neither appears to have been published in the refereed literature. In the absence of systematic testing with whole grain products, no conclusions can be drawn regarding the presence or absence of unintended secondary metabolites with potential human health effects.

5. No feeding trials are reported dealing with longer term exposures representative of the risks expected from chronic consumption of GM foodstuffs. Conclusions derived from acute toxicity studies are not predictive of chronic risks.

6. Evidence accepted by Health Canada for approval varies inconsistently among submissions, with 10 of 15 GM corn crops and 1 of 4 potato crops presenting lab and purified protein feeding trial evidence, while the other 5 corn and 3 potato crops did not. Actual livestock feeding trial evidence, however limited, is provided for only 1 of 5 cotton crops and for one soybean, but not for other crops destined largely for livestock feed. Doses, durations, and all other aspects of experimental design appear to vary at the discretion of the industry sponsor, rather than under the direction of the regulatory bodies. Inconsistent standards detract from the perception of a meaningful, enforceable risk assessment process.

CONCLUSIONS

Genetically modified foods may be safe for human consumption. However, critical analysis of the evidence publicly available on the Health Canada website for GM crops does not support this conclusion for risks of either toxicity nor allergenicity. The presented evidence demonstrates that when tested, the purified proteins coded for by transgenic insertions do not cause acute toxicity, and are expected to be neither toxic or allergenic in and of themselves. However, in the absence of long term whole food feeding trials and other more integrative (less narrowly targeted) risk assessment studies, extrapolating the safety of single purified proteins to entire crops, or results of acute testing to chronic risk, is unwarranted. The analysis presented in this report supports the need for a fundamental reassessment of the process by which the safety of GM food is tested in Canada.

Appendix Table 1. Health Canada “Novel Food Information Decisions” for 40 of 42 GE crops approved for use in Canada (2 are not yet available on the website) between 1994 and present. Numbers are from 1-22 (94-96), 23-34 (97), and 35-42 (98/99, through May 99)

Chron-ological No.	Proprietor - Event	Toxicity	Allergenicity
1	Pioneer Hi-Bred Imidazolinone tolerant Corn (34171R)	Not measured; “The mutation within the ALS enzyme is not judged to add any potential for human toxicity”	Not measured; “not possess characteristics typical of known rotein allergens...no regions of homology...unlikely to be allergenic”
2	Plant Genetic Systems Canola lines MS1, RF1 or hybrids derived therefrom, tolerant to glufosinate ammonium	Neither parameter is measured “since only processed oil...there are no additional toxicity or allergenicity concerns”	
3.	Monsanto Glyphosate tolerant Canola , GT73	Neither parameter is measured, as per #2	
4	Calgene Flavr Savr Tomato	Neither parameter is measured; “is not judged to have any potential for additional human toxicity or allergenicity”	
5	Pioneer Hi-Bred Imidazolinon tolerant Canola (NS738, NS1471, and NS1473)	Neither parameter is measured, as per #2	
7	(as #2, but for RF2)	Neither parameter is measured, as per #2	
8	Monsanto CPB resistant Potato lines BT06, BT10, BT12, BT16, BT17, BT18, and BT23	Acute oral toxicity studies with purified CryIIIA protein...no reveal any deleterious effects when mice were administered a dose of 5220 mg/kg body weight”; “amino acid sequence of inserted CryIIIA protein ... not show homologies with known mammalian protein toxins”	Not tested; “no regions of homology..compared to amino acid sequences of known protein allergens...rapidly degraded...the CryIIIA protein is extremely unlikely to be allergenic”
9	DNA Plant Technology Corp Delayed Ripening Tomato Line 1345-4	Neither parameter is measured; “not judged to have any potential for additional toxicity or allergenicity”	
10	CIBA-Geigy (now Novartis) Corn Line 176	“ Direct toxicity studies conducted using CryIA(b) and PAT test material did not reveal any deleterious effects” (no info on the actual studies); aa sequence of CryIA(b) protein closely related to the sequence of same	not measured; CryIA(b) protein and PAT enzyme do not possess characteristics typical of known protein allergens; no regions of homology with known protein allergens

Chronological No.	Proprietor - Event	Toxicity	Allergenicity
		proteins in Bt - used for 30 years; aa for CryIA(b) and PAT not show homologies with known mammalian protein toxins	
11	Monsanto Soybean lines GTS 40-3-2, glyphosate-tolerant	aa sequence - not show homology to known mammalian protein toxins; “acute oral toxicity studies with purified CP4 EPSPS not reveal any deleterious effects when mice were administered a dose of 572 mg/kg body weight” (1200X greater than highest potential from CP4 EPSPS soybean)	not measured; CP4 EPSPS enzyme not possess characteristics typical of known protein allergens; no regions of homology with known protein allergens
12	Monsanto - Insect Resistant Cotton Lines 531 and 757	Neither parameter is measured, as per #2	
13	Calgene High Lauric Acid Canola Lines 23-198, 23-18-17	Neither parameter is measured, as per #2	
14	Zeneca Suppressed Polygalacturonase Activity Tomato Hybrids 1401F, H282F, 11013F, 7913F	Neither parameter measured; “The reduced synthesis of native PG arising...truncated PG gene sequence is not judged to have any potential for additional human toxicity or allergenicity”	
15	Northrup King (now Novartis) Insect Resistant and Herbicide Tolerant Corn Line BT11	“Mammalian toxicity studies conducted using CryIA(b) and PAT..did not reveal any deleterious effects.”(no info on the actual studies); aa sequence..closely related to ...same proteins ..presentn in..B.t. used for over 30 years” aa sequence for CryIA(b) and PAT not show homologies with known mammalian protein toxins	Not measured; CryIA(b) and PAT not possess characteristics typical of known protein allergens; no regions of homology to known protein allergens
16	Pioneer hi-Bred High Oleic Acid/Low Linolenic Acid Canola Lines 45A37, 46A40	Neither parameter measured, as per #2	
17	Calgene Bromoxynil Tolerant Cotton Line BXN	Neither parameter measured, as per #2	
18	Monsanto Insect Resistant Cotton Lines 531 and 757	Neither parameter measured, as per #2	
19	Monsanto CPB Resistant	Not measured; “amino acid sequence	Not measured; “not possess

Chronological No.	Proprietor - Event	Toxicity	Allergenicity
	Potato Lines ATBT04-6, ATBT04-27, ATBT04-30, ATBT04-31, ATBT04-36, SPBT02-5, SPBT02-7	of CryIIIa protein...is closely related to the sequence of the same proteins...used for 30 years...not show homologies with known mammalian protein toxins”	characteristics typical of known protein allergens; no regions of homology...rapidly degraded”
20	DeKalb Glufosinate Resistant Maize DLL25	“compared to ..known protein toxins..no homology to known protein toxins” “protein rapidly degraded...simulate mammalian digestion” “acute mouse toxicity performed” (no details)	Not measured; “extremely unlikely to be an allergen” “compared to known allergens...not share homology” not possess “characteristics of known food allergens”
21	Monsanto Glyphosate Tolerant Cotton (Line 1445) (transformed with EPSPS, nNPTII) marker, and aad marker	Neither parameter measured; as per #2	
22	Pioneer Hi-Bred Insect Resistant Maize , MON 809	“should pose no risks...” “introduced proteins compared..to known protein toxins, neither shows any significant amino acid..similarity” “rapidly broken down.. under simulate mammalian digestion”. “neither protein was toxic when fed to mice” (no details)	Not measured; “extremely unlikely to be allergens ...compared to known allergens....neither.... comes from an allergenic source or is structurally similar to known allergens” do not possess “characteristics of known allergens”
23	BASF Sethoxydim Tolerant Corn (DK412SR and DK404SR)	Neither parameter is measured; no comment or evidence given	
24	AgrEvo Glufosinate Ammonium Tolerant Canola (T45)	Neither parameter is measured; no comment or evidence given	
25	Monsanto Insect Resistant Corn , MON 810	“..human safety..confirmed in experiments that included protein characterization, digestive fate in simulated gastric and intestinal fluids, and acute oral toxicity in mice. ” “No..toxicity..in mice administered CryIA(b) protein by oral gavage.” “compared to databases of known protein toxins...history of safe use”	Not measured; “compared to known allergens.....the CryIA(b) protein did not show meaningful amino acid sequence homology to known allergens” “potential allergenicity assessed based upon other characteristics of known allergens (stability to digestion)
26	Plant Genetics Systems Novel Hybridization	Neither parameter is measured, as per #2	

Chronological No.	Proprietor - Event	Toxicity	Allergenicity
	Systems for Canola (Ms8/RF3)		
27	AgrEvo Glufosinate Ammonium Tolerant Corn (T14 and T25)	“nucleotide sequence of the pat gene and amino acid sequence of the PAT protein...compared with sequences available for known toxins...no significant homology” “ acute oral toxicity study ...PAT protein administered to rats at up to 50,000 ppm for 14 days”	Not measured; “nucleotide sequence...compared with sequences for known allergens...no significant homology” “potential for allergenicity assessed based upon characteristics of known food allergens”
28	DeKalb Insect-Resistant and Glufosinate-Tolerant Maize DBT418	“CryIA(c) and PAT proteins compared to databases...no homology to known mammalian protein toxins” “ acute mouse toxicity ...using microbially produced, purified CryIA(c) or PAT protein...doses 3325 mg of CryIA(c) or 2500 mg of PAT/kg body weight by oral gavage”	Not measured; “extremely unlikely to be allergens..not share homology to known allergens” “potential based on characteristics of known food allergens”
29	Zeneca Imazethapyr Tolerant Corn EXP1910IT	Not measured; “amino acid sequence...is identical except for a single amino acid substitution...”	Not measured; “Does not possess characteristics typical of known protein allergens...no homology”
30	Plant Genetics Systems Novel Hybridization System for Corn	Neither parameter is measured; no comment or evidence given	
31	Rhone-Poulenc Bromoxynil Tolerant Canola (Westar-Oxy-235)	Neither parameter is measured; no comment or evidence given	
32	Monsanto Glyphosate Tolerant Canola GT200	Neither parameter is measured, as per #2	
33	Monsanto Insect-Protected Roundup Ready Corn Line MON 802	“protein characterization, digestive fate studies in simulated gastric and intestinal fluids and acute oral toxicity in mice ” “administered CryIA(b), CP4 EPSPS or GOX proteins by oral gavage.” “computerized search capabilities...not show meaningful homology” “history of safe use...”	Not measured; no comment
34	Monsanto Roundup Ready Corn Line Mon 832	“protein characterization, digestive fate studies in simulated gastric and intestinal fluids and acute oral toxicity ”	Not measured; no comment

Chronological No.	Proprietor - Event	Toxicity	Allergenicity
		<p>in mice “administered CP4 EPSPS or GOX proteins by oral gavage.” “..not show meaningful homology when compared to known allergens or protein toxins” “history of safe use..”</p>	
35	Crop Development Centre Flax Line CDC Triffid - FP967 resistant to sulfonylureas	Not measured; “amino acid sequence identical except for a single amino acid substitution...not show homologies with known mammalian protein toxins”	Not measured; “not possess characteristics typical of known protein allergens...no regions of homology”
36	Seminis Vegetable Seeds Virus Resistant Squash Line ZW-20	Not measured; “coat protein sequences...did not show homologies with known mammalian protein toxins....history of known safe consumption of these proteins from virus-infected plant products...evidence of lack of toxicity”	Not measured; “not possess characteristics typical of known protein allergens...no regions of homology...to the amino acid sequences of known protein allergens...coat proteins extremely unlikely to be allergens”
37	Seminis Vegetable Seeds Virus Resistant Squash Line CZW-3	Not measured; “coat protein sequences...did not show homologies with known mammalian protein toxins....history of known safe consumption of these proteins from virus-infected plant products...evidence of lack of toxicity”	Not measured; “not possess characteristics typical of known protein allergens...no regions of homology...to the amino acid sequences of known protein allergens...coat proteins extremely unlikely to be allergens”
38	Pioneer Hi-Bred Imidazolinone Tolerant (IT) Corn	Not measured; “no toxicity concerns are associated with the expression of the imidazolinone tolerance trait in IT corn hybrids”	Not measured; “no new protein or significantly altered protein is produced”
40	Monsanto NewLeaf-Plus Potato cultivars Russet Burbank (RBMT21-129, RBMT21-350 and RBMT22-082)	Not measured; “amino acid sequence of the CryIIIa protein...closely related to the sequence of the same proteins that are present in strains of <i>B. thuringiensis</i> ...used for over 30 years....not show homologies with known mammalian protein toxins...not judged to have any potential for human toxicity...history of known safe consumption”	Not measured; “The CryIIIa protein and PLRV replicase do not possess characteristics typical of known protein allergens...no regions of homology to amino acid sequences of known protein allergens...extremely unlikely to be allergenic”
41	Monsanto NewLeaf-Y Potato cultivars Shepody	Not measured; “amino acid sequence of the CryIIIa protein...closely related	Not measured; “The CryIIIa protein and PLRV replicase do

Chronological No.	Proprietor - Event	Toxicity	Allergenicity
	(SEMT15-02, SEMT15-15, and Russet Burbank (RBMT15-101)	to the sequence of the same proteins that are present in strains of <i>B. thuringiensis</i> ...used for over 30 years....not show homologies with known mammalian protein toxins...not judged to have any potential for human toxicity...history of known safe consumption”	not possess characteristics typical of known protein allergens...no regions of homology to amino acid sequences of known protein allergens...extremely unlikely to be allergenic”
42	Monsanto Glyphosate tolerant Corn, GA21	“amino acid sequence of the mEPSPS enzyme...is 99.3% identical to the sequence of the endogenous corn enzyme...not show homologies with known mammalian protein toxins...not judged to have any potential for human toxicity.... Safety has also been demonstrated in an acute mouse gavage study by feeding with high doses of the purified protein.	Not measured; “the mEPSPS enzyme...does not possess characteristics typical of known protein allergens...no regions of homology to amino acid sequences of known protein allergens...extremely unlikely to be allergenic”

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