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A comparative risk assessment of genetically engineered, mutagenic, and conventional wheat production systems

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Abstract

Wheat (*Triticum aestivum* L.) varieties produced using modern biotechnologies, such as genetic engineering and mutagenic techniques, have lagged behind other crop species, but are now being developed and, in the case of mutagenic wheat, commercially grown around the world. Because these wheat varieties have emerged recently, there is a unique opportunity to assess comparatively the potential environmental risks (human health, ecological, and livestock risks) associated with genetically engineered, mutagenic, and conventional wheat production systems. Replacement of traditional herbicides with glyphosate in a glyphosate-tolerant (genetically engineered) wheat system or imazamox in an imidazolinone-tolerant (mutagenic) wheat system may alter environmental risks associated with weed management. Additionally, because both systems rely on plants that express novel proteins, the proteins and plants themselves may impose risks. The purpose of our study was to examine comparatively the multiple aspects of risk associated with different wheat production systems in the US and Canada using the risk assessment paradigm. Specifically, we used tier 1 quantitative and qualitative risk assessment methods to compare specific environmental risks associated with the different wheat production systems. Both glyphosate and imazamox present lower human health and ecological risks than many other herbicides associated with conventional wheat production systems evaluated in this study. The differences in risks were most pronounced when comparing glyphosate and imazamox to herbicides currently with substantial market share. Current weight-of-evidence suggests that the transgenic CP4 EPSPS protein present in glyphosate-tolerant wheat poses negligible risk to humans, livestock, and wildlife. Risk for mutated AHAS protein in imidazolinone-tolerant wheat most likely would be low, but there are not sufficient effect and exposure data to adequately characterize risk. Environmental risks for herbicides were more amenable to quantitative assessments than for the transgenic CP4 EPSPS protein and the mutated AHAS protein.

Introduction

Modern biotechnologies, such as genetic engineering and mutagenic techniques, are rapidly creating numerous new crop varieties within many plant species. Currently, maize (*Zea mays* L.), soybean (*Glycine max* (L.) Merrill), cotton (*Gossypium hirsutum* L.), and canola (*Brassica* spp.) varieties that have been produced using modern biotechnologies are grown on millions of hectares around the world. Wheat (*Triticum aestivum* L.) varieties produced using these biotechnologies have lagged

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behind other crop species, but are now being developed in the case of genetic engineering and are being grown commercially in the case of mutagenic techniques.

Because these wheat varieties are just now emerging, there is a unique opportunity to assess comparatively the potential environmental risks (human health, ecological, and livestock risks) associated with the different biotechnology and conventional wheat production systems. Even though multi-system comparative risk assessments typically are not conducted by regulatory agencies or researchers, they clearly are warranted given that each system imposes risks to the environment.

Herbicide-tolerant wheat varieties have been produced using both genetically engineered (DNA recombination) and chemically induced DNA mutation techniques. The glyphosate-tolerant trait (Roundup Ready® wheat) was introduced into the spring wheat variety, Bobwhite, using an *Agrobacterium tumefaciens* mediated transformation system (Hu et al., 2003). The EPSPS gene, which confers tolerance to the herbicide glyphosate, was inserted into the wheat genome from *A. tumefaciens* strain CP4. In a wheat plant that does not have the inserted CP4 EPSPS gene, the glyphosate binds to the plant's native EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) enzyme and blocks the biosynthesis of aromatic amino acids, which deprives the plant of essential growth components. All plants, bacteria, and fungi have the EPSPS enzyme to allow synthesis of aromatic amino acids; therefore, transgenic glyphosate-tolerant wheat plants still contain their native EPSPS enzyme but the native enzyme is not functional when the plants are treated with glyphosate. Insertion of the CP4 EPSPS gene confers a reduced binding affinity for glyphosate and allows the plant to function normally by allowing synthesis of aromatic amino acids when the herbicide is applied (Pilacinski, 2002).

At the beginning of 2004, glyphosate-tolerant spring wheat was actively being developed for commercialization in the United States. Regulatory approvals and marketing of seed in the US were expected by the 2005 or 2006 growing season. In April 2004, Monsanto Company, the producer and registrant of glyphosate-tolerant spring wheat, announced that it was indefinitely deferring development of the product (D. Gigax, personal communication).

The development of imidazolinone-tolerant wheat (CLEARFIELD™) was accomplished by chemically induced mutagenesis of the DNA in the wheat varieties Gunner, Fidel, and Teal (CFIA, 2003, 2004). The chemicals ethylmethane sulfonate (EMS) and diethyl sulfate (DES) were used to create genetic mutations in the wheat varieties. However, imidazolinone-tolerant wheat generally is not considered a genetically modified organism because recombinant DNA technology or transgenes were not used to create herbicide resistance. The genetic mutation in wheat that is responsible for the tolerance to imidazolinone herbicides is due to a point mutation of a single nucleotide in the acetohydroxyacid synthase (AHAS) gene, resulting in a single amino acid change of serine to asparagine in the AHAS enzyme (CFIA, 2003, 2004). Imidazolinone herbicides are active against the AHAS enzyme, also known as acetolactate synthase (ALS). The AHAS enzyme is found in a wide variety of bacteria and plants. This enzyme catalyzes the biosynthesis of the essential branched chain amino acids isoleucine, leucine, and valine. The amino acid change in the mutated AHAS protein alters the binding site for imidazolinone herbicides such that they no longer inhibit the AHAS enzyme. The herbicide, imazamox (Beyond™) currently is registered for use on imidazolinone-tolerant wheat in the US (BASF, 2003). Imidazolinone-tolerant winter wheat is currently available, and spring wheat varieties are being developed (L. Talbert, personal communication).

Even though glyphosate-tolerant spring wheat currently is not being grown commercially and further development is uncertain, the public has expressed concerns about ecological, agronomic, and human health risks from the technology (Northern Plains Resource Council, 2002; Center for Food Safety, 2003). Current regulatory processes identify and assess many potential risk issues associated with genetically engineered crops before the crop has been commercialized. However, in the scientific literature most genetically engineered risk issues and potential risks have occurred after the crop has been commercialized. This situation provides a unique opportunity to present a comparative risk assessment of both genetically engineered and non-genetically engineered wheat systems before the genetically engineered wheat product is commercialized. This type of risk assessment approach can provide baselines

and appropriate comparators which may result in better management of, and communication about, new biotechnologies.

Wolt and Peterson (2000), Wolt et al. (2003), and Peterson and Hulting (2004) argued that science-based risk assessment can provide a valuable framework from which to measure, communicate, and make decisions about the environmental impacts from agricultural biotechnology. Risk assessment is a formalized basis for the objective evaluation of risk in which assumptions and uncertainties are clearly considered and presented (NRC, 1983, 1996). Human health and ecological risk can be described in quantitative terms as a function of effect and exposure (NRC, 1983; USEPA, 1999). Risk assessment typically utilizes a tiered modeling approach extending from deterministic models (tier 1) based on conservative assumptions to probabilistic models (tier 4) using refined assumptions (SETAC, 1994). In risk assessment, 'conservative assumptions' in lower-tier assessments represent overestimates of effect and exposure; therefore, the resulting quantitative risk values typically are conservative and err on the side of environmental safety.

Replacement of traditional herbicides with glyphosate in a glyphosate-tolerant wheat system or imazamox in an imidazolinone-tolerant wheat system may alter human health and ecological risks associated with weed management (Peterson & Hulting, 2004). Additionally, because both systems rely on plants that express novel proteins, the proteins and plants themselves may impose risks. Therefore, the objective of this study was to use tier 1 quantitative and qualitative risk assessment methods to compare specific environmental risks associated with genetically engineered, mutagenic, and conventional wheat production systems (specifically herbicide and protein risks) in Canada and the United States.

Materials and methods

Problem formulation and conceptual model

For our risk assessment, we developed a model to conceptualize the nature of the problem (Figure 1). The sources of the risks are the conventional, glyphosate-tolerant, and imidazolinone-tolerant wheat cropping systems. (Organic wheat cropping systems are not included in this assessment because of the paucity of data on stressors and effects. However, it should be noted that organic wheat systems pose environmental risks and a comprehensive risk assessment of wheat production systems would need to include organic systems.)

Herbicides, transgenic protein, and mutated protein are the primary stressors. However, all wheat systems do not exhibit the same stressors. For the conventional wheat system, herbicides are the only stressor (in our assessment). Therefore, the conventional wheat production system served as a baseline in our analysis. For the glyphosate-tolerant wheat system, herbicides and the transgenic protein are the stressors. For the imidazolinone-tolerant wheat system, herbicides and the mutated protein are the stressors. The primary stressors then potentially affect the systems through human health, livestock, and ecological effects (Figure 1). The effects we considered in this assessment reflect primary impacts. Therefore, we present only direct effects of the stressors on the human and ecological receptors. Indirect effects, such as the ecological consequences of improved weed control with glyphosate or imazamox, were not considered. Additionally, we did not consider economic risks or agronomic risks, such as pollen-mediated and mechanical mixing of wheat grain from different production systems, pollen-mediated gene flow to wild or weedy relatives of wheat, fallow management with herbicides, herbicide resistance in target weeds, and herbicide rotation risks to alternate crops.

Herbicide risk assessment

The herbicide active ingredients evaluated in this study included 2,4-dichlorophenoxy acetic acid (2,4-D), bromoxynil, clodinafop, clopyralid, dicamba, fenoxaprop, flucarbazone, MCPA, metsulfuron, thifensulfuron, tralkoxydim, triallate, triasulfuron, tribenuron, and trifluralin. These active ingredients were chosen because they are used on a relatively large percentage of spring wheat acres in the US and Canada. Risk associated with glyphosate and imazamox also was evaluated because of their role in glyphosate-tolerant and imidazolinone-tolerant wheat.

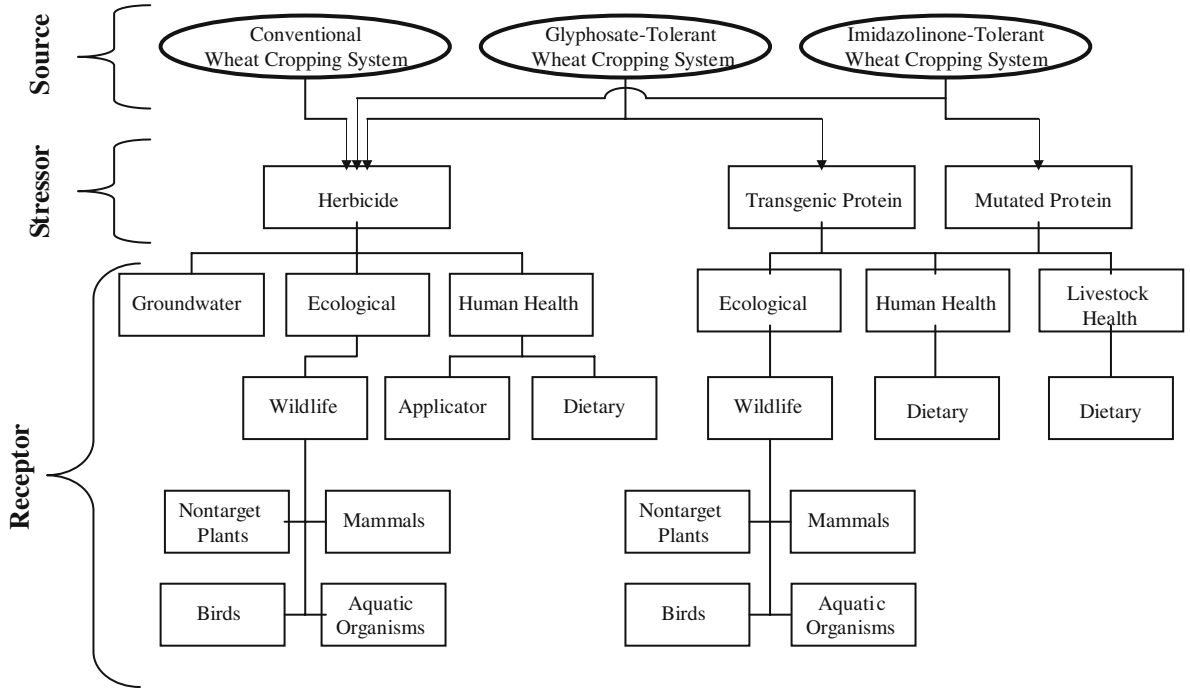

Figure 1. Conceptual model of the scope of the risk assessment.

Human dietary risk assessment

Toxicity

For all of the herbicides, the United States Environmental Protection Agency (USEPA) chronic reference dose (RfD) was used as the human toxicity endpoint. The RfD typically is the greatest estimated chronic exposure level believed to have no adverse impact on human health (Whitford et al., 1999). The chronic RfD (also termed the acceptable daily intake) is the exposure level to which humans can be exposed every day for a lifetime without experiencing adverse effects. The chronic RfD is usually calculated by dividing uncertainty factors into the most sensitive chronic no-observed-adverse-effect-level (NOAEL) observed in laboratory animal testing. For example, if the lowest NOAEL from a battery of required chronic toxicity studies was 10 mg kg^{-1} d^{-1} and a 100-fold uncertainty factor was used to account for intraspecies and interspecies uncertainty, then the chronic RfD would be 0.1 mg kg^{-1} d^{-1}. The RfD's used in this study are presented in Table 1.

Exposure

Consistent with tier 1 standardized methods, dietary exposures to the herbicides were determined by assuming that all dietary portions of wheat contained tolerance level residues of the herbicide. Many of the herbicides are registered for use on more crops than wheat, making comparisons difficult. Therefore, dietary risk calculations were standardized to only reflect risks associated with residues on wheat. The tolerance residue (also termed maximum residue limit) is the legal threshold for residues of a pesticide on a crop. For our risk assessment, we used the Theoretical Maximum Residue Contribution (TMRC) technique to determine reasonable worst-case potential exposures to each herbicide. We assumed that for each herbicide the tolerance level residue was present on wheat and 100% of the wheat crop was treated with the herbicide. Therefore, all foods derived from wheat contained the herbicide at tolerance levels, regardless of processing.

The amount of pesticide ingested was estimated as the product of the residue concentration and the average quantity of food consumed (the TMRC). Food consumption patterns were determined using the Dietary Exposure Evaluation Model (DEEM-FCID™ v. 2.03, Exponent, Washington, DC U.S.A.). The model determines dietary consumption for the U.S. population and several subpopulations by using individual food consumption

Table 1. Human dietary risk from wheat herbicides

Active Ingredient[a]	Application rate (g ai ha^{-1})	RfD[b] (mg kg^{-1} d^{-1})	Tolerance[c] (wheat, ppm)	% Chronic RfD (US Population)	% Chronic RfD (most sensitive subpopulation)	Rank	Q^*[d]	Lifetime cancer risk	RfD and Q^* Data Source
Glyphosate	840	2	5	0.4	1	11			USEPA (1993)
Bromoxynil	560	0.015	0.1	1.1	2.7	7	0.103	1.75×10^{-5}	USEPA (1998b)
2,4-D	1100	0.01	0.5	8.5	20.5	4			USEPA (2005a)
Clodinafop	67	0.00003	0.1	565.5	1336.8	1	0.129	2.19×10^{-5}	USFR (2000a)
Clopyralid	146	0.05	3	10.2	24.6	3			USFR (1995a)
Dicamba	280	0.045	2	7.5	18.2	5			USFR (1999)
Fenoxaprop	90	0.0025	0.05	3.4	8.2	6	0.091	7.72×10^{-6}	USFR (1998)
Flucarbazone	34	0.36	0.01	0.005	0.01	16			USFR (2000b)
MCPA	1457	0.0005	0.1	33.9	82	2			USEPA (2005b)
Metsulfuron	9	0.25	0.1	0.1	0.2	15			USFR (2002)
Thifensulfuron	22	0.013	0.05	0.7	1.6	9			USFR (2004)
Tralkoxydim	280	0.005	0.05	0.7	1.6	9	0.0168	5.70×10^{-7}	USFR (2003b)
Triallate	1100	0.025	0.05	0.3	0.8	13	0.0717	6.08×10^{-6}	USEPA (2001)
Triasulfuron	34	0.01	0.02	0.3	0.8	13			USFR (1995b)
Tribenuron	16	0.008	0.05	1.1	2.6	8			USFR (1996)
Trifluralin	1100	0.024	0.05	0.4	0.9	12	0.0077	6.53×10^{-7}	USEPA (1996)

[a] Imazamox was not assessed for dietary risk because it is exempt from the requirement of a tolerance in the United States (USFR, 2003a).
[b] RfD = reference dose (acceptable daily intake).
[c] Maximum allowable residue in the United States.
[d] Tumor potency factor.

records collected by the USDA Continuing Surveys for Food Intake by Individuals (CSFII) surveys for 1994–1998. Translation factors used to convert foods-as-eaten to commodities, and direct and indirect water consumption into source components, are based on an EPA/USDA FCID recipe set. For this assessment, we determined chronic food consumption patterns using mean consumption values (3 day average).

Risk characterization

For each herbicide, dietary risk was determined using the DEEM-FCID™ model. The model was run to perform a chronic exposure analysis and risk characterization for each herbicide. A TMRC (exposure) was determined for each herbicide and then risk was characterized by determining the estimated exposure as a percentage of the chronic RfD (toxic endpoint). We evaluated dietary risk to the total U.S. population and the most sensitive subgroup (Table 1).

Carcinogenicity

Cancer risk assessments for chronic dietary exposure also were determined for those herbicides that have a reported tumor potency factor (Q^*). The DEEM-FCID™ model also was used for this assessment. Lifetime excess cancer risk for the overall U.S. population was determined using the following formula:

$$\begin{aligned}&\text{Lifetime cancer risk}\\&= (\text{chronic dietary exposure, mg kg BW}^{-1}\text{d}^{-1}) *\\&\quad (Q^*, (\text{mg kg BW}^{-1}\text{d}^{-1})^{-1})\end{aligned} \tag{1}$$

Applicator risk

Another aspect of human risk associated with herbicides is risk to applicators. To determine applicator exposures to the herbicides, we used the USEPA Pesticide Handler Exposure Database (PHED, v. 1.1) (USEPA, 1998a). The PHED contains pesticide-handler scenarios derived from field studies and the exposure estimates based on physical factors such as application rate, hectares treated per day (standardized to 97), type of clothing worn, methods of application, and formulation type. For our assessment, we assumed the mixing, loading, and application of the herbicide was done by the same person. We assumed that the person was mixing and loading with single-layer clothing (long sleeve shirt and long pants or coveralls) and chemical resistant gloves (Scenario 3). We also assumed that the person was operating an open-cab groundboom applicator (Scenario 13). Further, we assumed that the applicator wore single-layer clothing and gloves. Some of the herbicides require more stringent personal protective equipment. In those cases, risks would be lower than presented here. However, to standardize risk estimates we applied the same assumptions to all herbicides.

We modeled only total dermal exposures (mg kg BW^{-1} d^{-1}) because inhalation exposures with the herbicides in our study would be negligible. To determine total dermal exposure, we first used PHED to estimate cumulative exposures in mg kg^{-1} active ingredient (ai) herbicide handled per day (unit exposure) by adding exposures to head, neck, upper and lower arms, chest, back, thigh, lower legs, and hands. Total dermal exposure for each herbicide (mg kg BW^{-1} d^{-1}) was determined by using the following formula:

$$\begin{aligned}&\text{Total dermal exposure}\\&= (\text{PHED unit exposure, mg kg ai}^{-1})\\&\quad * (\text{maximum label rate, kg ai ha}^{-1})\\&\quad * (\text{maximum ha treated d}^{-1})\\&\quad * (100\%\ \text{dermal adsorption})\\&\quad \div 70\ \text{kg body weight}\end{aligned} \tag{2}$$

We used the chronic toxicity NOAEL from which the RfD is based to provide a conservative toxicity endpoint. The most appropriate toxicity endpoint is the dermal toxicity NOAEL. However, that value is not as publicly available as the chronic toxicity NOAEL; dermal toxicity NOAEL's only could be obtained for nine of the 17 herbicides. Risk was determined by determining the exposure as a percentage of the chronic toxicity NOAEL (Table 2).

Ecological risk assessment

Peterson and Hulting (2004) presented a tier 1 ecological risk assessment of herbicides used in spring wheat in the US and evaluated the potential changes in risk associated with a glyphosate-tolerant wheat system. In the present study, we included imazamox in our analysis.

Table 2. Applicator risk from wheat herbicides

Active ingredient[a]	Aplication rate (g ai ha^{-1})	Label (REI)[b]	Total exposure (mg d^{-1})	Total exposure (mg kg BW^{-1} d^{-1})	RfD NOEL[c]	% RfD NOEL[d]	Rank
Glyphosate	840	4	6.66	0.095	175	0.054	14
Bromoxynil	560	12	4.44	0.063	1.5	4.225	6
2,4-D	1100	48	8.71	0.124	1	12.450	3
Clodinafop	67	12	0.53	0.008	0.03	25.371	2
Clopyralid	146	48	1.15	0.016	5	0.330	9
Dicamba	280	24	2.22	0.032	45	0.070	13
Fenoxaprop	90	24	0.71	0.010	0.25	4.059	7
Flucarbazone	34	12	0.27	0.004	36	0.011	15
MCPA	1457	12	11.54	0.165	0.15	109.943	1
Metsulfuron	9	4	0.07	0.001	25	0.004	16
Thifensulfuron	22	12	0.18	0.003	1.3	0.195	12
Tralkoxydim	280	12	2.22	0.032	0.5	6.343	4
Triallate	1100	12	4.03	0.058	2.5	2.304	8
Triasulfuron	34	4	0.27	0.004	1.2	0.317	10
Tribenuron	16	12	0.12	0.002	0.8	0.225	11
Trifluralin	1100	12	8.88	0.127	2.4	5.286	6

[a] Imazamox was not assessed for applicator risk because it regulated no-observed-effect-level in the United States (USFR, 2003a).
[b] Minimum re-entry interval (hours).
[c] No-observed-effect-level (NOEL) from which the reference dose (RfD) is based.
[d] Applicator risk = exposure as a percentage of the RfD NOEL.

Peterson and Hulting (2004) characterized risks to the following ecological receptors: wild mammals, birds, non-target terrestrial plants, non-target aquatic plants, aquatic vertebrates, aquatic invertebrates, and groundwater. In this study, ecological effects, exposures, and risks from direct exposure to herbicides were evaluated using the approach of Peterson and Hulting (2004). Ecological receptors and effects evaluated in the present study were aquatic vertebrates (acute risk), aquatic invertebrates (acute risk), aquatic vascular plants (acute risk), non-target terrestrial plants (seedling emergence and vegetative vigor), and groundwater exposure. Acute and chronic risks to wild mammals and birds are not presented here because the previous assessment revealed that the risks are negligible. Additionally, chronic risks to aquatic vertebrates and invertebrates and insect pollinators are not presented here because of similar reasons.

Ecological risks in this study were assessed by integrating toxicity and exposure. To do this, risks to ecological receptors were assessed using the risk quotient method (RQ). For each ecological receptor, an RQ was calculated by dividing the estimated environmental concentration (EEC) by the appropriate toxicity endpoint (e.g., the LC_{50}). The general equation used was:

$$\mathrm{RQ} = \mathrm{EEC} \div \text{toxicity endpoint} \quad (3)$$

(See Peterson and Hulting (2004) for a detailed discussion of methodology, including toxicity endpoint selection, environmental exposure estimates, and risk quotient values).

Transgenic protein risk assessment

Risks for the glyphosate-tolerant CP4 EPSPS protein were determined primarily using a qualitative weight-of-evidence approach. Effect and exposure information for humans, livestock, and wildlife (such as mammals, birds, and fish) were obtained from the scientific literature. Other information was obtained from regulatory reports and submissions.

Mutated protein risk assessment

As with the CP4 EPSPS protein, risks for the mutated AHAS protein were determined using a qualitative weight-of-evidence approach. However, effect and exposure information for the mutated AHAS protein is not available in the scientific literature. Further, because it is a mutagenic trait and not a genetically engineered trait, regulatory approvals are not required in the US. The regulatory status of imidazolinone-tolerant wheat also limits the availability of public information. In Canada, imidazolinone-tolerant wheat is regulated as a novel trait by the Canadian Food Inspection Agency (CFIA) and Health Canada. Therefore, we used the decision documents produced by these two agencies for our risk assessment (CFIA, 2003, 2004; Health Canada, 2004a, b).

Results

Herbicide risk assessment

Human dietary risk

Imazamox was the only herbicide evaluated that is exempt from having a USEPA tolerance (USFR, 2003a); therefore, there is no established chronic RfD and no dietary risk estimate. Dietary risk as a percentage of the chronic RfD for the other herbicides ranged from 0.005 to 565.5 for the U.S. population (Table 1). Children from 3 to 5 years of age represented the most sensitive U.S. subpopulation for each herbicide. Dietary risk for that subpopulation ranged from 0.01 to 1336.8% of the respective chronic RfD. Clodinafop was the only herbicide active ingredient to exceed 100% of the chronic RfD. Ten of the 16 herbicides had greater dietary risks than glyphosate. All of the herbicides had greater dietary risks than imazamox.

Cancer risk. Six active ingredients (bromoxynil, clodinafop, fenoxacarb, tralkoxydim, triallate, and trifluralin) have reported tumor potency factors. Tier 1 lifetime excess cancer risk estimates for the U.S. population ranged from 5.7×10^{-7} to 2.19×10^{-5}(Table 1).

Applicator risk

Applicator risk ranged from 0.004 to 109.9% of the chronic toxicity NOAEL (Table 2). Thirteen of the 15 herbicides had greater applicator risks than glyphosate. As with dietary risk, all herbicides had greater applicator risks than imazamox.

Another factor which provides an estimation (and comparison) of human risk is the USEPA-regulated reentry interval (REI) after treatment of a

field. Table 2 includes REI's for each herbicide and indicates that two herbicides have the same REI's as glyphosate (4 h), whereas all other herbicides have REI's which exceed glyphosate. Imazamox also has an REI of 4 h (BASF 2003).

Ecological risk

Non-target terrestrial plants. All 13 active ingredients for which data were available had seedling emergence risk quotients (RQ's) greater than glyphosate, which was not unexpected given that glyphosate was the only herbicide assessed which is practically non-toxic with respect to seedling emergence (Table 3). Seven of the 14 herbicides for which data were available had vegetative vigor RQ's greater than glyphosate (Table 3).

Aquatic organisms. Fourteen of the 15 herbicides for which toxicity data were available had vascular aquatic plant RQ's greater than glyphosate. Four of the 16 active ingredients had aquatic invertebrate RQ's greater than glyphosate. Five of the 16 active ingredients had aquatic vertebrate RQ's greater than glyphosate.

Herbicide concentrations in groundwater. Eleven of 16 herbicides had higher predicted groundwater concentrations than glyphosate. Seven herbicides with lower maximum single use rates than glyphosate had higher predicted groundwater concentrations.

Transgenic protein risk assessment

Human and livestock dietary risk assessment

Most of the information summarized here is from studies conducted on glyphosate-tolerant soybeans, glyphosate-tolerant corn, and glyphosate-tolerant canola. However, the CP4 EPSPS protein is the same in all systems that confer glyphosate resistance (Sidhu et al., 2000; Kan & Hartnell 2004; Taylor et al., 2004); therefore, data from other transgenic crops can be considered when evaluating the risks associated with CP4 EPSPS protein in glyphosate-tolerant wheat.

Homology of the CP4 EPSPS protein with native EPSPS protein. Lee et al. (2001) sequenced the modified EPSPS protein in glyphosate-tolerant maize and observed that it was 99.3% identical in its amino acid sequence to the native maize EPSPS protein. The only difference in the protein was its tolerance to glyphosate (Sidhu et al., 2000; Lee et al., 2001).

The sequence of CP4 EPSPS for glyphosate-tolerant wheat event MON 71800 has been completely coded and as a mature protein it is substantially similar (with respect to amino acid sequence homology) to native EPSPS proteins consumed in a variety of human food and animal feed sources (Pilacinski, 2002).

Sidhu et al. (2000) characterized the expression of transgenic EPSPS protein because it catalyses a step in the aromatic amino acid biosynthetic pathway and the levels of aromatic compounds could be altered because of the presence of the transgenic EPSPS. Their results indicated that the levels of the aromatic amino acids phenylalanine and tryptophan were unchanged when compared to a non-glyphosate-tolerant maize line.

Toxicity

Acute and subchronic effects. Harrison et al. (1996) examined the acute toxicity of CP4 EPSPS protein in mice by feeding high doses of soybean meal or seed containing the CP4 EPSPS protein at 572 mg kg BW^{-1} administered once by gavage, which exceeds by 1000-fold the estimated consumption level for food products containing CP4 EPSPS protein. No adverse effects occurred in the mice dosed with CP4 EPSPS protein. Their body weight, cumulative body weight, and food consumption did not show significant differences between the control groups and CP4 EPSPS protein treated groups.

Hammond et al. (2001) compared the responses of rats fed grain for 13 weeks from glyphosate-tolerant maize to the parental variety of non-transgenic maize and commercial varieties of non-transgenic maize. There was no observed toxicity and no differences in organ weight among all maize varieties. Lee et al. (2001) performed an *in vitro* test on mice to determine the toxicity of CP4 EPSPS protein in maize. A high dose of 45.6 mg of transgenic EPSPS protein per kg body weight was administered orally each day for 90 days. There was no observed toxicity from the protein (Lee et al., 2001).

Chang et al. (2002) subjected Sprague Dawley rats to a toxicity test by orally administering 0.5 or 2.0 mg kg BW^{-1} of CP4 EPSPS protein in saline solution three times per week for three weeks.

Table 3. Ecological risk quotients for wheat herbicides

Active ingredient	Application rate (g ai ha^{-1})	Seedling emergence RQ[a]	Rank	Vegetative vigor RQ	Rank	Duckweed RQ	Rank	Groundwater concentration[b]	Rank	Waterflea RQ	Rank	Fish RQ	Rank
Glyphosate	840	0.005	14	0.08	8	0.00012	14	0.0005	12	0.0003	5	0.00055	6
Bromoxynil	560	0.25	8	0.29	4	0.0142	9	0.0004	13	0.097	3	0.06	2
2,4-D	1100	2	3	1	2	0.0532	8	0.005	9	0.69	1	0.0003	9
Clodinafop	67	0.12	11	0.03	10	0.0002	13	0.000025	16	0.000008	14	0.002	4
Clopyralid	146	0.78	4	0.13	7	NA		0.06	4	0.00003	10	0.00006	11
Dicamba	280	0.375	6	0.25	5	0.0038	10	0.1	3	0.00012	7	0.0001	10
Fenoxaprop	90	NA		NA		NA		0.000006	17	0.00007	9	0.00046	8
Flucarbazone	34	0.18	10	0.03	10	0.00011	15	0.2	2	0.000013	12	0.000015	12
Imazamox	70	0.28	7	0.05	9	0.168	5	0.024719	7	0.000014	11	0.000014	13
MCPA	1457	7.8	1	1.3	1	0.2774	4	0.26	1	0.0003	5	0.00052	7
Metsulfuron	9	NA		NA		1.03	2	0.004	10	0.000002	15	0.000002	16
Thifensulfuron	22	NA		NA		0.5082	3	0.0001	14	8.1E-07	16	0.0000081	15
Tralkoxydim	280	0.75	5	0.25	5	0.0036	11	0.001	11	0.0001	8	0.001	5
Triallate	1100	3	2	0.3	3	0.0011	12	0.04	6	0.11	2	0.009	3
Triasulfuron	34	0.09	12	0.03	10	6.4	1	0.05	5	0.00001	13	0.0000121	14
Tribenuron	16	0.084	13	0.007	14	0.1371	6	0.00003	15	0.0000008	17	0.0000006	17
Trifluralin	1100	0.182	9	0.01	13	0.0763	7	0.009	8	0.006	4	0.08	1

[a] RQ = risk quotient.
[b] Groundwater concentration in ppb.

The dosages of CP4 EPSPS protein in this study were considered to be the approximate amounts of EPSPS protein in soybean consumed annually by humans. No toxicity was observed when compared to the saline control (Chang et al., 2002).

Allergenicity. An accepted measure of the potential for a protein to be an allergen is its degree of degradation in a simulated human gastric system. In a lamb's gastric system, the half-life of CP4 EPSPS protein produced by glyphosate-tolerant canola was less than 15 s and in the intestinal system it was less than 10 min (Stanford et al., 2003). The human stomach is estimated to empty 50% of solid food in 2 h, and liquid empties in 25 min. This indicates that if the CP4 EPSPS protein does not degrade in the human gastric system, it most likely would degrade in the intestinal system. The CP4 EPSPS protein also was inactivated by heating at 65°C for 15 min (Stanford et al., 2003).

Stanford et al. (2003) observed complete digestibility of the CP4 EPSPS protein in gastric fluid in 60 s. *In vivo*, *in vitro*, and *ex vivo* tests were performed on Sprague Dawley rats using glyphosate-tolerant soybean seeds. All three tests gave negative results, indicating that the allergenic potential of the CP4 EPSPS protein is very low (Stanford et al., 2003). The CP4 EPSPS protein shows no homology with known allergens; therefore, glyphosate-tolerant crops containing the novel protein have a low potential of causing allergies among humans and other animals. Native EPSPS proteins are normally present in foods and feeds derived from plant and microbial sources and the CP4 EPSPS protein is similar to these (Stanford et al., 2003).

Nutritional effects. Feeding studies have been conducted on rats, broiler chickens, catfish, and dairy cows for glyphosate-tolerant soybean (Hammond et al., 1996). All the studies have shown that glyphosate-tolerant soybean was not statistically different than non-transgenic varieties. The variables observed in poultry were body weight, live weight gain, feed intake, gain-to-feed ratio, breast muscle and fat-pad weights. In catfish, the variables were weight-gain-to-feed ratios, body composition, moisture, protein, fat, and ash content. Gross pathology observations were made, as well as organ weight, when studying the effects of glyphosate-tolerant soybean and non-transgenic soybean meal to rats. Milk production, milk composition, rumen fermentation, and nitrogen digestibility were observed in dairy cattle fed glyphosate-tolerant soybeans and non-transgenic soybeans (Hammond et al., 1996). In a 6-week feeding study on young chickens, Sidhu et al. (2000) determined that there were no significant differences in growth, feed efficiency, adjusted feed efficiency, and fat-pad weights in chickens fed either glyphosate-tolerant maize and non-transgenic maize.

Donkin et al. (2003) conducted a nutritional study using glyphosate-tolerant, insect-protected, and non-transgenic maize hybrids by feeding the silage and grain to dairy cattle to determine their effects on milk production, milk composition, and ruminal digestion. The diets contained 42–60% silage and 20–34% grain from glyphosate-tolerant, insect-protected, and non-transgenic maize hybrids. The cows were fed *ad libitum* and milked two times per day. The results revealed no differences in dry-matter intake (DMI), milk production, milk composition, efficiency of 4% fat-corrected milk production, and digestible energy coefficients. Average daily gain, DMI, feed/gain, and ruminal digestion were also unaffected by the transgenic grain fed to the dairy cows.

Ash et al. (2003) examined the fate of CP4 EPSPS protein in laying chicken hens after being fed glyphosate-tolerant soybean. According to immunoassay tests, there was no presence of the CP4 EPSPS protein in whole egg, egg albumen, liver, or feces, which suggests that the digestive system of laying hens completely breaks down the CP4 EPSPS protein from glyphosate-tolerant soybean meal.

Compositional analysis was conducted on wheat forage of transgenic wheat (event MON 71800), a non-transgenic parental control (event MON 71900), and commercially grown wheat varieties (Pilacinski, 2002). The analysis measured protein, fat, ash, moisture, acid detergent fiber, neutral detergent fiber, calcium, and phosphorous. Compositional analysis was also conducted on the wheat grain of the same three wheat lines. The analysis measured protein, fat, ash, moisture, gluten and gliadin, sugars (fructose, galactose, glucose, maltose, mannose, raffinose, stachyose, sucrose, and xylose), total dietary fiber, amino acids, fatty acids (C8–C22), vitamin B6, vitamin E, niacin, riboflavin

(vitamin B2), thiamin (vitamin B1), minerals (cadmium, calcium, copper, iron, magnesium, manganese, phosphorous, potassium, selenium, sodium, and zinc), starch, and phytic acid. There were no statistically significant differences between the varieties.

Kan and Hartnell (2004) conducted a study on broiler chickens using glyphosate-tolerant wheat. The broiler weight, feed conversion, carcass yield, and breast meat showed no statistically significant change when fed glyphosate-tolerant wheat (event MON 71800) non-transgenic wheat (event MON 71900), and commercial wheat varieties. Birds fed event MON 71900 (non-transgenic control) showed a lower carcass yield at 41 days. There were no significant treatment by sex interactions, except for evisceration yield between birds fed two commercial wheat varieties.

Exposure. Pilacinski (2002) found that the CP4 EPSPS protein levels in wheat were greater in forage than in grain tissues. The average CP4 EPSPS protein level in forage was 106 $\mu g\ g^{-1}$ fresh-weight, and in grain it was 13 $\mu g\ g^{-1}$ fresh-weight. Currently, there is no publicly available information on the degradation of CP4 EPSPS protein between harvest and human consumption. However, even if the protein did not denature in human food processing and preparation, exposures would still be very low because of rapid digestibility in simulated human and other mammalian digestive systems.

Risk characterization. Our risk characterization for the CP4 EPSPS protein for humans, livestock, and wildlife (e.g., mammals, birds, and fish) relies primarily on a qualitative weight-of-evidence approach. First, the homology of CP4 EPSPS protein is very similar to native EPSPS protein. Native EPSPS proteins are normally present in human foods and animal feeds derived from a large variety of plant and microbial sources. There is no evidence that these proteins have any toxic effects. Second, despite dosages of CP4 EPSPS protein far in excess of estimated dietary exposures, studies to date have revealed no acutely toxic, allergenic, or nutritional/chronic effects on numerous test animals, including rats, mice, chickens, swine, cattle, and catfish. Finally, the transgenic CP4 EPSPS protein does not seem to alter the composition of wheat forage or grain.

Dietary exposures of humans and livestock to CP4 EPSPS protein most likely would not be zero, especially for livestock consumption of wheat grain and forage. However, studies to date indicate that CP4 EPSPS protein would degrade rapidly in mammalian and avian digestive systems. Because we could not identify a deleterious effect associated with exposure to CP4 EPSPS protein, the dietary risk to humans, livestock, and wildlife was determined to be negligible.

Mutated protein risk assessment

Human and livestock dietary risk assessment

Homology. Because the AHAS enzyme catalyzes the first step in synthesis of the essential branched chain amino acids isoleucine, leucine, and valine, the levels of those amino acids produced with the altered AHAS protein were examined. The altered AHAS protein did not affect isoleucine, leucine, and valine levels when compared to unmodified wheat plants (CFIA, 2003, 2004)

The protein components of the mutated wheat plants were not altered when compared to an unmodified wheat line. Further, no new proteins or increases in protein expression were observed. A comparative compositional analysis of imidazolinone-tolerant wheat and the parent line, Gunner, has been conducted. The content of the anti-nutrients, phytic acid and trypsin inhibitor, was not statistically different among varieties. Other factors, such as amino acids, fatty acids, potassium, zinc, iron, niacin, pantothenic acid, pyridoxine, and vitamin E were not statistically different among varieties. Thiamine, oleic acid, and palmitic acid levels in imidazolinone-tolerant wheat were statistically lower than the parent line (CFIA, 2003, 2004; Health Canada, 2004a, b).

Toxicity. The AHAS protein, which is a common, yet minor plant protein, is not a known toxin. The altered AHAS protein is inactivated within 1 min at 100°C and is completely degraded within 30 min of treatment with trypsin. The altered AHAS protein is unlikely to be an allergen for the following reasons: (1) AHAS is not a known allergen, (2) altered AHAS degrades rapidly in a simulated human gastric system, (3) altered AHAS is inactivated rapidly at high temperatures, (4) altered AHAS is found in small amounts in plant tissues, and (5) the amino acid sequence of altered

AHAS has a high degree of homology to AHAS, which is not a known allergen.

Risk characterization. As with the glyphosate-tolerant CP4 EPSPS protein, the risk characterization for the altered AHAS protein is based largely on a qualitative weight-of-evidence approach. The key difference is that we could find no evidence that toxicity testing has been conducted on the mutated AHAS protein. Current information suggests that the altered AHAS protein is most likely not toxic or allergenic. Further, dietary exposures to the altered AHAS protein most likely would be low because the protein is found in small amounts in plant tissue. However, the lack of specific toxicity testing and quantification of altered AHAS protein levels in wheat grain limits our ability to characterize risk.

Discussion

Herbicide risk

Human risk

Both glyphosate and imazamox present lower risks than many other herbicides associated with conventional wheat production systems evaluated in this study. Because no acute or chronic toxicity for imazamox has been identified, dietary risk for it is essentially zero. The dietary risk for glyphosate also is low; 7 of the 17 herbicides had lower tier 1 dietary risk.

Applicator risks also are low for imazamox and glyphosate, compared to the other herbicides. Imazamox and glyphosate represented the lowest and fourth lowest applicator risks in our assessment, respectively. In an epidemiological study, De Roos et al. (2005) suggest that glyphosate exposure among pesticide applicators in the US was correlated with multiple myeloma. However, the study did not quantify exposures and current toxicological data do not support the hypothesis that glyphosate is a mutagen or carcinogen (Williams et al., 2000). Additional research is needed to determine if the correlation between myeloma and glyphosate reflects actual causation.

The differences in human risks were most pronounced when comparing glyphosate and imazamox to herbicides currently with substantial market share, such as 2,4-D, MCPA, dicamba, fenoxaprop, triallate, and bromoxynil (USDA, 2003) (Tables 1, 2). Currently, 2,4-D, MCPA, dicamba, fenoxaprop, triallate, and bromoxynil are used on approximately 58, 26, 23, 58, 12, and 13%, respectively, of total spring wheat acres in the US (USDA, 2003). Imazamox had lower dietary and applicator risks than all of these herbicides. Glyphosate had lower applicator risks than all of these herbicides (except for imazamox) and lower dietary risks than all of these herbicides (except for imazamox and triallate) (Tables 1 and 2).

Ecological risk

Ecological RQ's for the 17 herbicides were highly variable, ranging from 0.0000006 to 7.8 across all ecological receptors (Table 3). Despite the variation in RQ's, ranking each herbicide with respect to RQ for each ecological risk category allows us to draw several conclusions. For risks to duckweed, groundwater, and non-target plant seedling emergence, glyphosate had less risk than most other active ingredients. For risks to fish and waterflea, imazamox had less risk than most other active ingredients. Imazamox had greater risk than glyphosate for non-target plant seedling emergence, duckweed, and groundwater.

As with human risks, the differences in ecological risks were most pronounced when comparing glyphosate and imazamox to active ingredients with substantial market share, such as 2,4-D, MCPA, triallate, dicamba, and bromoxynil (USDA, 2003). Currently, the broadleaf herbicides 2,4-D, MCPA, dicamba, tribenuron, and bromoxynil are used on approximately 58, 26, 23, 20, and 13%, respectively, of total spring wheat acres in the US (USDA, 2003). The herbicides 2,4-D, MCPA, and bromoxynil had greater RQ's than glyphosate for five, five, and four of the six ecological receptors evaluated. Dicamba and tribenuron had greater RQ's than glyphosate for four and two of the six receptors, respectively. The herbicides 2,4-D, MCPA, bromoxynil, dicamba, and tribenuron had greater RQ's than imazamox for four, six, three, five, and zero of the six ecological receptors evaluated (Table 3).

The grass weed management herbicides fenoxaprop, triallate, trifluralin, and tralkoxydim currently are used on approximately 58, 12, 9, and 6%, respectively, of spring wheat acres in the US (USDA, 2003b). Triallate, trifluralin, and

tralkoxydim had greater RQ's than glyphosate for six, five, and five of the six ecological receptors evaluated in this study, respectively. Triallate, trifluralin, and tralkoxydim had greater RQ's than imazamox for five, two, and four of the six ecological receptors, respectively. Fenoxaprop did not have any RQ's greater than glyphosate. However, effects data were only available for three of the six ecological receptors (Table 3).

Transgenic protein risk

Despite not having the same set of toxicity and exposure studies as those of the herbicides, the current weight-of-evidence suggests that CP4 EPSPS protein present in glyphosate-tolerant wheat poses negligible risk to humans, livestock, and wildlife. Acute toxicity, allergenicity, and animal feeding (chronic toxicity) studies have been conducted for CP4 EPSPS protein and glyphosate-tolerant grain. In all studies, despite very large dosages, toxic effects were not observed. We did not determine risk quantitatively because a toxic effect level has not been identified. Further, for humans, exposures to CP4 EPSPS proteins were expected to be low because of rapid digestibility in simulated human digestive systems. Consequently, because risk is the joint probability of effect and exposure, the risk to humans from exposure to CP4 EPSPS would be negligible.

Mutated protein risk

Conclusions about risk are more problematic for the altered AHAS protein present in imidazolinone-tolerant wheat. This is because, to our knowledge, there are no toxicity data for the altered AHAS protein. Unlike CP4 EPSPS, acute toxicity, allergenicity, and animal feeding studies have not been conducted for altered AHAS protein. Because there is no information on toxicity of the altered AHAS protein, we could not quantitatively determine risk for humans, livestock, or wildlife. However, based on the alteration of the protein, familiarity with its function, and the qualitative data on degradation, it seems likely that the risk is low.

Uncertainty

Tier 1 risk assessment approaches have limited value for accurate quantifications of risk because of their simplistic hazard and exposure assumptions (Peterson & Hulting, 2004). These assumptions, which are highly conservative and err on the side of environmental safety, typically are used for highlighting significant vs. negligible risks during preliminary decision-making and not for determining actual site-specific risks. Higher-tier more refined assessments for these technologies should use more realistic effect and exposure assumptions, especially for the herbicides. However, because of their standardized effects and exposure assumptions we believe quantitative and qualitative tier 1 approaches are valuable for making direct comparisons between environmental stressors.

In this study, environmental risks for herbicides were more amenable to quantitative assessments than for the transgenic CP4 EPSPS protein and the mutated AHAS protein. This was because of two reasons: (1) effects and exposure data for the proteins are lacking or not as complete as for the herbicides, and (2) the data are not as publicly available as herbicide data. Because of specificity and familiarity with their native counterparts, evolving regulatory requirements, and the fact that they are not pesticides, these proteins do not have the same completeness of toxicity testing data as herbicides. We are not suggesting that these proteins should be required to have the same raft of effect and exposure studies as pesticides. However, we believe it is important that minimum effect and exposure data (such as acute mammalian toxicities to altered or inserted proteins) are generated and made publicly available for all novel plant traits, including non-genetically engineered approaches. These data would allow for independent, third-party tier 1 assessments of risk and proper communication of those risks to the public.

Current novel organism or trait regulations within the USEPA and the U.S. Department of Agriculture (USDA) focus entirely on genetically engineered organisms and not on other processes used to produce novel traits; hence, glyphosate-tolerant wheat is regulated before commercialization, but imidazolinone-tolerant wheat is not. To properly and logically assess risks, we believe similar effect and exposure data should be available for all novel organisms, regardless of the process used to produce them. Currently, Canada regulates all novel traits – regardless of process – but for imidazolinone-tolerant wheat effect or toxicity data for the altered AHAS protein do not seem to have

Table 4. Weight-of-evidence analysis of effect and exposure uncertainty by stressor and effect type

Effect	Herbicide		Transgenic protein		Mutated protein	
	Effect uncertainty	Exposure uncertainty	Effect uncertainty	Exposure uncertainty	Effect uncertainty	Exposure uncertainty
Human Health	Low	Low	Medium	Low	High	Medium
Ecological	Low	Low	Medium	Low	High	Medium

been generated. This adds considerable uncertainty to our risk assessment (Table 4).

Risk assessment is characterized by a systematic, transparent process in which effect and exposure information is presented in a transparent manner. Even though we employed this process in our assessment, we could not analyze all of the risks in the same manner. Therefore, we used a mix of quantitative and qualitative approaches. Both approaches, however, are valid for characterizing risk (NRC, 1996).

Overall, our tier 1 comparative assessment of environmental risks for CP4 EPSPS would be negligible. Risk for mutated AHAS protein most likely would be low, but there are not sufficient effect and exposure data to adequately characterize risk. Tier 1 human health and ecological risks for herbicides indicate that both glyphosate and imazamox represent lower risk compared to many other herbicides typically used in wheat production systems in the US and Canada. Consequently, based on our analysis, glyphosate-tolerant and imidazolinone-tolerant wheat may pose less ecological and human health risks than current conventional production systems. Although beyond the scope of this paper, characterization of agronomic risks from these production systems would add valuable information to a comparative assessment and may allow for a more refined delineation of risks between glyphosate-tolerant and imidazolinone-tolerant wheat systems. Although our assessment is not comprehensive, we believe the approach presented here demonstrates the potential risk trade-offs (especially for herbicides) when implementing the newer biotechnologies.

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