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Prospective formulation of environmental risk assessments: Probabilistic screening for Cry1A(b) maize risk to aquatic insects

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ABSTRACT

A critical first step for environmental risk assessment is problem formulation to identify environmental entities of concern and determinants of exposure that place these entities at risk. A conservative screening level approach was used to evaluate the potential risk to sensitive aquatic species from maize-expressed Cry1A(b) protein occurring in a representative agroecosystem. Estimated environmental concentrations for Cry1A(b) were compared to threshold concentrations of concern for putative sensitive aquatic organisms as estimated from species sensitivity distributions. The high-end risk expressed as the combined probability of short-term exposure and acute effects to a sensitive species indicated no concern in 99% of cases with limited opportunity for chronic effects due to the rapid decline of Cry1A(b) from the environment. Addressing uncertainties in the distribution of Cry1A(b) in soil, water, and sediment clarify the need for expanded ecotoxicity testing for aquatic effects.

1. Introduction

The use of *in planta* expression of *Bacillus thuringiensis* (Bt) delta-endotoxins to protect maize from insect pests has become a widespread agronomic practice. In 2009, approximately 63% of planted maize in the USA represented genetically engineered (GE) varieties expressing one or more Bt toxins (USDA, 2009) and worldwide adoption of this technology is moving forward at a rapid pace (James, 2009). The non-target organism (NTO) risks of Bt maize have been extensively assessed as exhibiting reasonable certainty of no harm as part of their regulatory clearance before commercialization (see, for example, Wolt et al., 2008). These findings are supported by comprehensive meta-analyses of both field and laboratory findings (Marvier et al., 2007; Naranjo, 2009). Despite these assessments, as well as experience gained in more than two decades of commercial use, inevitable questions remain regarding the broad-scale environmental impacts of Bt maize, including non-target effects to aquatic insects (Rosi-Marshall et al., 2007).

These questions of broad-scale impacts can be addressed using recognized frameworks for environmental risk assessment (ERA) (USEPA, 1992, 1998), which have been adapted for use with GE crops (Romeis et al., 2008). Successful application of an ERA paradigm for a GE crop entails several key attributes including (1) the case-specific use of proper problem formulation for design of the risk assessment; (2) the recognized need to establish a causal relationship of stressor exposure to receptor resulting in a measurable consequence of exposure (for instance, exposure of an insect species of concern to maize-expressed Bt toxin results in an adverse effect, such as lethality or growth reduction); and (3) conducting the risk assessment on comparative terms through use of appropriate comparator plants, environments, and managements (Wolt et al., 2010). This ERA paradigm has been applied to varying degrees for terrestrial non-target risk assessments of Bt maize (Sears et al., 2001; Wolt et al., 2003, 2005; Peterson et al., 2006; Romeis et al., 2006). In the case of aquatic risks, the causal relationship of exposure to effect has not been established because of the lack of relevant information on the environmental occurrence and concentration of the stressor (a specific plant-expressed Bt protein) and toxicity to specific aquatic species of concern.

Analysis of the aquatic risks of Bt maize requires problem formulation as an important first step for an effective ERA (Wolt et al., 2010), where a primary consideration is identification of the environmental entities of concern and determinants of exposure that place these entities at risk. Of particular importance in arriving at the appropriate design for the ERA is determining through problem formulation the needs for exposure assessment, which is the process establishing the likelihood, magnitude, route, and duration of exposure to a population of concern. For terrestrial non-target risk assessments of Bt maize, estimated environmental concentrations (EECs) have been developed from

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models and/or measurements to estimate risk as the probability for harm to be manifested under relevant conditions of exposure (Wolt et al., 2003, 2005) and this approach can be similarly applied for aquatic risk assessments. When direct evidence of toxicity is lacking, these EECs have been used in conjunction with sensitivity estimates for putative susceptible organisms as a means to arrive at a preliminary assessment of risk for formulation of more robust, targeted testing and assessment (Wolt et al., 2003, 2005; Peterson et al., 2006). These screening level assessments, using highly conservative exposure estimates and effects thresholds, are a useful prospective tool for formulation of the appropriate analytical plan for the ERA.

Presented here is a consideration of Cry1A(b) protein accumulation and loss when maize expressing this protein is grown in a representative agroecosystem. The quantities and partitioning of Cry1A(b) protein generated throughout the crop growing cycle are synthesized using conservative environmental fate models into EECs for aquatic species of concern in or near maize fields because these estimates are a current unanswered consideration for aquatic NTO risk assessments. Species sensitivity distributions are used to estimate the threshold concentrations of concern for putative sensitive aquatic organisms.

2. Methods

A reasonable worst-case scenario was developed for temperate zone maize production on the basis of a high-end exposure estimate (USEPA, 1997). This estimate of exposure is greater than the 90th percentile of incidences for that case, but less than the exposure at the highest percentile. The high-end risk descriptor is an estimate of the risk level where risk is based on a combined probability of the high-end exposure and susceptibility to the stressor.

2.1. Environmental loading of Cry1A(b)

The spatial and temporal distribution of Cry1A(b) produced in a standing crop of Bt maize provides the starting point for estimates of exposure to aquatic receiving environments. For the case presented here, we have used the data of Nguyen (2004) and Nguyen and Jehle (2007), which present an internally consistent description of the season-long pattern of Cry1A(b) expression, distribution, and dry matter partition[1] for MON810 maize grown over five location-years. The reported concentrations, variance, and relative distribution of Cry1A(b) are corroborated with values reported elsewhere (Mendelsohn et al., 2003; Székács et al., 2010a, 2010b). The data were considered to be independent and identically distributed and therefore were combined by tissue type and growth stage to generate the mean, variance, and range of Cry1A(b) tissue concentration and relative dry matter partition (Table 1). The summary statistics for Cry1A(b) concentrations were used in the generalized beta distribution with the form

$$P(x) = \frac{(x-a)^{(p-1)}(b-x)^{(q-1)}}{\beta(p,q)(b-a)^{(p+q-1)}} \quad (1)$$

where a and b are location parameters (minimum and maximum, respectively) representing the range in observations and p and q represent shape parameters (Wang, 2005), which were estimated by the method of moments using minimum, maximum, mean (m), and variance (v) (NIST, 2006) as

$$p = \left(\frac{m-a}{b-a}\right)\left(\frac{(m-a)(b-m)}{v} - 1\right) \quad (2)$$

and

$$q = p\left(\frac{b-m}{m-a}\right). \quad (3)$$

The derived beta distributions were used to estimate the distribution of Cry1A(b) concentration for each tissue and growth stage (Table 1). These data and average dry matter partition were supplemented with published values of maize residue and protein dissipation rates and dry matter partitioning indices (stover:grain=0.082, Pordesimo et al., 2004; root:shoot=0.15, Echarte et al., 2008) to estimate the in-crop and post-harvest pool of maize-derived Cry1A(b) within the production environment (Table 2). The decline in post-harvest residues was established on the basis of a zero-order decomposition rate for maize residues ($-0.2505\%/d$, Lehman et al., 2008), the apparent first-order degradation rate for Cry1A(b) protein from root tissue ($-0.0189/d$, calculated from the in-field data of Nguyen and Jehle, 2007), and an apparent first-order degradation rate for Cry1A(b) protein from leaf tissue ($-0.0099/d$, estimated from litter bag decomposition, Zurbrügg et al., 2010). For these calculations, total plant biomass was scaled for maize grain yield of 10 Mg ha^{-1}, representative of average yield for the upper Midwestern USA in 2008–2009 (USDA, 2009).

To evaluate aggregate protein production over time as affected by variance for in-crop Cry1A(b) concentrations, forecasts were generated from Latin hypercube sampling of the beta distributions describing Cry1A(b) concentrations (Table 1). This partially stochastic analysis was performed to achieve 3% resolution of mean results with 95% confidence (4100 iterations for convergence) using @Risk software (Palisade, Ithaca, NY). Forecast results were represented as an output distribution of the total protein present in the standing crop by growth stage and in post-harvest plant residues (Fig. 1).

2.2. Aquatic estimated environmental concentrations

The screening level exposure assessment used the USEPA GENEEC and FIRST tier 1 models for ecological risk assessment of pesticides (USEPA, 2001, 2007). Aquatic EECs for Cry1A(b) were determined for three standard default scenarios: the GENEEC pond (a static pond of 20,000 m^3 volume, 2 m deep, draining a 10 ha field planted to maize); a shallow semi-aquatic wetland (represented as a 3600 m^3 volume, 0.15 m deep, draining a 10 ha field planted to maize); and the FIRST index reservoir (a 144,000 m^3 water body, 2.74 m deep catching runoff from a 172.8 ha watershed with 56% of area planted to maize, and with annual flow of twice the reservoir volume). The screening level models allow for estimates of the concentration of Cry1A(b), which ostensibly occurs in receiving waters due to the presence of residual plant materials in fields where GE maize is grown. These aquatic EECs are considered to represent Cry1A(b) to which aquatic micro- and macro-invertebrates may be exposed.

The following case-specific parameters were used to develop the aquatic EEC for maize-expressed Cry1A(b) for each scenario. The soil adsorption coefficient (K_d) for maize-expressed Cry1A(b) was 1 L/kg (as indicated from studies with the maize-expressed protein, Hopkins and Gregorich, 2003; studies with microbial-derived Cry1A(b) suggest values ranging from 0.1 to 14 L/kg, Sundaram, 1996; Koskella and Stotzky, 1997; Stotzky, 2000). The aerobic soil half-life for maize-produced Cry1A(b) was 2.6 days (based on a DT_{50} of 1.6 to 3.6 days, Sims and Holden, 1996; Hopkins and Gregorich, 2003). The aerobic aquatic half-life was 1.3 days (based on observations that Cry-proteins from green tissue degrade approximately twice as fast in unfiltered surface water as in soil, Douville et al., 2005; Prihoda and Coats, 2008a, 2008b). The computations did not consider degradation due to aqueous photolysis or anaerobic metabolism and assumed high water solubility of the protein. As a worst-case assumption, total Cry1A(b) from maize biomass, including root biomass, was assumed to be instantaneously present for runoff or erosion (i.e., the total Cry1A(b) produced in maize within the modeled agroecosystem was immediately available to the receiving water body).

2.3. Sensitive species thresholds of concern

Species sensitivity distributions (Solomon and Takacs, 2002) were used to develop a probabilistic profile of acute effects for arthropod exposure to Cry1A(b). Since the Cry1A(b) protein is selectively toxic to Lepidoptera (Glare and O'Callaghan, 2000), acute LD_{50} values for larvae of lepidopteran species ingesting the purified protein (cited in Wolt et al., 2008) were used as surrogate data describing toxicity for a putative susceptible aquatic arthropod. The cumulative distribution in sensitivity was used to project the threshold of concern for a putative susceptible aquatic species.

3. Results

3.1. Environmental loading of Cry1A(b)

The average measured Cry1A(b) concentration and biomass distribution in maize tissue over time (Nguyen, 2004; Nguyen and Jehle, 2007) was used to deterministically estimate mean Cry1A(b) production from MON810 maize (Table 2). The mean total quantity of Cry1A(b) present in the standing crop showed a linear increase from planting through early dough stage, where it reached a maximum of 62 g/ha and then declined to 41 g/ha at harvest. Grain removal at harvest further reduced the total Cry1A(b) present to 39 g/ha. Subsequent degradation of residual

[1] Dry matter partition is the distribution of biomass on a dry weight basis among various plant parts at a given stage of development.

Table 1
Summary of measured concentrations of Cry1A(b) in maize tissues at various growth stages (Nguyen and Jehle, 2007) and calculated beta parameters describing the modeled distribution in Cry1A(b).

Tissue	Growth stage[a]	[Cry1A(b)] (μg/g fw)				Beta parameter	
		Mean *m*	Variance *v*	Minimum *a*	Maximum *b*	*p*	*q*
Root	19	1.52	0.031	0.28	3.95	32.57	63.58
	30	1.53	0.026	0.27	4.17	41.04	85.40
	61	1.51	0.011	0.59	2.69	41.75	53.13
	83	1.50	0.013	0.34	2.79	53.64	59.30
Stalk	19	0.43	0.002	0.13	1.10	33.70	74.30
	30	0.38	0.001	0.08	0.85	38.41	58.82
	61	1.00	0.011	1.24	8.58	26.08	56.45
	83	1.18	0.011	1.88	11.07	40.06	68.78
Upper leaf	19	2.89	0.029	0.32	4.71	94.74	66.75
	30	3.12	0.078	0.70	6.59	43.75	62.79
	61	3.89	0.089	1.24	8.58	49.95	88.49
	83	6.08	0.124	1.88	11.07	76.63	91.08
Lower leaf	30	4.50	0.080	1.14	7.76	68.91	66.98
	61	3.37	0.061	1.27	7.04	45.77	79.68
	83	4.86	0.148	1.36	9.60	47.32	64.03
Inflorescence	61	2.43	0.079	0.30	6.65	37.71	74.80
Ear[b]	83	0.57	0.015	0.02	1.15	50.09	52.97
Kernel	99	0.30	0.006	0.01	0.61	6.29	6.65

[a] 19, 9 leaves; 30, stem elongation; 61, beginning of flowering, anthesis; 83, early dough; 99, harvested product (BBA, 2001).
[b] Average for kernel and shank tissues at early dough stage.

Cry1A(b) present in the field would leave a total of 3.4 g/ha at the beginning of the next growing season (6 months after harvest).

Stochastic evaluation of Cry1A(b) concentrations using modeled beta distributions resulted in more conservative estimates of the total Cry1A(b) protein (Fig. 1) as compared to the deterministic result (Table 2). For example, the deterministic estimate for maize in post-harvest residues was 41 g/ha, whereas the mean stochastic result was 47 g/ha. Output variance for total protein production was normally distributed about the mean for each growth stage sampled. Based on the stochastic results, a high-end environmental load of Cry1A(b) is represented by the 90th percentile of total Cry1A(b) protein present in the field immediately post-harvest (50 g/ha). This value was used to develop the tier 1 EEC.

3.2. Aquatic estimated environmental concentrations

The peak EEC, occurring immediately following harvest, for the farm pond (GENEEC) and reservoir (FIRST) scenarios were very similar (1.3 and 1.2 μg/L, respectively), since under the assumption of near instantaneous loading of Cry1A(b) to surface water there was little impact of physical and chemical processes to dissipate the protein. The GENEEC average EEC, however, rapidly fell to 0.9 μg/L at 4 days and 0.1 μg/L at 60 days, due largely to degradation. The FIRST average EEC similarly declined due to chemical degradation and physical dilution by flowing water, so that the predicted average annual EEC was <0.01 μg/L. The average EEC for the semi-aquatic wetland scenario ranged from an instantaneous peak value of 7.2 μg/L to 0.6 μg/L at 60 days and represents the worst-case exposure at the immediate near field edge where residues may accumulate.

3.3. Sensitive species thresholds of concern

The distribution of terrestrial lepidopteran larvae sensitivity to acute Cry1A(b) exposure ranged over four orders of magnitude as shown in the log-probability plot (Fig. 2). As a first estimate of sensitivity of a putatively susceptible aquatic arthropod to Cry1A(b), the multi-species distribution indicates 96% of species will be less acutely sensitive than the EEC for the standard pond and reservoir scenarios, and 90% of species will be less sensitive than the EEC for the semi-aquatic wetland (hatched areas in Fig. 2).

4. Discussion

Conceptual models are used in problem formulation to describe potential routes of exposure to key arthropod functional groups within aquatic ecosystems. The use of a screening level assessment provides insight as to which taxa and exposure paths within these general conceptual models will be relevant to a specific plant-expressed toxin. The case presented here forecasts high-end exposure for Cry1A(b) entering aquatic environments through maize residues. The environmental loading estimates (Table 2) show the logical focus for a prospective analysis is the immediate post-harvest period when the presence of Cry1A(b) in plant residuals is at its highest level. Other than for the immediate post-harvest interval (modeled here as all Cry1A(b) in the standing crop occurring instantaneously in water at 1 day following harvest), there was negligible potential for substantive Cry1A(b) occurrence in the aquatic environment either during in-crop or post-harvest phases of production. The conservatism of this estimate is confirmed in monitoring of streams adjacent to maize fields, which showed considerably less debris contribution than estimated here and with the larger portions entering waterways several months following harvest after Cry1A(b) protein had degraded to negligible levels (Jensen et al., 2010). Pollen, especially, proves to be a highly limited source of protein to the environment and would only be of consequence for sensitive organisms directly consuming this food source immediately following its environmental release.

Our focus on the immediate post-harvest interval was based on in-field measurements of overwintering maize debris decomposition (200-day half-lives for either GE or non-GE plant

Table 2
Predicted deterministic mean Cry1A(b) concentration, maize biomass, and calculated total mass of Cry1A(b) protein summarized by plant tissue and growth stage as determined from field-measured values (Nguyen, 2004; Nguyen and Jehle, 2007).

Growth stage	BBCH[a]	Tissue								
		Reproductive			Root			Stalk		
		[Cry1Ab] (μg/g)[b]	Biomass (Mg/ha)[c]	Protein (g/ha)[c]	[Cry1Ab] (μg/g)	Biomass (Mg/ha)	Protein (g/ha)	[Cry1Ab] (μg/g)	Biomass (Mg/ha)	Protein (g/ha)
Dry seed (caryopsis)	00	0.30	0.02	0.01						
9 leaf	19				1.52	1.95	3.49	0.43	0.89	0.46
Stem elongation	30				1.53	2.25	4.07	0.38	0.67	0.30
Flowering, anthesis	61	2.43	4.27	12.21	1.51	2.39	4.25	1.00	2.20	2.59
Early dough	83	0.57	5.29	3.56	1.50	2.71	4.78	1.18	2.56	3.56
At harvest	99	0.30[d]	8.54	3.02	1.50[e]	1.05[f]	1.86	4.38[g]	7.01[h]	36.08
Post-harvest residue[i]	1-d PH	0.30	2.05[j]	0.72	1.50	1.05	1.86	4.38	7.01	36.08
	6-mo PH				0.05[k]	0.58[l]	0.03	0.74[m]	3.85[l]	3.33

Growth stage	BBCH	Tissue										
		Upper leaf			Lower leaf			Pollen				
		[Cry1Ab] (μg/g)	Biomass (Mg/ha)	Protein (g/ha)	[Cry1Ab] (μg/g)	Biomass (Mg/ha)	Protein (g/ha)	[Cry1Ab] (μg/ha)	Biomass (Mg/ha)	Protein (g/ha)	Total Biomass (Mg/ha)	Total Cry1A(b) protein (g/ha)
Dry seed (caryopsis)	00										0.02	0.01
9 leaf	19	2.89	1.83	6.23							4.67	10.18
Stem elongation	30	3.12	2.29	8.39	4.50	2.06	10.92				7.28	23.68
Flowering, anthesis	61	3.89	3.63	16.60	3.37	2.67	10.61				15.93	46.31
Early dough	83	6.08	3.59	25.71	4.86	4.23	24.17	0.05	0.77	0.05	18.37	61.78
At harvest	99										16.60	40.95
Post-harvest residue	1-d PH										10.11	38.66
	6-mo PH										4.42	3.37

[a] BBA (2001), PH, post-harvest.
[b] Fresh weight basis, 15% moisture assumed.
[c] Dry weight basis.
[d] Equivalent to dry seed.
[e] Value from early dough stage is used.
[f] Calculated from stover biomass at harvest (root:shoot=0.15 for maize at physiological maturity, Echarte et al., 2008; see also Amos and Walters, 2006).
[g] Stover; weighted average for stalk and leaf tissue at early dough stage.
[h] Stover; calculated from grain biomass at harvest (stover:grain=0.082 at physiological maturity, Pordesimo et al., 2004).
[i] Unless otherwise noted, at harvest values are used.
[j] Calculated for a worst-case where 24% of grain remains in the field at harvest (Wolt et al., 2004).
[k] $[Cry1A(b)]^{root}_{1-dPH}e^{-kt}$, where k is the apparent first-order rate constant (-0.0189 day^{-1}) and $t=180$ days, calculated from the data of Nguyen and Jehle (2007).
[l] $Biomass_{at\ harvest}(100-kt)(0.01)$, where k is the apparent zero-order rate constant (-0.251% day^{-1}) and $t=180$ days, Lehman et al. (2008).
[m] $[Cry1A(b)]^{leaf}_{1-dPH}e^{-kt}$, where k is the apparent first-order rate constant (-0.0099 day^{-1}) and $t=180$ days, estimated from the data of Zurbrügg et al. (2010).

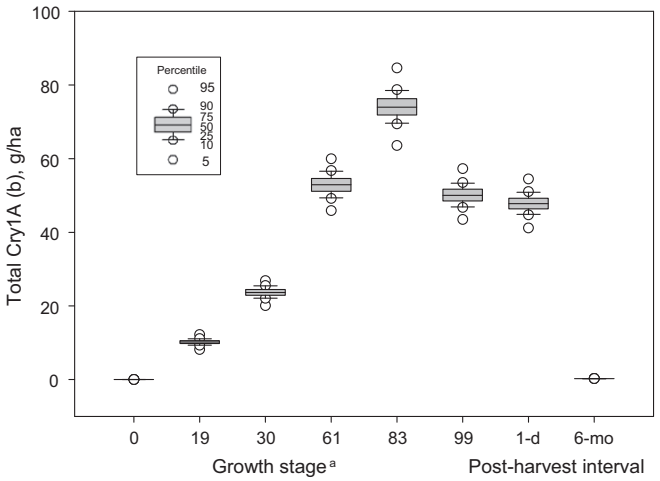

Fig. 1. Variance in predicted Cry1A(b) protein present in the standing maize crop and in post-harvest residues as forecast through stochastic analysis of Cry1A(b) expression as a function of plant part and growth stage. [a]BBCH growth stage (BBA, 2001).

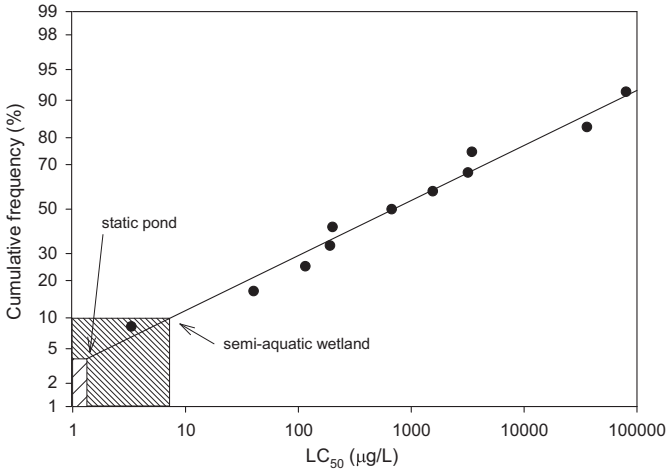

Fig. 2. Species sensitivity distribution for acute toxicity of lepidopteran larvae to Cry1A(b) protein, from Wolt et al. (2003), compared to peak aquatic EEC developed from high-end (90th percentile) exposure estimates. Putative susceptible species are at risk from high-end exposures in 0.4% and 1% of cases, respectively, for static pond and semi-aquatic wetland scenarios.

residues, Lehman et al., 2008) and much shorter dissipation for plant-made Cry1A(b). Our exposure estimates assume Cry1A(b) protein half lives of approximately 37 and 70 days for roots and stover, respectively (Nguyen and Jehle, 2007; Zurbrügg et al., 2010); and are consistent with the studies of Hopkins and Gregorich (2003) who observed loss of Cry1A(b) was much more rapid than the decay of whole plant materials and was more in-line with the mineralization of the water-soluble fraction of plant residues. The Cry1A(b) half-life estimates we have used are consistent with the data of Zwahlen et al. (2003) who measured Cry1A(b) declines from buried maize litter in both conventional and no-till systems where dissipation half-times were less than 60 days. Jensen et al. (2010), however, found Cry1A(b) protein bioactivity declined to undetectable levels within 14 days for maize leaf exposure in full-sun on stream banks.

The use of the species sensitivity distribution to estimate an adverse effect threshold assumes potentially affected species will be as sensitive to Cry1A(b) as are Lepidoptera; and, therefore, represents a conservative surrogate value for trichopteran (caddisfly) species because of the selectivity of Cry1A(b) to lepidopteran species and the close phylogenetic relationship between Lepidoptera and Trichoptera (Wheeler et al., 2001; Whiting, 2002). These projections are even more highly conservative when applied to more distantly related aquatic insect taxa, such as dragonflies, mosquitoes, black flies, and midges, and crustacean taxa, such as waterfleas (*Daphnia* spp.).

Using highly conservative first-tier screening models and high-end estimates of the Cry1A(b) entering aquatic systems, this analysis projected that for the aquatic arthropods in the most highly exposed scenario (a semi-aquatic wetland within a field of Bt maize), the 90th percentile acute aquatic EEC (7.2 µg/L) would be below the multi-species susceptibility for 90% of potentially susceptible species (Fig. 2). Thus, risk expressed as the combined probability of exposure and effect occurring at or below 7.2 µg/L would be manifested in 1% of cases $[(1-0.9)\times(1-0.9)=0.01]$. In other words, for 99% of cases where there is a potential for exposure to a susceptible species, there is reasonable certainty of no harm. These results are cast in terms of the effective concentrations of Cry1A(b) in the water column and are based on decline rates for the freely solubilized Cry1A(b); whereas, the rate of Cry1A(b) degradation from plant debris within the water column may be somewhat slower (Swan et al., 2009). Thus, debris feeders have the potential for exposure to more concentrated amounts of the toxin through direct feeding, but given the aforementioned considerations of susceptibility, protein degradability, window of exposure, the use of a high-end loading estimate, and the large margins of exposure shown, this screening level assessment remains conservative.

This analysis suggests that studies targeting effects of Cry1A(b) in aquatic ecosystems would need to identify aquatic species (putative susceptible NTOs) having the possibility of being as sensitive to Cry1A(b) as the most sensitive terrestrial lepidopteran species tested to date and subject to exposure in the immediate post-harvest interval. This approach is reasonable based on the selectivity of Cry1A(b) to Lepidoptera as confirmed by lack of effect to other insect or arthropod NTOs (Glare and O'Callaghan, 2000; Wolt et al., 2008). Because Cry1A(b) concentrations rapidly decline after the immediate post-harvest interval, chronic adverse effects would likely be even less evident. The extreme conservatism of this screening level assessment is evident from the biomass loading used to project the aquatic EEC for the semi-aquatic wetland. For this case, the biomass loading is three orders of magnitude greater than that observed in agricultural streams (Rosi-Marshall et al., 2007).

This prospective analysis indicates that before initiating ecotoxicity testing of aquatic species, exposure analysis in conjunction with surrogate data for terrestrial species is useful for determining where and at what levels in the aquatic environment GE crop residues might reasonably impact environmental entities of concern. Ecotoxicity findings for susceptible terrestrial species (including targets for control) provide a reasonable first estimate of toxicity to a putative sensitive aquatic species. The need for exposure characterization may be addressed through site-restricted field monitoring (e.g., Swan et al., 2009) or through development of refined environmental fate parameters, which allow for modeling of impacts throughout use environments. The prospective use of screening level assessments demonstrated in the current analysis suggests that more exacting measurements of Cry1A(b) fate in soil, water, and sediment would allow for more realistic considerations of exposure, which may negate the need for expanded ecotoxicity testing for aquatic effects because of the already low EECs based on worst-case assumptions. For instance, in the present analysis the adsorption of Cry1A(b) to soil and sediment is highly uncertain. If the K_d was

10-fold higher (representing a high-end estimate as suggested from Sundaram, 1996; Koskella and Stotzky, 1997; Stotzky, 2000), the various EECs reported here would be approximately halved; whereas if the K_d was 10-fold lower, the EEC is minimally affected.

The need for and design of either laboratory or field studies to evaluate non-target risks of plant-expressed toxins should be driven by a prospective analysis that considers the casual relationship of exposure and effect. As shown here for Cry1A(b) maize, joint consideration of specificity of the toxin and potential for exposure indicates negligible potential risk when making very conservative assumptions. Previous research inferring risk to a leaf-shredding trichopteran (*Lepidostoma liba*) (Rosi-Marshall et al., 2007) made the implicit assumption that overwintering accumulation of plant debris in low-order agricultural drainage ways was consistent with substantial plant-derived Cry1A(b) loading into headwater streams in agroecosystems leading to a focus on toxicity testing. This assumption disregarded the clear potential for protein degradation from plant debris. Consequently, recent non-target bioassays of aquatic leaf-chewing invertebrates, including trichopterans, showed no effect when larvae were fed Cry1A(b) maize leaf tissue *ad libitum* for 30 days (Jensen et al., 2010). In a similar vein, Bøhn and co-workers (Bøhn et al., 2008, 2010) expended considerable effort to demonstrate reduced fitness of *Daphnia magna* from exposure to Cry1A(b) maize in laboratory studies at doses many orders of magnitude higher than environmental exposures anticipated on the basis of exposure characterization. These studies confuse hazard (the outcome of an ecotoxicity test) with risk (the manifestation of toxicity under field conditions) and, thus, contribute to uncertainties in the ERA process (Ricroch et al., 2010).

5. Conclusions

If used in a prospective manner, the outcomes of the screening assessment described here, which uses literature data and extremely conservative modeling assumptions, would point to clarification of the exposure assumptions rather than ecotoxicity testing as the appropriate first step for a more robust ERA. These outcomes are clearly conservative when considering they assume that all Cry1A(b) present in the field in plant debris at harvest will be available to the putative susceptible species. If refined exposure analysis were employed to determine more realistically the exposure due to protein degradation as well as the feeding habits of the non-target aquatic species, one would anticipate substantially lower levels of exposure and, therefore, even lower probable risk.

A further critical aspect of ERA for GE insect resistant crops is that studies with plant expressed insecticidal proteins clearly distinguish exposure and effect for the stressor (Cry1A(b) in this instance) from that of the plant residue. This allows for distinguishing the direct effect of the stressor from possible indirect effects occurring as study artifacts or due to the baseline composition of the GE-crop (as distinguished from its non-GE comparator) (Romeis et al., 2006, 2008). Through the screening level ERA reported here, we provide guidance as to the appropriate problem formulation and analysis for addressing this need in future studies that evaluate risks to aquatic environments.

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