# A Screening Level Approach for Nontarget Insect Risk Assessment: Transgenic Bt Corn Pollen and the Monarch Butterfly (Lepidoptera: Danaidae)

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**ABSTRACT** Quantitative risk assessment affords an objective approach for assessing ecological risk from crops produced using biotechnology. Ecological risk assessment for plant-incorporated insecticidal proteins necessitates consideration of risks to nontarget insects when species-specific hazard information may be lacking. Screening-level risk assessment methods afford a means by which risks to species of concern may be evaluated conservatively using exposure estimates, host-range information, and a probabilistic estimate of toxicity to sensitive species. This approach was applied to the special case of Bt corn pollen risk to monarch butterfly, *Danaus plexippus* (L.), populations; the results were compared with more highly refined risk assessment techniques in terms of the risk conclusions which can be developed with more highly certain information. Exposure analysis based on readily available literature showed pollen interception by the host for monarch butterfly larvae (common milkweed, *Asclepias syriaca* L.) declined exponentially with distance from the pollen source. Intra- and inter-genera sensitivity of lepidopteran species was used to project effect to monarch butterfly larvae. When the $90^{th}$ percentile of effect ($LC_{50}$) was used to estimate monarch butterfly sensitivity to Bt corn pollen expressing Cry1A(b) protein, the risk of lethality to individual larvae was negligible at >1 m from the edge of source corn fields. Subsequent field measurements of pollen distribution, interception by milkweed, and especially effects determinations for monarch butterfly larvae exposed to Cry1A(b) toxin indicate that the screening-level approach was effective in focusing the scope of the problem to exposure from high-expressing Cry1A(b) events occurring within source cornfields or at the near-field edge. Screening level risk assessment conservatively identifies the scope of concern and the uncertainties that need clarification so that subsequent research can be appropriately focused.

**KEY WORDS** Cry1A(b) protein, *Bacillus thuringiensis*, *Danaus plexippus*, corn, pollen, ecological risk assessment

THE GOAL OF ECOLOGICAL risk assessment is to account for the magnitude and probabilities of adverse effects to nontarget species resulting from the presence of environmental stressors (USEPA 1999). Considerable attention has been directed toward the use of probabilistic risk assessment techniques that statistically quantify ecological risks as well as the associated uncertainty and variability in the subsequent risk conclusions (SETAC 1994, USEPA 1999). Probabilistic risk assessment effectively and transparently links science to the overall societal decision-making frameworks whereby risks are analyzed within an analytical-deliberative process (NRC 1983, 1996). A growing body of scientific literature attests to the utility of probabilistic approaches for ecological risk assessment (e.g., Kendall et al. 1996, Klaine et al. 1996, Solomon et al. 1996, Giesy et al. 1999, Hall et al. 1999, Giddings et al. 2000).

Risk assessment paradigms for genetically modified pest-protected plants do not differ in principle from those for other technological risks. Therefore, quantitative risk assessment using probabilistic approaches should be the ultimate goal for crop biotechnology risk assessments (NRC 2000, Wolt and Peterson 2000).

Losey et al. (1999) and Jesse and Obrycki (2000) reported adverse nontarget effects for monarch butterfly, *Danaus plexippus* (L.), larvae feeding on milkweed containing surface-deposited pollen originating from transgenic corn expressing *Bacillus thuringiensis* (Bt) Berliner derived δ-endotoxin. Corn expressing Bt toxin for protection against European corn borer, *Ostrinia nubilalis* (Hübner), was planted on ≈25% of corn acres in the Midwestern United States at the time of their reports. Resulting uncertainties in risk to monarch butterfly populations, especially to populations on milkweed outside of corn fields, engendered significant research activity to understand the risks to

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monarch butterfly populations (Shelton and Sears 2001).

In this paper, we use a common screening-level exposure and risk assessment methodology to evaluate quantitatively nontarget effects of Bt corn pollen on the monarch butterfly for corn events expressing the Cry1A(b) insecticidal crystalline protein. Then, we compare the utility of this generalized assessment approach to specific effects on monarch butterfly and exposure data recently generated for higher-tier ecological risk analyses.

## Approach

Conventional risk assessment first considers the nature and conceptual formulation of the problem and then proceeds through the characterization of effect, exposure, and the resulting risk and uncertainties (NRC 1983). In this paper, these elements of risk assessment are considered as they relate to risk of Bt corn pollen to monarch butterfly larvae.

### Problem Formulation

*Bacillus thuringiensis* subspecies are differentiated by their insecticidal activity. Generally, only insect species within a given order are susceptible to a given insecticidal Bt δ-endotoxin protein. Therefore, insect susceptibility results provide general information about the δ-endotoxin(s) expressed by particular *B. thuringiensis* strains. In the case of *B. thuringiensis kurstaki* (source of Cry1A(b) protein toxin) the greatest activity is shown for the order Lepidoptera. Toxicological studies on nontarget beneficial insect species using Cry1A(b) corn pollen, or bacterially expressed protein, support selectivity within the Lepidoptera, given the margins of safety shown for representative species from other major orders (Hymenoptera, Diptera, and Coleoptera) (Glare and O'Callaghan 2000).

The utility of corn expressing Cry1A(b) protein arises from the toxicity of the expressed protein to a specific lepidopteran pest, European corn borer, which is of economic importance in corn production. Because this plant-expressed Bt protein is active against lepidopteran species, an assessment of the risk to nontarget lepidopteran species inhabiting corn production systems is warranted.

Toxicological hazard has been demonstrated for monarch butterfly larvae consuming milkweed (*Asclepias* spp.) leaves containing surface-deposited pollen from corn expressing Bt protein (Losey et al. 1999, Hansen and Obrycki, 2000). Thus, the monarch butterfly is a logical focus for assessment.

Ecological risk can be described in quantitative terms as a function of exposure (environmental dose) and effect (toxicological hazard) (USEPA 1999). Exposure and risk assessment uses a tiered modeling approach extending from deterministic field-scale models (Tier 1) based on very conservative assumptions to probabilistic regional-scale models (Tier IV) using refined assumptions (SETAC 1994). In environmental risk assessment, "conservative assumptions" in lower-tier assessments represent likely overestimates of hazard and exposure. Consequently, the resulting quantitative risk value typically is itself conservative and therefore errs on the side of environmental safety.

The availability of relevant data has a bearing on the way risk may be characterized. When the nature, quality, or quantity of data are limited, single-point estimates are used in a manner that conservatively characterizes risk in a way that accounts for the upper bounds of uncertainty of sensitive elements governing exposure and effects determinations. When key descriptors of exposure and effect can be characterized in terms of distributions, deterministic risk assessments can be clarified through probabilistic approaches that characterize risk in statistical terms and that quantitatively describe variable and uncertain aspects of the risk conclusions.

From a screening-level perspective, the hazard, exposure potential, and consequent risk associated with Bt corn pollen effects on monarch butterfly populations can be described from knowledge of pollen dispersal, milkweed distribution, protein expression levels in pollen, toxin bioavailability, timing and duration of pollen shed, timing and proximity of larval appearance, larval dose-response, and spatial-temporal distributions of Bt corn pollen and sensitive larval populations. As a first step, a deterministic risk assessment can be developed with discrete estimates for pollen dispersal, milkweed distribution, protein expression levels in pollen, and toxin bioavailability. This field-scale estimate, if conservatively bounded to account for uncertainties in the broader characterization of regional exposure and population-level effects, should provide a reasonable worst-case characterization of risk for the purpose of risk management decision-making. [A "reasonable worst case" is an estimate of the individual dose, exposure, or risk level received by an individual in a defined population that is greater than the 90th percentile, but less than that received by an individual in the 98th percentile in the same population (USEPA 2002).] More involved probabilistic assessments go beyond the deterministic assessment to inform the research enterprise as to sensitive, variable, and uncertain components of the risk characterization. This allows research to be focused on those aspects of the problem that will clarify uncertainties for sensitive components, thus allowing fuller interpretation of the risk conclusions with regard to overall monarch butterfly ecology.

The initial exposure and risk assessment reported here is based on reasonable worst-case input assumptions in the absence of specific effects and exposure data. It projects effect levels on the basis of statistical evaluation of overall lepidopteran sensitivity to Bt toxin and considers exposure to monarch butterfly larvae at the field-edge with pollen dissemination empirically described from published data. The conclusions are subsequently compared with refined effects and exposure data recently reported as part of a concerted effort specifically to assess risk of Bt corn pollen to monarch butterfly populations (Hellmich et al.

**Table 1.** Acute sensitivity of lepidopteran species to Cry1A(b) δ-endotoxin as determined in artificial diet studies

| Species (Common name) | $LC_{50}$ (μg/g) | Reference |
|---|---|---|
| *Manduca sexta* (L.) (tobacco hornworm) | 0.04 | MacIntosh et al. 1990 |
| *Diatraea grandiosella* Dyar (southwestern corn borer) | 0.08–0.15 | Song et al. 2000[a] |
| *Trichoplusia ni* (Hübner) (cabbage looper) | 0.19 | MacIntosh et al. 1990 |
| *Heliothis virescens* (F.) (tobacco budworm) | 0.2 | Luttrell et al. 1999 |
| *Pseudoplusia includens* (Walker) (soybean looper) | 0.67 | Luttrell et al. 1999 |
| *Helicoverpa armigera* (Hübner) (old world bollworm) | 1.55 | Chakrabarti et al. 1990 |
| *Spodoptera exigua* (Hübner) (beet armyworm) | 3.18 | Luttrell et al. 1999 |
| *Helicoverpa zea* (Boddie) (corn earworm) | 3.45 | Luttrell et al. 1999 |
| *Ostrinia nubilalis* (Hübner) (European corn borer) | 3.6 | MacIntosh et al. 1990 |
| *Agrotis ipsilon* (Hufnagel) (black cutworm) | >80 | MacIntosh et al. 1990 |
| *Spodoptera frugiperda* (Smith) (fall armyworm) | 95.89 | Luttrell et al. 1999 |

[a] Song, Q., C. Luppens, and X. Gan, 2000. Monitoring the susceptibility of the southwestern corn borer, *D. grandiosella,* to *B. thuringiensis* toxin Cry1Ab. Unpublished study submitted to EPA (part of Monsanto's 2000 IRM report). MRID # 453205-02.

2001, Oberhauser et al. 2001, Pleasants et al. 2001, Sears et al. 2001, Stanley-Horn et al. 2001).

## Effects Characterization

Specific data describing Bt protein effect on sensitive stages of the monarch butterfly have only recently become available (Hellmich et al. 2001). Because these data were not available during the screening-level risk assessment process described here, a conservative lower bound effect level is determined from the overall distribution of acute sensitivity for lepidopteran species. [The use of distributional analysis of effects in this manner to arrive at an effect endpoint is described in SETAC (1994).]

A database for lepidopteran sensitivity to bacterially expressed Cry1A(b) δ- endotoxin fed in an artificial diet provides the $LC_{50}$ for 11 species (Table 1). The lepidopteran species demonstrate >3 orders of magnitude variation in sensitivity to Cry1A(b). The most sensitive species are the tobacco hornworm, *Manduca sexta* (L.), and the southwestern corn borer, *Diatraea grandiosella* Dyar. The toxicities to species listed in Table 1 are used here as indicators for nontarget species of concern.

Lepidopteran species susceptibility (intra- and inter-genera sensitivity) to Cry1A(b) protein is graphically represented as a probability-log plot (Fig. 1). The high-end effect, representing the 90th percentile of sensitivity, is 0.03 μg/g (90% of species are less sensitive to this dose). The 90th percentile sensitivities are 31-fold lower than the species geometric mean $LC_{50}$ (0.95 μg/g).

## Exposure Characterization

Exposure characterization describes the physical, chemical, and biological factors governing the effective dose of Bt protein in the environment and leads to the development of an estimated environmental concentration (EEC). In lieu of actual pollen densities measured in and around cornfields, as recently reported by Pleasants et al. (2001), the approach used in this assessment is to estimate the concentrations of Cry1A(b) protein occurring on pollen receptors (milkweed leaf, the food source for the larvae) with

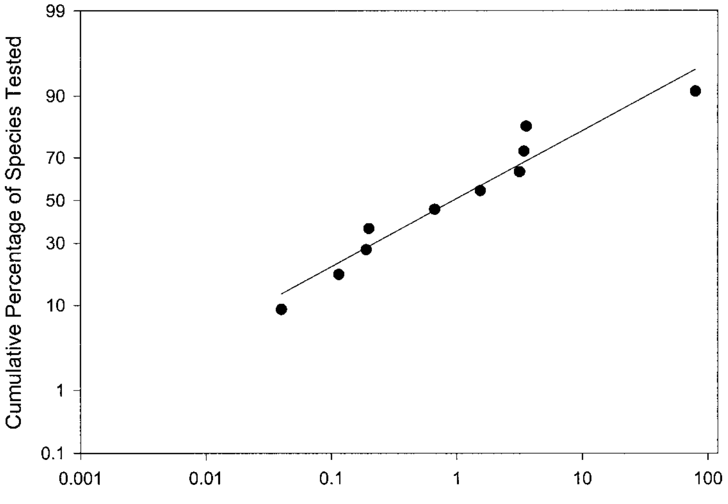

**Fig. 1.** Distribution of acute susceptibility of lepidopteran species to Cry1A(b) δ–endotoxin.

distance from the edge of corn fields on the basis of assumptions regarding (1) corn pollen dispersal with distance from the field edge, (2) levels of Cry1A(b) protein expression in pollen, (3) milkweed distribution and leaf biomass at the field edge, (4) physical properties of the pollen grain, and (5) spatial-temporal availability of toxin to monarch butterfly larvae.

**Off-Site Pollen Dispersal.** Corn pollen disseminates largely by gravitational settling because of its large size and density. Therefore, although corn pollen movement is typically quite limited, wind dispersal may lead to off-field transport of pollen within the immediate vicinity of corn fields. A robust body of published research quantitatively describes the degree of off-field corn pollen transport anticipated under a variety of environmental conditions within corn production regions (Mudra 1943, Jones and Newell 1946, Jones and Brooks 1950, Buller 1951, Haskell and Dow 1951, Raynor et al. 1970, 1972, Paterniani and Stort 1974). These data serve as the basis of pollen dispersal estimates in the current assessment and demonstrate that differing amounts of off-source corn pollen dispersal arise from differences in source size and wind speed (Raynor et al. 1972). We also can anticipate that the field microenvironment and corn genotype considered would further influence the timing, duration, and quantity of pollen shed.

Timing of pollen shed can be based on timing of tasseling (the VT growth stage) as predicted on the basis of growing degree-days. Timing of pollen shed is important relative to the temporal overlap of pollen shed and off-field dispersal with the occurrence of monarch butterfly larvae in the proximity of Bt corn fields. For the purposes of this assessment, we make the assumption that there will be significant overlap in the timing of pollen shed and the occurrence of second and third generation monarch butterfly larvae; therefore, timing of pollen shed is not considered consequential to exposure and risk.

Duration of pollen shed typically occurs over a 10–14 d period within a given field. Pollen shed usually begins 2–3 d before silk emergence (R1 stage) and continues for 5–8 d; peak shed occurs on approximately the third day. Pollen shed on a typical midsummer day occurs from 9 to 11 a.m. Cool, cloudy, or humid conditions may delay the daily onset of pollen shed. An entire field may take as long as 14 d to complete pollen shed because of variability in plant development (Lauer 1998). Under favorable environmental conditions, the vast majority of pollen will be shed in a 1–2 d period at the middle of this interval. In this assessment, corn pollen is considered to be conserved and accumulated (the environmental dose is not diluted by surface wash off of pollen or degradation of toxin) such that monarch butterfly larvae in the near-field zone are exposed to the maximum accumulated dose over time.

**Quantity of Pollen Shed.** Estimates of pollen production of normal corn plants range from $4.5 \times 10^6$ to $25 \times 10^6$ pollen grains/plant (Pohl 1937, Ogden et al. 1974, Paterniani and Stort 1974, Poehlman and Sleper 1995). As many as $10 \times 10^6$ pollen grains/d are produced for a plant at the peak of the flowering period (Coe et al. 1988), again emphasizing that pollen shed is concentrated over a very narrow time interval.

**Screening-Level Estimation of Pollen Dispersal.** The off-source flux data of Raynor et al. (1972) provide representative values of corn pollen dissemination for screening level exposure assessment. The data considered are for 20 pollen sampling events of 1.5–9 h duration over a 14-d interval. Three varieties of corn were interplanted (to increase duration of pollen shed) and plantings occurred on widely spaced hills (two plants/hill on 0.8 m spacing = 31,250 plants/ha). The experimental plot was circular (18.3-m diameter, area = 263 $m^2$) and off-plot pollen deposition and air flux were measured by receptors arranged along an 80° arc in the downwind direction. These data indicate that rapid pollen settling occurs as corn pollen is released from its source. Air flux measurements of corn pollen dispersion indicate <2% of corn pollen grains transport to distances >60 m from the field edge.

For the purposes of this assessment, data of Raynor et al. (1972) describing off-source air flux of pollen are scaled upward to represent conservatively off-source pollen deposition. Upper-bound estimates of dispersion were obtained by scaling these data to reflect $35 \times 10^6$ pollen grains/plant at a density of five plants/$m^2$ versus $17 \times 10^6$ pollen grains/plant and 3.1 plants/$m^2$ as reported by Raynor et al. (1972). Additional upward scaling of these data adjusts air flux measurements for actual ground deposition recoveries and season-long pollen release. When combined, these adjustments to the air flux data result in upward scaling by a factor of 9.4-fold to assure that pollen deposition estimates in the current assessment are sufficiently conservative. The estimated upper-bound scaling of pollen deposition as a function of offset from the field edge shows rapid declines in pollen deposition. Pollen deposition estimates range from $6.4 \times 10^7$ grains/$m^2$ in field to $3.0 \times 10^7$ grains/$m^2$ at a 1-m offset from the field edge and $1.9 \times 10^3$ grains/$m^2$ at 60 m. This represents a decline in environmental loading of pollen of >4 orders magnitude extending from radial distances of 1–60 m from the field edge.

**Toxin Concentration in Corn Pollen.** Three commercial corn events express Cry1A(b) protein. Event 176 corn has high pollen expression typically ranging in concentration from 1.14 to 2.35 μg/g of Cry1A(b) protein in pollen (CFIA 1996, Fearing et al. 1997), but levels up to 7.1 μg/g have been reported (Sears et al. 2001). Events Mon810 and Bt11 have negligible Cry1A(b) expression (≈0.09 μg/g; Hellmich et al. 2001). In the present assessment, a concentration of 2 μg/g Cry1A(b) is used, and represents a typical high-end estimate for Event 176 and >20-fold the expression level of Cry1A(b) in pollen of Mon810 or Bt11.

The distribution in pollen Cry1A(b) protein concentrations among pedigree lines as reflected in these data are used in the refinement of exposure and in sensitivity analysis. Commercial Bt corn lines are hybrid crosses of a line expressing Cry protein with an elite nonexpressing inbred line, thus toxin occurrence

**Table 2. Input assumptions and equations describing screening-level estimates of pollen-derived Cry1A(b) protein occurrence on milkweed**

| Input parameter | Value | Unit | Rationale |
|---|---|---|---|
| Pollen characterization | | | |
| Relative spherical diameter | 100 | μm/grain | High-end estimate |
| Density | 1.1 | g/cm$^3$ | Typical for bioaerosol |
| Cry1A(b) expression | 2 | μg/g (fw) | High-end estimate |
| Pollen deposition | | | |
| Total pollen | $8.9 \times 10^9$ | grains | Raynor et al. 1972 |
| Off-plot movement | 37 | % of production | Raynor et al. 1972 |
| Mass flux with distance from source | varies | % of off-plot movement | Raynor et al. 1972 |
| Milkweed characterization | | | |
| Leaf weight | 135 | g (fw)/plant | Typical value |
| Plant density | 1.5 | Plants/m$^2$ | High-end estimate |
| Pollen interception | 30 | % of pollen deposition | Typical value |
| Scaling factors | | | |
| Pollen production (SF1) | 2.06 | | $35 \times 10^6$ grains/plant |
| Air flux to ground deposition conversion (SF2) | 2.09 | | Raynor et al. 1972 |
| In-test to full anthesis conversion (SF3) | 1.37 | | Raynor et al. 1972 |
| Plant density (SF4) | 1.60 | | 5 plants/ha |

| Equation | Unit |
|---|---|
| Protein concentration | |
| (Expression) (Density) (Volume)[a] | μg/grain |
| Pollen deposition (with distance) | |
| (ΔMass flux) (Off-plot movement) (Total pollen) (Scaling factors)[b] | Grains/m$^2$ |
| Estimated Environmental Concentration (EEC) | μg/g (fw) |
| (Protein concentration) (Pollen deposition)/(Leaf weight) (Milkweed density) | |

[a] Volume = $(4/3)\pi$(Relative spherical diameter/2)$^3$.

[b] Scaling factors = (SF1) (SF2) (SF3) (SF4) = 9.6.

in 50% of the haploid corn pollen grains is considered in this assessment.

**Milkweed Distribution and Leaf Biomass.** Monarch butterflies oviposit on milkweed (*Asclepias* spp.) which constitute the sole food source for larvae. Common milkweed, *A. syriaca* L., is the dominant monarch butterfly food source throughout the high intensity corn production region of the Midwestern United States and Canada, where it frequently infests corn fields (Bhowmik and Bandeen 1976, Cramer and Burnside 1982). Early season milkweed infestation of corn fields at populations >8.8 plants/m$^2$ represents an economic threshold for control. When actively managed for weed control, corn fields have milkweed populations of ≈0.14 plants/m$^2$ at the time of pollen shed (Yenish et al. 1997). Agronomic practices are therefore assumed to maintain milkweed populations at <1.5 plants/m$^2$ in the near field edge. Although milkweed occurs in clusters at the field edge, the milkweed population is assumed to distribute evenly within the field edge zone considered in this assessment. This is conservative because all milkweed plants in the near field zone are assumed to receive pollen deposition when recent field studies show pollen deposition is dominantly in the prevailing wind direction (Pleasants et al. 2001).

Early season data collected from two field locations in 1999 are used to estimate the mass of milkweed leaves and their relationship to leaf surface area (data not reported). The leaf mass used for this model (135 g fresh weight/plant) is based on extrapolation from the early season measurements to mature milkweed plants (assumed to be ≈150-cm tall). The estimated values of milkweed plant density and leaf fresh weight at the near field edge are equivalent to a leaf area index (LAI) of 0.5 and leaf biomass of two metric tons/ha.

Thirty percent of pollen occurring within an off-field zone is assumed to deposit onto milkweed leaves. In this assessment, milkweed populations are fixed at 1.5 plants/m$^2$ uniformly distributed about the near-field edge. All milkweed plants are assumed to receive 30% of available pollen irrespective of location.

**Physical Properties of Pollen Grains.** The exposure assessment model requires conversion of Bt protein concentration in corn pollen from a mass toxin per mass pollen basis to a mass toxin per pollen grain basis. This requires assumptions regarding corn pollen density and volume. Pollen density ranges from just over one to ≈1.5 g/cm$^3$ (Pohl 1937, Funkhouser and Evitt 1959). A density of 1.1 g/cm$^3$ is considered representative of bioaersols and is used here (Cox and Wathes 1995). The effective spherical diameter of corn pollen is ≈90 μm (Jones and Newell 1948). In this assessment, a pollen grain volume of $5.24 \times 10^5$ μm$^3$ is assumed on the basis of a relative spherical diameter of 100 μm.

**Spatial-Temporal Availability of Toxin.** The present assessment assumes 30% of all corn pollen released off-source from a production field is intercepted and accumulated on milkweed leaves with no degradation or wash-off. This conservatively represents the availability of pollen containing Bt protein to monarch butterfly larvae.

**Environmental Loading and Estimated Environmental Concentration (EEC).** The foregoing assumptions (summarized in Table 2) allow for the computation of off-field environmental loading and EEC relevant to monarch butterfly larval exposure [con-

**Table 3. Environmental loading of corn pollen and Estimated Environmental Concentration (EEC) for Event 176 derived Cry1A(b) protein on milkweed leaves with distance from the field edge**

| Distance from source edge, m | Pollen load, grains/cm$^2$ milkweed leaf[a] | EEC, $\mu$g Cry 1A(b)/g (fw) milkweed leaf |
|---|---|---|
| 0 | 2111 | 0.060 |
| 1 | 890 | 0.025 |
| 2 | 21 | 0.0006 |
| 3 | 10 | 0.0003 |
| 4 | 6 | 0.00017 |
| 5 | 4 | 0.00011 |
| 6 | 2 | 0.00007 |
| 7 | 2 | 0.00005 |
| 8 | 1 | 0.00004 |
| 9 | 1 | 0.00003 |
| 10 | 1 | 0.00002 |
| 11 | 1 | 0.00002 |
| 12 | 0 | 0.00001 |
| 13 | 0 | 0.00001 |
| 14 | 0 | 0.00001 |
| 15 | 0 | 0.00001 |
| 20 | 1 | 0.00002 |
| 30 | 1 | 0.00002 |
| 40 | 0 | 0.00001 |
| 50 | 0 | 0.00000 |
| 60 | 0 | 0.00000 |

[a] Calculated pollen load rounded to the nearest whole number.

centrations of Cry1A(b) protein occurring on milkweed leaves] (Table 3). The pollen grain estimates on milkweed predict pollen loads on milkweed that fall rapidly from 2111 grains/cm$^2$ milkweed leaf at the field edge to 890 grains at a 1-m offset from the field edge and 10 grains/cm$^2$ at 3 m. At the field edge (as well as within the field), the EEC is 1/33rd the protein concentration expressed in corn pollen. The EEC falls off rapidly with distance from the field edge. It is 1/100th of pollen concentrations at slightly more than a 1-m offset from that field edge, and <1/1000th at 2 m.

## Risk Characterization

Second and third generation monarch butterfly larvae are expected to be present to varied degrees within corn production areas at the time of pollen shed. If larvae occurring near corn fields uniformly distribute within the immediate 60 m of the field edge, 99% of larvae will be present on milkweed occurring at radial distance >2 m from the field edge. Interposition of log-probability plots for lethal concentration ($LC_{50}$) of Cry1A(b) protein to lepidopteran species with the exposure-distance plot for regression of field offset on EEC in the near field zone shows limited risk (Fig. 2). The slope of the exposure-distance plot for EEC shows rapid fall-off in exposure with distance. Assumptions in this field-scale assessment for Cry1A(b) are event-neutral other than for differences in pollen expression level; therefore, the variation in EEC with distance for Mon810 and Bt11 parallels that for Event 176, but scales ≈22-fold lower because of the lower pollen expression of these events.

Risk quotients (RQ, the ratio of exposure concentration to a relevant effects concentration) express the relative magnitude of ecological risk associated with a stressor in the environment. In this assessment, the RQ's represent the EEC in and near the edge of Bt corn fields (Table 4) relative to the intra- and inter-genera 90th percentile $LC_{50}$ as a sensitive surrogate for effect to the monarch butterfly. These RQ's are $\leq 0.1$ for the monarch butterfly larvae occurring beyond 1 m from the field edge.

**Probabilistic Modeling of Monarch Butterfly Larval Exposure and Risk.** The screening-level assessment for Cry1A(b) protein occurrence on milkweed at the

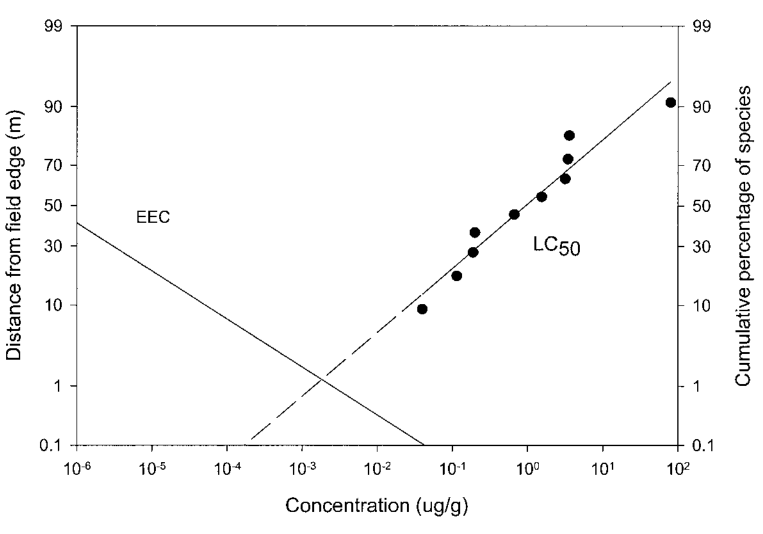

**Fig. 2.** Log-probability plots describing Cry 1A(b) protein toxicity to lepidopteran species in relation to the exposure-distance profile for milkweed leaves with surface deposits of *Bt* corn pollen at the near-field edge (0–60 m). EEC are < $LC_{50}$ for >90% of lepidopteran species at distances greater than ≈0.2 m off-source; EEC are < $LC_{50}$ for >99% of lepidopteran species at distances greater than ≈2 m off-source.

**Table 4. Comparative risk quotients (RQ = EEC/$LC_{50}$) for lethality of Event 176 pollen to neonate monarch butterfly larvae as developed from the deterministic screening-level assessment where exposure and effect are estimated, and from refined data which explicitly measure effect levels to the monarch butterfly and corn pollen densities on milkweed**

| Distance from field edge (m) | Risk Quotient (RQ) | |
|---|---|---|
| | Screening level[a] | Refined data[b] |
| in-field | — | 0.44–1.05 |
| 0 | 2.0 | 0.02–0.39 |
| 1 | 0.83 | 0.09–0.22 |
| 2 | 0.02 | 0.04–0.09 |
| 4–5 | 0.003–0.006 | 0.03–0.05 |

[a] $LC_{50}$ = 90th percentile of lepidopteran sensitivity, 0.03 $\mu$g/g artificial diet. EEC = $\mu$g Cry1A(b)/g milkweed leaf (Table 3).

[b] $LC_{50}$ = direct measurements for neonate monarch butterfly larvae; either 161 pollen grains/cm$^2$ milkweed leaf (Sears et al. 2001) or 389 pollen grains/cm$^2$ milkweed leaf (Sears and Stanley-Horn 2000). EEC = average pollen grains/cm$^2$ milkweed leaf (Table 2 of Pleasants et al. 2001).

near-field edge was considered further using Monte Carlo analysis (Crystal Ball Pro, Decisioneering, Inc, Denver, CO) to better evaluate input assumptions and their effect on exposure and risk estimates. Ten thousand iterations were performed for distributional analysis using the input assumptions shown in Table 5 to forecast the distributions in exposure (EEC on milkweed leaf) predicted at a 2-m off-set from the field edge. The results predicted a mean EEC of 0.0010 $\mu$g/g as compared with the screening estimate of 0.0006 $\mu$g/g at 2 m. The forecast showed significant positive skewness and high kurtosis, indicative that high-end estimates of pollen loading to the off-field environment, and the resulting EEC, are atypical events.

**Sensitive Components in the Assessment.** Clearly, the relevant effect level is a key uncertainty when assessing risk to monarch butterfly larvae in the absence of species-specific toxicity. The Monte-Carlo analysis indicated that further uncertainty in the screening-level assessment arises from the most sensitive components governing predictions of EEC concentration on milkweed leaves at the near-field edge (pollen interception by milkweed, pollen Bt concentration, and pollen shed/plant). Additionally, off-site pollen deposition, corn stand density, and physical properties of pollen, had a significant, but lesser, effect on these estimates of EEC. The conclusions arising from the screening-level assessment must, therefore, be couched in terms of the degree of uncertainty manifested in sensitive inputs. Recent research geared toward clarification of uncertainties in the risk assessment for the monarch butterfly has focused on these sensitive components.

## Comparison of the Screening-Level Risk Assessment with Refined Data

The high-end effect, representing the 90th percentile of lepidopteran sensitivity on artificial diet, as used in this screening-level assessment, is 0.03 $\mu$g/g. Hellmich et al. (2001) recently reported monarch butterfly neonate $LC_{50}$ on artificial diet of 0.0033 $\mu$g/g, a value 9.1-fold more sensitive than the estimate based on 90th percentile lepidopteran sensitivity. Indeed, the monarch butterfly is more sensitive to Cry1A(b) than any other lepidopteran species tested to date (Table 1 and Fig. 1). Although the 90th percentile of lepidopteran sensitivity on artificial diet in itself underestimates

**Table 5. Input distributions for probabilistic analysis of Bt corn pollen environmental dispersion and interception by milkweed leaves in the near field edge**

| Input distribution | Distribution type | Parameter | Value | Unit |
|---|---|---|---|---|
| *Pollen characterization* | | | | |
| Relative spherical diameter | normal | Mean | 100 | $\mu$m/grain |
| | | std dev | 10 | |
| Density | truncated normal | Mean | 1.1 | g/cm$^3$ |
| | | std dev | 0.1 | |
| | | lower bound | 1.0 | |
| Pollen production (SF1) | triangular | minimum | $1.0 \times 10^7$ | grains/plant |
| | | likeliest | $1.8 \times 10^7$ | |
| | | maximum | $4.0 \times 10^7$ | |
| Cry1A(b) expression | triangular | minimum | 1.00 | $\mu$g/g (fw) |
| | | likeliest | 2.00 | |
| | | maximum | 5.00 | |
| *Pollen deposition* | | | | |
| Off-plot movement | normal | mean | 37 | % of production |
| | | std dev | 4 | |
| Air flux to ground deposition conversion (SF1) | truncated normal | mean | 2.41 | Scaling factor |
| | | std dev | 0.91 | |
| | | lower bound | 1.00 | |
| *Milkweed characterization* | | | | |
| Pollen interception | triangular | minimum | 0 | % of pollen deposition |
| | | likeliest | 30 | |
| | | lower bound | 1.00 | |
| *Corn production system* | | | | |
| Plant density | triangular | minimum | 45,000 | plants/ha |
| | | likeliest | 50,000 | |
| | | maximum | 70,000 | |

toxicity to the monarch butterfly, the assumptions we use here in the screening-level risk assessment are scaled conservatively in consideration of exposure and effect as manifested in the field. This can be shown through conversion of the 90th percentile of lepidopteran effect, used here as a surrogate value for monarch butterfly sensitivity into a pollen density in the field. Hellmich et al. (2001) present bridging calculations for conversion of an $LC_{50}$ determined from dietary incorporation of pure protein to an $LC_{50}$ expressed on the basis of pollen grains/$cm^2$ of receptor leaf. On the basis of these calculations, the 90th percentile effects level used herein for monarch butterfly neonate larvae is 391 grains/$cm^2$ leaf [(0.03 $\mu$g Cry1A(b)/g diet)(0.0033 g diet/d)(1,500,000 grains pollen/g pollen)/((2 $\mu$g Cry1A(b)/g pollen)(0.19 $cm^2$ leaf/d))]. Estimated effects are of the same order as reported for direct feeding of Event 176 (approximate $LC_{50}$ of 389 and 161 grains/$cm^2$; Sears and Stanley-Horn 2000, Sears et al. 2001).

Furthermore, the corn pollen exposure concentrations predicted in this screening-level assessment can be compared with recent field data of Pleasants et al. (2001), who made >5,000 individual measurements of corn pollen on leaves of milkweed plants from throughout the Corn Belt. Pollen density averaged 170.6, 63.1, 35.4, 14.2, and 8.1 grains/$cm^2$ inside the source corn field, 0, 1, 2, and 4–5 m from the edge of the field, respectively (Pleasants et al. 2001). The screening-level approach used here predicts exposure concentrations of 2111, 890, 21, and 5 grains/$cm^2$ at 0, 1, 2, and 4–5 m from the field edge, respectively.

When the RQ's for the screening level estimates of exposure and effect and are compared with actual measurements of exposure and effect from Event 176 pollen (Table 4), the screening-level estimates somewhat over-estimate risk at the near-field field edge (0 and 1 m from the field edge) and slightly underestimate risk at two and 4–5 m from the field edge. Both assessments show that risk to the monarch butterfly is restricted to the field or immediate field extremity, because the RQ's are <0.1 beyond 1 m from the field edge. The screening-assessment, which attempts to estimate reasonable worst-case exposure and effects, seems sufficiently conservative to identify the focal area for concern as the field and the immediate field extremity. This has indeed proven to be the focus for refined measurements of Bt corn pollen effects on monarch butterfly (Sears et al. 2001).

## Discussion

Risk associated with nontarget exposure to Cry1A(b) corn pollen has been assessed for monarch butterfly larvae feeding on milkweed (*Asclepias* spp.) in the near-field edge of cornfields using a deterministic screening-level assessment based on conservative input assumptions which considered exposure at the field-edge. Pollen dissemination to the field edge and estimated environmental concentrations were empirically described on the basis of published data. Corn pollen characterization and deposition patterns pose methodological uncertainties in this assessment; therefore, the exposure assessment was subsequently modified to allow for a probabilistic analysis of uncertainties and sensitivities in the assessment. Recent field research supports the validity of the estimated environmental concentrations developed to date (Pleasants et al. 2001).

Effects characterization based on the distribution of acute susceptibility for lepidopteran species underestimated hazard when compared with the $LC_{50}$ measured for neonate monarch butterfly fed artificial diet. The published susceptibility data we used focused on targets for insect control. Therefore, it was a limitation in our approach. Broadening of toxicity testing to consider relevant nontargets in addition to targets likely would help to improve estimates. Activity of Cry proteins on insects within an order is not easily predictable; therefore, further research to better understand selectivity of this class of insecticidal protein can benefit nontarget risk assessment. However, our effect estimate presented here, when scaled to the field environment, produced an estimate of toxicity in terms of corn pollen density on milkweed that is comparable to values measured using Event 176 corn pollen (Sears and Stanley-Horn 2000, Sears et al. 2001). In addition, we have used an effect to neonate larvae, which will be the most sensitive larval stage exposed to pollen.

A recent risk assessment of Bt corn pollen effects on monarch butterfly supports that Cry protein expressed in corn pollen does not pose a serious risk to monarch butterfly populations (Sears et al. 2001). This is because only a small fraction of the population will be exposed to pollen at a level that affects developing larvae. The much lower potency of Mon810 and Bt11 pollen compared with Event 176 pollen further reduces the risk posed by exposure of Cry1A(b) protein to monarch butterfly larvae.

Formalized approaches to ecological risk assessment using quantitative analysis to evaluate statistically the probability of exposure, effect, and consequent risk show that susceptibility of monarch butterfly larvae to Bt corn pollen is a field-scale concern restricted to the corn field or near-field edge. The screening-level assessment developed here is formulated around the problem of determining the scale of concern (at most a field scale versus a landscape or regional scale concern) sufficiently for risk management decision-making. The methodology is shown to be robust in that the risk conclusions are supported by actual measurements of effect and exposure. Public and scientific interest in the impacts of Bt corn on the monarch butterfly led to reformulation of the risk assessment problem to consider no effect levels within Bt cornfields (Shelton and Sears 2001), necessitating substantial generation of data to afford higher level probabilistic assessment of risk (Sears et al. 2001).

The methods used in this assessment are widely adaptable to a variety of nontarget exposure and risk concerns and are consistent with well-recognized scientific and policy frameworks. This approach can be employed usefully to evaluate risks to other nontarget species, provided there is a body of data allowing estimates of hazard and exposure for the stressor of concern. Conservatively employed quantitative risk assessment overcomes the constraint of limited data for exposure and effects characterization and allows for resources to be used in a timely and effective manner to support well-informed risk management decision-making.

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