



Ecological Safety Considerations

The Environmental Risk Assessment (ERA)

BIGMAP

Biosafety Institute for Genetically
Modified Agricultural Products

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ERA

This module is comprised of three components –

.ERA Framework

.Gene Flow

.Non Target Organisms

Environmental Risk Assessment

The release of a **Genetically Engineered** plant to the environment requires consideration of the environmental safety of the GE plant within the context of the scale, nature, and region of deployment.

Environmental Risk Assessment (ERA) is the process which evaluates risk as the likelihood for an undesired consequence to be manifested under realistic conditions of exposure.



ERA considers the impact of introduction of a GE plant into a given environment.

Specific questions that are commonly addressed in the ERA for most GE plants:

- Does the modification of the plant cause it to have attributes commonly associated with weeds in managed environments? Invasiveness in natural environments?
- Will the transgenic element in the GE plant move into native plant populations? And so what if it does?
- Will the GE plant adversely impact non-target organisms that may be of special interest because they are beneficial, endangered, threatened, or charismatic?



Principles of ERA

The well-established principles of ERA are applied to the potential environmental risks of biotechnology through a process that

- Defines the regulatory need (problem context)
- Describes relevant concerns for analysis (problem definition);
- Estimates the likelihood of exposure (exposure characterization);
- Evaluates the consequence of exposure (effects characterization); and
- Formulates an understanding of degree of risk (risk characterization).

<http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?did=12460>



Risk—Likelihood of an unwanted outcome

- Risk is the likelihood that there will be an undesired consequence when the GE plant is present in the environment.
- Fully quantitative ERA describes risk as a probability of exposure to the GE plant and the undesired consequence of the exposure.
 - A probability ranging from zero to one
- More frequently risk is described as a likelihood or degree of concern based on a comparison of the GE plant and its uses to similar non GE plants and their uses.
 - There are high, low, or negligible concerns regarding the GE plant and its proposed use

There is always some degree of risk

Risk of anything is zero only in the absolute absence of exposure

Thus for the GE plant risk is comparative – it asks

Is the GE plant and the way it will be used riskier than the comparable non-GE plant and its uses with which we are familiar?



ERA focuses on change

The undesired consequence we evaluate in the ERA is focused on a specific change that has been brought about with genetic transformation

This change may be do to

- A stressor
 - the changed attribute of the GE plant
 - for instance, an expressed protein
- Or an action
 - environmental release of the transgenic plant
 - for instance, release of a GE plant into a particular environment

ERA is a tiered process

The ERA ideally proceeds in tiers of increasing complexity

- lower tiers focus on stressor-mediated effects in laboratory and glasshouse settings with well-controlled conditions
- higher tiers focus on action-mediated effects in semi-field and field environments which are more realistic but less well-controlled.

Problem Formulation

Problem formulation is a formal process whereby relevant considerations for risk assessment are determined.

Problem Formulation considers –

- **Problem context – establishes the parameters for the risk assessment, including policy goals, scope, assessment endpoints and methodology.**
- **Problem definition – distills risk questions into tractable problems for analysis**

Comparability

The problem formulation develops the plan for the ERA by first developing a baseline of comparability

- To what degree are the host crop and the expressed attribute familiar?
- Is the GE plant substantially equivalent to the non-GE plant in its composition and intended use?
- If yes, the ERA can proceed with a focus on the changed attribute of the GE plant

Are the GE and non-GE crops the same?

The problem formulation should establish that the particular GE plant is substantially equivalent to the comparable non-GE plant as it is encountered and used in present-day agriculture

Data (found in the literature and/or generated and submitted by the product developer) provides the basis for the determination of substantial equivalence.

In most regulatory schemes, precursor information has already been considered in the regulatory dossier; the ERA is found as an annex to the dossier that considers ecological safety *only once the substantial equivalence has been demonstrated*. A good example of this process can be found in EFSA guidance for GE plant risk assessments. http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620775747.htm

Precursor information for use in Problem Formulation

Precursor information establishes that other than of an changes the GE plant is equivalent to non-GE comparators.

Once equivalence is established on the basis of the GE plant characterization, the ERA is conducted with emphasis on the change.

For instance, in the problem formulation for a non target insect ERA, precursor information describes

- the characteristics of the donor and recipient organisms;
- the genes inserted and their expression;
- agronomic performance and characteristics;
- equivalence of the plant expressed protein to the wild counterpart;
- compositional characteristics (nutrients and antinutrients).

This information is found in published studies and data submitted from product developers and is integrated with expert opinion and stakeholder deliberations to determine the risk hypothesis to be tested, the endpoints for consideration, and the scope of the analysis plan.

An example of using precursor information to focus the ERA

Cry1 Bt toxin expressed in corn for which an ERA is needed for approval of an unconfined environmental release.

The problem considers the degree to which the host crop (corn) and the expressed product (a Cry1 protein) and their combination are familiar (well-understood) in terms of

- history of use;
- scientific knowledge;
- prior regulatory considerations; and
- unique aspects of the environmental release that is being considered.

In this case,

- Corn biology, production, and use are well-understood
- The GE corn will not alter corn biology, production, and use
- The change involved is to produce Cry1 proteins which are selectively active on Lepidoptera,
- The specific selectivity of the Cry1 protein can be established from literature and/or developer data
- The history of use of Cry1 proteins in other GE plants and sprayable biopesticides is well-understood
- There is 10+ years of experience in the environmental release of Cry1 Bt corn throughout various regions of the world

Risk hypothesis

- The risk hypothesis represents an assumption regarding the cause-effect relationships between sources, changes, exposure routes, endpoints, responses and measures relevant to the ERA.
- A tentative explanation taken to be true for the purpose of argument or investigation
- Not to be confused with scientific hypotheses which are specific, testable postulates (these are a part of the analytical phase of the ERA)
- The ERA process for GE plants is comparative, so the risk hypothesis considers the comparative difference as it relates to exposure and the undesired consequences of exposure

The analytical plan

Addresses the specific risk hypothesis

Describes various measures to be used in the assessment and the characterizations that form the body of the risk assessment in terms of

- proscribed studies to be conducted,
- the appropriate tier for analysis,
- the appropriate risk formulation, and
- specific decision criteria that will be used for risk characterization.

The analysis phase

Has three main parts

- characterization of exposure;
- characterization of effect (a consequence of exposure); and
- characterization of risk (an undesired consequence of exposure given that exposure occurs).

Effects characterization

The specific adverse effect of interest has been identified in the problem formulation.

In characterizing effects, the risk assessor seeks information establishing a specific adverse effect (or lack there of) of the transformation in the GE plant.

The effects characterization will use data generated at various tiers (Tiered process example) depending on the nature of the problem and the uncertainty.

Exposure characterization

Establishes the source, duration, intensity, and duration of exposure on the basis of expression data as well as knowledge of the crop, its management, and the environment where it will be released.

This phase of analysis can also proceed in tiers beginning with estimated environmental concentrations that are modeled from knowledge of the GE plant and the environment where it will be deployed, through to higher tiered field measurements of actual environmental concentrations

Environmental fate is critical to exposure characterization

Environmental fate describes what happens to the transgene and its expressed product in the environment.

Two relevant examples are soil degradation and gene flow.

- If soil degradation studies show that residues of the transformed plant are not likely to persist or accumulate in the soil, then there is negligible exposure and little reason for concern that soil organisms will be at risk due to long term exposures (this is the typical case for Cry proteins released to the environment).
- If gene flow studies show that there is no stable introgression of the transgene into a receiving population, then there is no route for environmental exposure due to gene flow and limited concern for long term effects from this route of exposure.

In both of these cases, exposure is unlikely and therefore risk is negligible.

Risk characterization

In the final stage of the analysis, risk is characterized from consideration of the effects and exposure characterizations.

The risk characterization makes a statement, with respect to the risk hypothesis, regarding the likelihood for an undesired consequence to be manifested under realistic conditions of exposure.

The risk conclusion compares the GE plant and its conditions of environmental release with the non-GE plant and the prevailing conditions of its use.

It is common in for GE plant ERA for the result to be a qualitative lines-of-evidence determination which will express risk as a likelihood using terms such as high, moderate, low, or negligible.

Risk conclusion

In the final phase of the ERA, risk conclusions are drawn on the basis of the specific problem formulation and analysis.

The risk conclusion makes explicit statements regarding what is known, variable, uncertain, and sensitive in the risk estimate.

The ERA at this point may additionally suggest mitigation options that can be implemented to further reduce the degree of risk identified – or the level of uncertainty in outcomes.

For instance, a common risk mitigation is to implement post-commercial monitoring to verify the integrity and adequacy of the risk estimate and to allow for reassessment should concerns be identified.

Regulatory considerations

Numerous and sometimes overlapping regulatory requirements may be involved in the ERA.

Depending on the type of environmental release and the political jurisdiction of the release, the competent authorities involved may include institutional to national biosafety committees;

And the regulatory standards may be country-specific, regional (as within the EU, NAFTA, or other regional compacts), or international (the Cartagena Protocol on Biosafety, CPB) in scope.

Despite this complexity, there are common elements.

A current important consideration for the design and conduct of the ERA is implementation of risk assessment that is consistent with Annex III of the CPB.

Scale considerations for the ERA

This module has specifically addressed the ERA process for GE plants intended for ‘releases into the environment’ which refers to field releases outside the confines of a highly contained environment such as a laboratory or greenhouse.

Such environmental releases range from small-scale confined field trials, to larger scale production releases, to unconfined commercial releases.

The size and nature of confinement influences the magnitude of potential exposure and therefore the type of ERA questions

For instance, confined field trials are of limited scale – both in terms of plot size and numbers of locations – and therefore may represent minimal potential for exposure, such that for a familiar host and donor, risk is negligible and trials may take place under notification using limited product characterization data.

On the other hand, a commercial release will require comprehensive product characterization along with specific information on the host, the introduced gene, and the environment where the plant is released and managed.

ERA is science-based

Risk assessment as a science-based activity occurring within the overall process of risk analysis (which also considers risk management and communication).

Many national and international regulatory standards tend to intermix risk assessment and risk management under guidance for risk assessment.

For example, the EFSA guidance for GE risk assessment includes provision for general surveillance monitoring as a risk management activity unrelated to the science-based evaluation of exposure and its consequence.

Ecological considerations

The ERA process is a flexible framework for addressing any nature of concern that arises from a case-specific instance of GE plant environmental safety assessment.

The problem formulation phase determines those concerns relevant to environmental safety and distills the concern into a risk hypothesis that can be characterized. The process therefore is highly flexible.

Experience with GE plants that have been assessed and commercialized to date identifies certain base ecological considerations that need be explicitly considered in undertaking the ERA, especially with respect to common regulatory concerns. These are gene flow, weediness, and adverse effects to non-target organisms. Each of these are described in the remaining presentation with examples of how they have been addressed through ERA.

Gene flow

Gene flow is a natural biological phenomenon important for maintaining genetic variation. In a strict sense it refers to gamete transfer between populations.

Gene flow represents a route of exposure and is addressed as part of the exposure characterization.

Gene flow is a route of exposure

Gene flow can disperse the product of transgene expression to other varieties of the same crop (crop-to-crop), to following crops (via dormancy and volunteering), to non-agricultural habitats (invasiveness or weediness), or to other species (crop-to-wild, horizontal gene transfer).

Relevant risk hypotheses developed within a given ERA would reflect one or more of these considerations. Crop-to-wild considerations are the most commonly evaluated concern with respect to environmental safety and transgene flow, therefore, they are the focus of this section.

Gene flow

Key questions asked when considering exposure via gene flow

- Will the GM plant become a weed in agroecosystems or invasive in natural ecosystems?
- Will gene flow to another plant make it weedy (or weedier) or (more) invasive?
- Will hybrids of the GM plant with a native plant cause native plant extinctions?
- Will gene flow into wild population have adverse impacts on non-target organisms?

Gene Flow in relation to AP

Adventitious presence (AP) refers to the unintended low level occurrence of transgenic elements in foods, feeds, or seed.

Regulations or permit conditions may seek to control AP, but these concerns are not of direct relevance to the ERA per se.

In some cases AP may relate to gene flow, but in most cases it is due to mixing of seeds and plant materials in distribution channels.

In practice some regulatory statutes may include AP considerations within the ERA. And, in fact, the nature of the data and assessment is in many ways similar to the concerns that are addressed for specific environmental considerations.

What is the consequence of gene flow?

Since gene flow is simply a route of environmental exposure, the demonstration of gene flow in terms of crop-to-wild gene transmission must be further addressed as to the consequences of the gene flow.

For there to be undesired consequences from exposure through gene flow; the gene flow must lead to the stable introgression of the gene into the wild population and the expression of the transgenic product must result in an adverse environmental impact.

Example chain of events for an undesired consequence of gene flow

confined field
release

pollen release
at source

pollen flow
to receptor

sexual
compatibility

niche

undetected
out-crossing

stable
introgression

expression

biomagnification

movement into
food supply

adverse
effect

concerns

consequences

A break anywhere
along the
exposure chain
means the adverse
consequence will not
occur

A gene flow example

Cultivated and wild sunflower commonly co-occur within the environment in the Midwestern and Western United States (sunflower's center of origin).

In fact, wild and feral sunflower, and domestic wild hybrids of sunflower commonly occur in and among fields of cultivated sunflower.

Sunflower is a highly out-crossing species; thus, gene flow is a consequential route of exposure. Therefore, environmental release of a transgenic sunflower would clearly result in the exposure of wild populations to the transgene via gene flow.

The consequences of this exposure can only be understood if in a stepwise fashion the risk assessment determines there is stable gene introgression into the wild population and that the introgressed gene results in expression of the stressor. Stressor expression may increase the weediness or invasiveness of the wild plant. For instance, gene flow leading to transfer of a lepidopteran-active Bt gene to a wild sunflower population might increase the ability of the wild population to be protected from natural pests (weevils). And this in turn could adversely impact non-target organisms.

For this hypothetical example, the effect to wild sunflower populations would become a consideration for the weediness assessment and the impact to non-target would be a consideration in the non-target organism assessment.

Further discussion of this sunflower example as well as other examples of gene flow considerations can be found here: [Ecological Effects of Pest Resistance Genes](#)

Weediness & Invasiveness

The concept of weediness relates to the potential for the transformed plant to establish as a weed in agricultural environments

Similarly, invasiveness relates to establishment in natural environments.

The assessment of weediness or invasiveness potential can be understood within the context of three categories of undesired consequences:

- competition;
- acting as hosts of disease when no crop is present (green bridges); and
- quality impacts on harvested crops (contamination).

What makes a weed a weed?

Crops plants have been highly domesticated and, therefore, do not exhibit the commonly understood attributes of a weed; namely, for weeds

1. Germination occurs in many environments;
2. Germination is discontinuous with great longevity of seeds;
3. There is rapid plant growth from vegetative phase to flowering;
4. Seed production is continuous throughout the growing season;
5. Plants are self-compatible, but not completely autogamous or apomictic;
6. If cross-pollinated, pollinators are unspecialized or pollen is windborne;
7. Very high seed production occurs in favorable environments;
8. Some seed will be produced under a wide range of environmental conditions;
9. Seed is adapted for short- and long-range dispersal;
10. If a perennial, has the plant has vigorous vegetative reproduction or regeneration from fragments;
11. If a perennial, the plant is not easily drawn from the ground; and
12. The plant is able to compete interspecifically by special means (such as, rosette, choking growth, allelochemical production).

Weediness assessment

During development of GE plants, greenhouse and field observations are taken over multiple environments and several generations. GE plants that have performance inconsistent with the comparable non-GE plant (including weediness traits that make them unsuitable as crops) will be withdrawn from development. There will be substantial data provided by GE plant developers for regulatory assessment of ecological safety, this data describes the phenotype and its variation over environments and generations as well as adaption to stress. These data serve as the base of information for the weediness assessment.

The weediness assessment should consider impacts on both managed ecosystems where there are implications for management strategies and for natural ecosystems where feral plants might become invasive by effectively competing to displace niche species.

Confinement to manage risk

Regulatory guidelines have established measures to be taken to reproductively isolate regulated plants from non-regulated reproductively compatible ones through a confinement management process.

Post-harvest monitoring and land use restrictions along with confinement measures seek to mitigate movement of the transgene into the environment.

Confinement to manage risk

A confined field trial is an activity done in an open field, which is an essential step in development of new varieties. It allows performance characterization of the new plant line in natural environment. The size of the trial field is usually less than 1 ha.

Confinement measures are taken to prevent persistence of the regulated test article or their progeny in the environment and in food supply by physical and reproductive isolation. These measures are based on reproductive features and seed dormancy characteristics of the plant species under question.

Confinement considerations

Site Selection: Conducting the trial in a sufficiently isolated location is critical. USDA-APHIS determines isolation distances on a case-by-case basis using isolation distances determined by Association of Seed Certifying Agencies (<http://www.aosca.org/>) for maintaining seed stock purity as starting point.

Important considerations are

- Ability of the plant species to cross pollinate
- Pollen-dispersal distance
- The mechanism of pollen dispersal
- The presence and distribution of wild species that could cross pollinate with the GE plant
- Whether any members of the genus of the GE plant species is a known weed

Ways pollen flow and seed dispersal are managed

Pollen flow:

1. Pollen or pollination proof caging
2. Bagging prior to flowering
3. Removing the flower before pollination
4. Use of border rows to dilute the transgenic pollen
5. Use of male sterile lines
6. Use of plant growth regulators to block reproductive development
7. Temporal isolation through dissimilar planting and flowering times.

Seed dispersal:

1. Isolation from crops of the same type
2. Netting or fencing to prevent access of animals to the trial site
3. Using screens or levees to prevent spreading in irrigation water

Source: Draft Guidance for APHIS Permits for Field Testing or Movement

Post-harvest confinement measures

Depending on the plant species used these may include

- Autoclaving or on-site burning and/or burial of any plant material removed from the field and brought to analysis or processing location after testing.
- Treating any remaining viable plant material after harvest with herbicide.
- Monitoring for volunteer plants in the next growing season.

Non-target organisms

Adverse effects to non-target organisms (NTOs) is an additional relevant ecological concern with respect to ecological safety.

NTOs are a special subset of ecological entities of concern.

The ERA process is designed to accommodate considerations of ecological entities of concern other than NTOs, but the assessment process is best described for NTOs

An example of ERA for GMOs and NTOs

The way that the NTO risk assessment is conceptualized and conducted has already been described for the example of a Cry1 Bt toxin expressed in corn.

This particular type of consideration is in fact the most thoroughly developed in terms of application of an ERA framework. Several examples where NTO ERA has considered risks to sensitive non-target butterflies and biocontrol organisms can be found in the published literature and extends to all tiers of study design and assessment.

Monarch Butterfly and Bt Corn

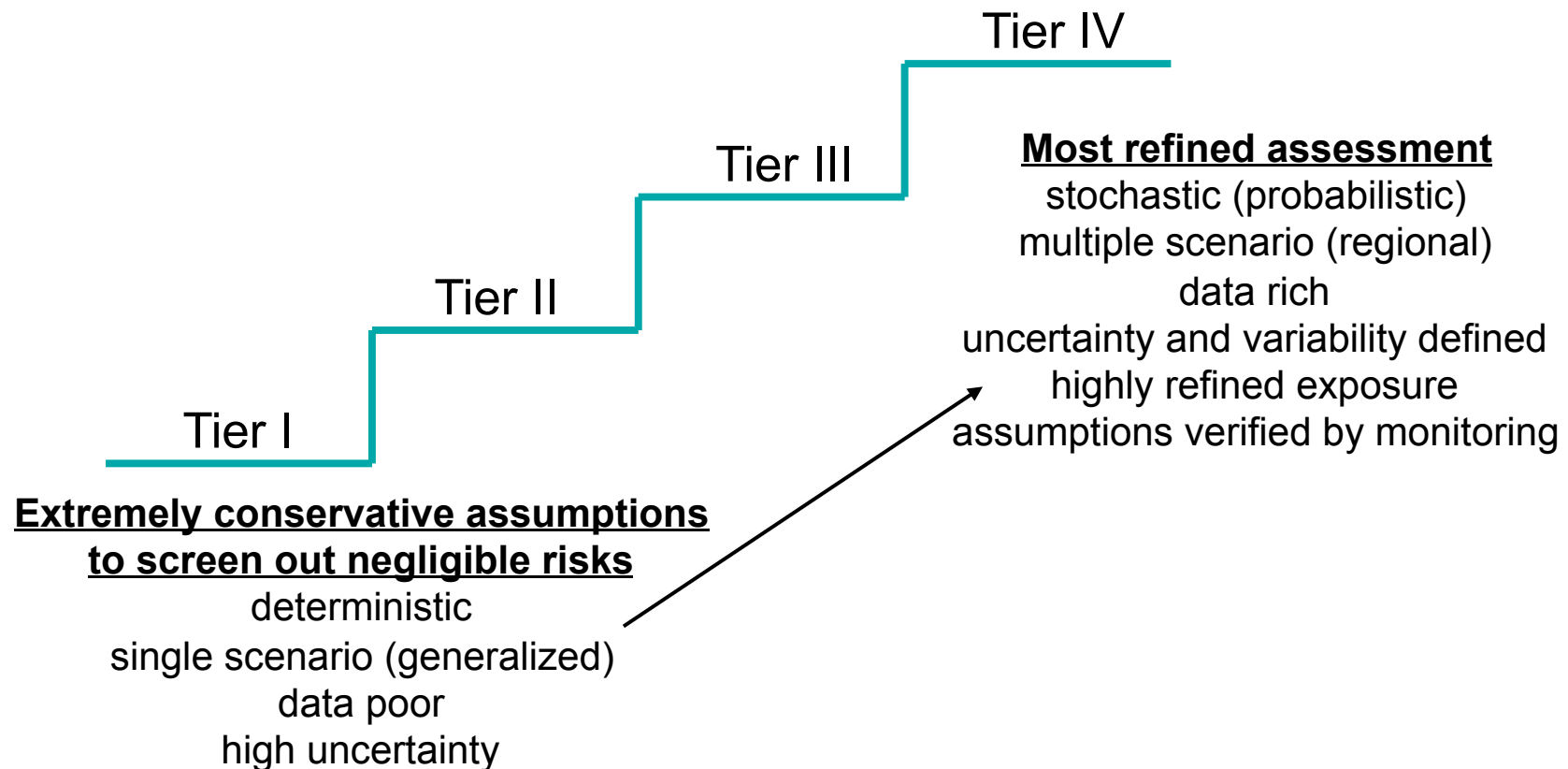
NTO risk assessments can take many forms depending on the nature of concern being evaluated.

A common attribute of these assessments is the use of a tiered process. This module presents the specific case of tiered assessment in NTO risk assessment.

This specific case uses quantitative information and therefore is termed a Quantitative Risk Assessment (QRA).

risk assessment is recursive and proceeds in tiers

example for non-target organisms (NTO)

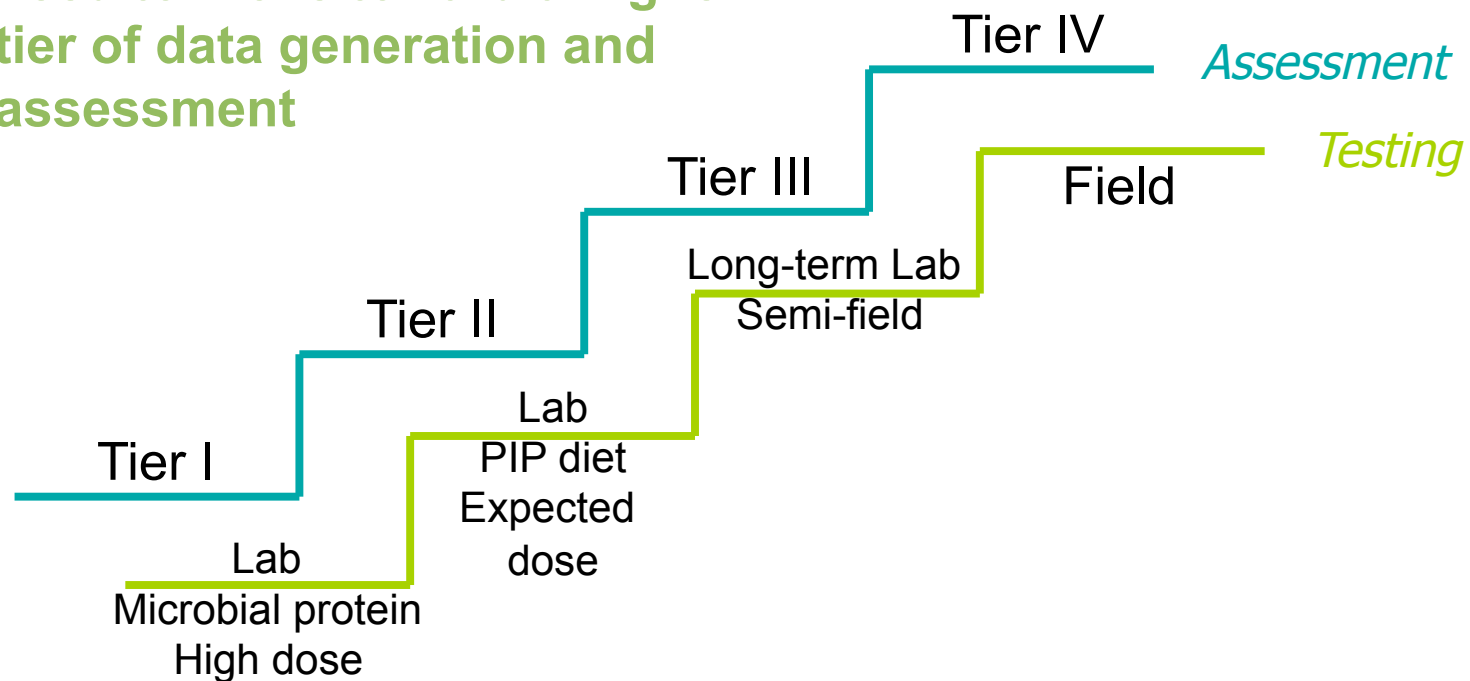


risk assessment is recursive and proceeds in tiers

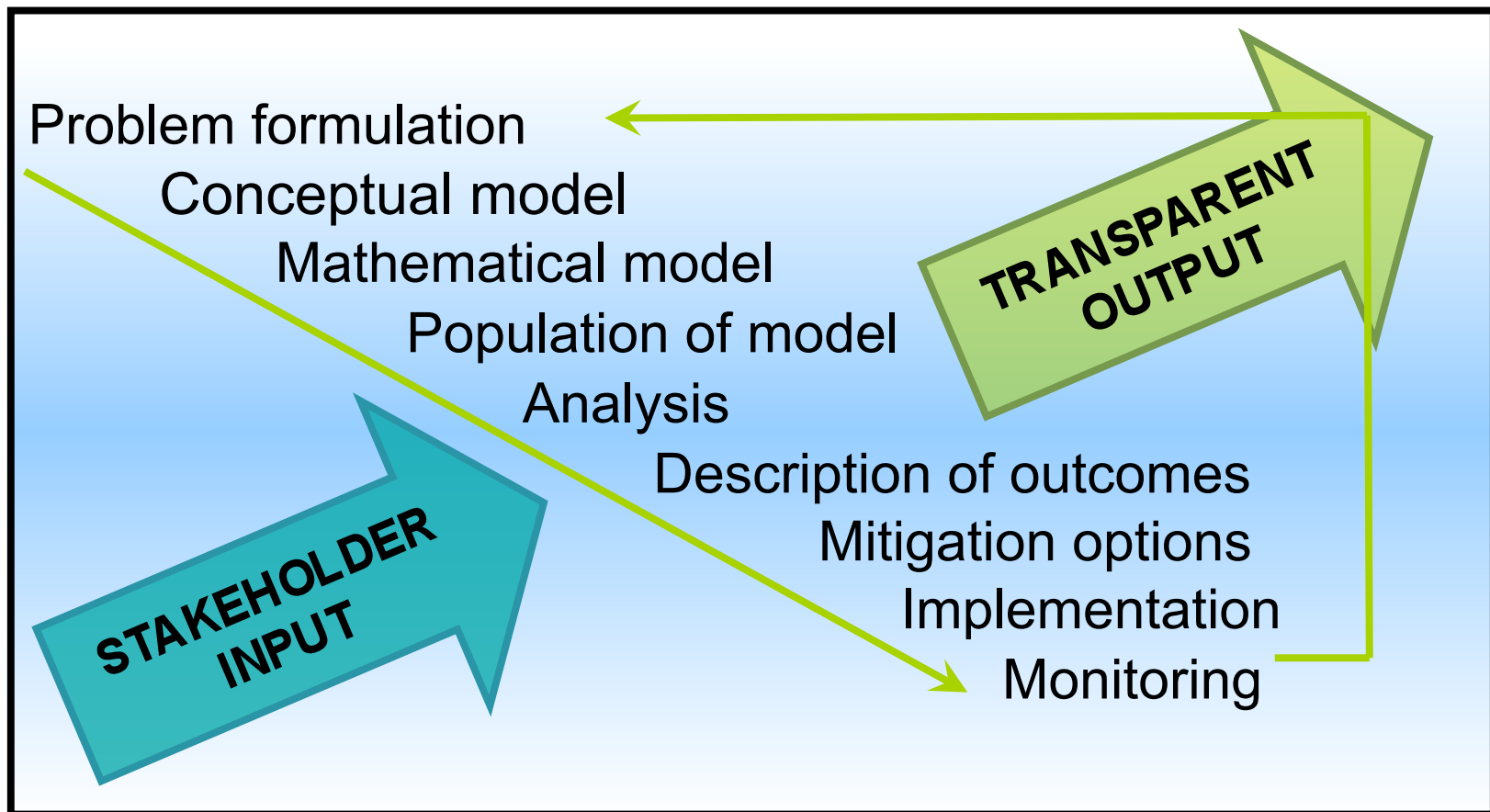
Tier	Description	Scenario(s)
I	<i>Screening case. Deterministic. Uses metamodels or empirical information</i>	Worst case assumptions: most vulnerable environment and inputs representing the upper 95-98th percentile of exposure and effect
II	<i>Partially stochastic. May use metamodel or physical model</i>	Reasonable worst case representing high end exposures and effects (90 to 95th percentile). Typical case w/ average values and/or distributions for the "typifying" environment.
III	<i>Fully stochastic. May incorporate the physical model into a patch model or a GIS.</i>	Multiple scenarios representing the breadth of anticipated uses. Geographic information reflects the use landscape or use region.
IV	<i>Field monitoring. May occur in conjunction with refined tier III model.</i>	Monitor and respond.

tiers of assessment & tiers of testing

- level of concern
- degree of uncertainty
 - ... arising from a lower tier of assessment drives the need to move toward a higher tier of data generation and assessment



flow path for a QRA



assessment considerations

At each tier of assessment, the risk assessor determines

- the nature of the **problem**;
- the nature of the effect (**hazard**);
- the nature and magnitude of the **exposure**;
and,
- the **risk**, which is a joint consideration of the exposure and effect

Retrospective case of tiered NTO risk assessment – monarch and Bt corn

- **Gist of EPA's original analysis (pre-1999):**
 - is there an adverse effect of Bt corn on monarch butterfly? (**problem**)
 - Bt (Cry1Ab) protein expressed in corn is toxic to lepidopteran insects
 - monarch butterflies are lepidopteran insects
 - \therefore Bt (Cry1Ab) protein expressed in corn is toxic to monarch butterflies (**hazard**)
 - there is limited **exposure**
 - **risk** is negligible
 - *risk formulation is a weight-of-evidence analysis*

tier 0 RA

Tier 0 [problem scoping]

Losey et al. 1999. *Nature*, 399, 214

- **hazard** (intrinsic toxicity) to monarch empirically established
- **exposure** route elaborated (indirect exposure via pollen)
- overarching **problem** formulated (manifestation of harm through indirect exposure)
- **risk** was not characterized

tier 0 QRA

Tier I/II

Wolt et al. 2003. *Environ Entomol*, 32:237-246

- **problem** considered harm to individuals at field scale
- potential **hazard** inferred from interspecies distribution of effect
- **exposure** estimates based on synthesis from peer review literature
- **risk** formulated as a simple empirical relationship
 - risk formulation

$$RQ = EEC_d / LC_{50}^{90}$$

where,

EEC_d is the estimated environmental concentration at distance (d) from the field

LC_{50}^{90} is the 90th centile probability of toxicity

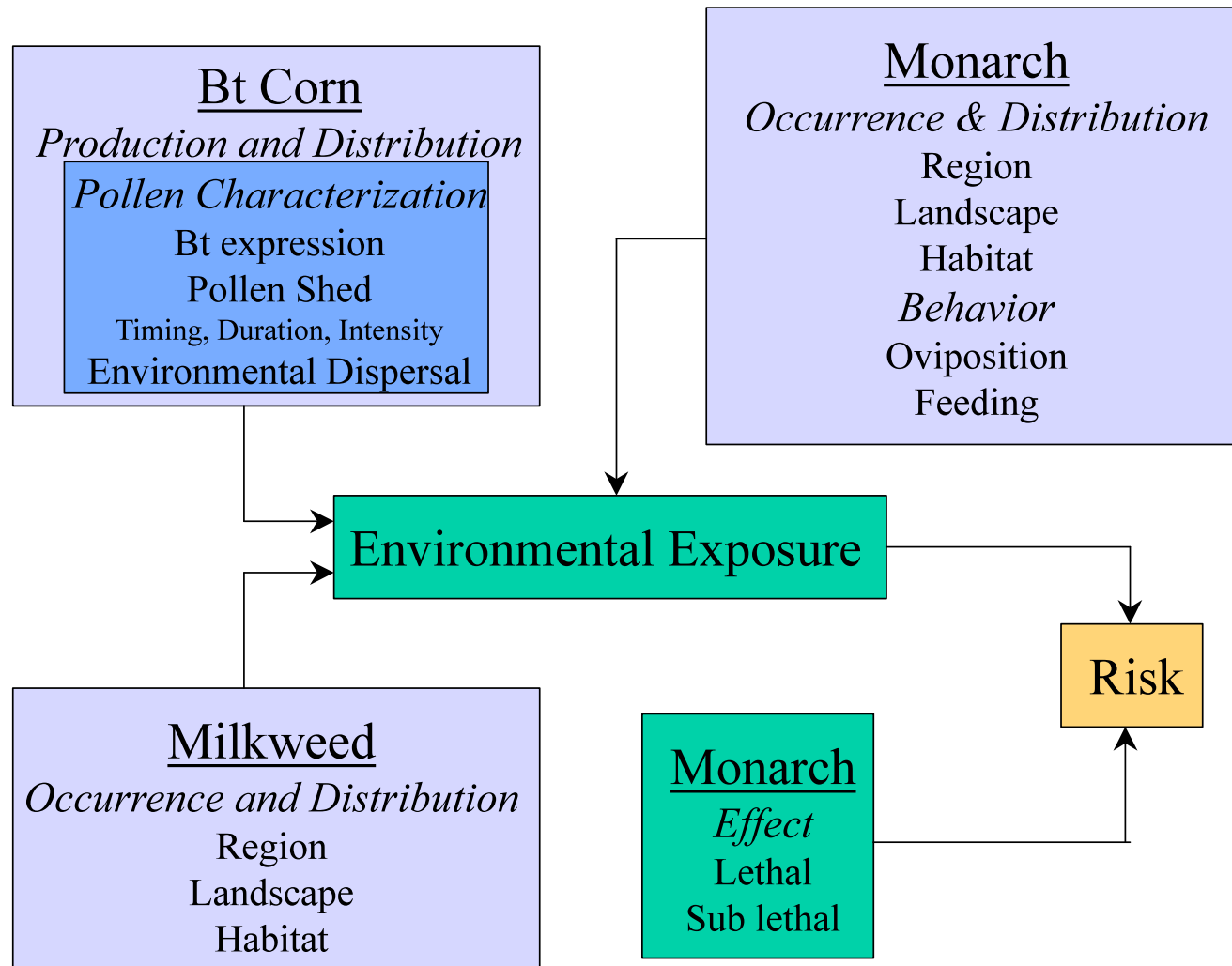
tier I/II QRA

1 problem formulation

- **address as field scale concern using available data**
- **utilize conservative assumptions to provide upper bounds on uncertainties**
- **goals**
 - do not to seek exact answers
 - seek conservative upper bound risk estimates
 - describe uncertain, variable, and sensitive components
 - verify approach on the basis of emerging science

tier I/II QRA

2 conceptual model



tier I/II QRA

3 determine modeling approach

4 identify sources of information

Use conventional approach for non target risk

$\text{Risk} = f(\text{exposure, effect})$ where $\text{RQ} = \text{EEC}/\text{Effect}$

Monarch-specific effect concentrations are lacking
use interspecies distribution of effect

Estimated Environmental Concentration (EEC)

Pollen dispersal - published literature

Milkweed distribution - published literature

Bt concentration in pollen - regulatory submissions

Effect concentrations

Bioavailability of Bt from pollen - assume available

Larval dose-response - **generalized Lepidoptera data**

Exposure refinements

Timing and Duration of pollen shed - assume instantaneous

Timing of larval appearance - assume sensitive larval state is present

Larval feeding behavior - assume consumption of pollen

Spatial-temporal distributions - assume co-occurrence

tier I/II QRA

3 determine modeling approach

4 identify sources of information

April 2003

WOLT ET AL.: BT CORN AND RISK TO NONTARGET INSECTS

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

Input parameter	Value	Unit	Rationale
Pollen characterization			
Relative spherical diameter	100	$\mu\text{m}/\text{grain}$	High-end estimate
Density	1.1	g/cm^3	Typical for bioaerosol
CryIA(b) expression	2	$\mu\text{g}/\text{g}$ (fw)	High-end estimate
Pollen deposition			
Total pollen	8.9×10^9	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		35×10^6 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			$\mu\text{g}/\text{grain}$
Pollen deposition (with distance)			
$(\Delta\text{Mass flux}) (\text{Off-plot movement}) (\text{Total pollen}) (\text{Scaling factors})^b$			Grains/ m^2
Estimated Environmental Concentration (EEC)			$\mu\text{g}/\text{g}$ (fw)
$(\text{Protein concentration}) (\text{Pollen deposition}) / (\text{Leaf weight}) (\text{Milkweed density})$			

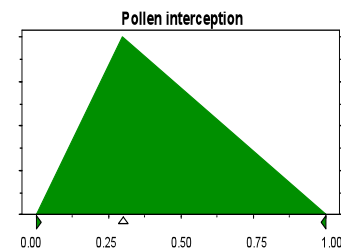
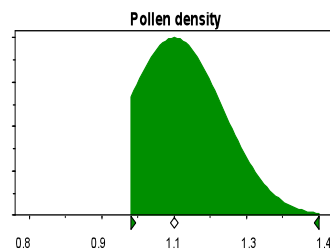
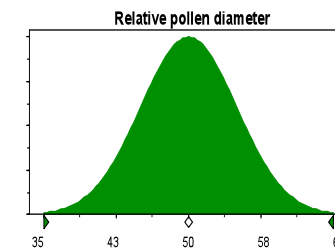
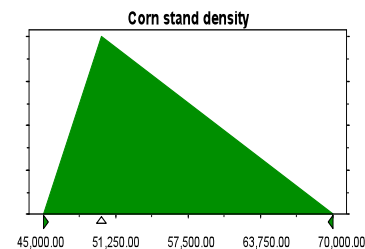
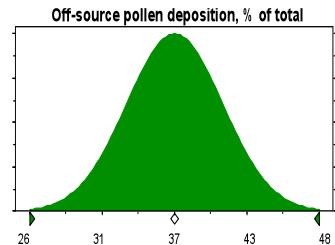
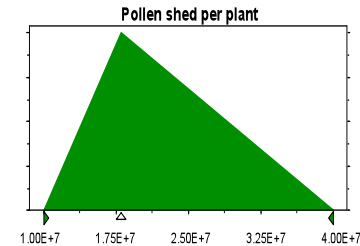
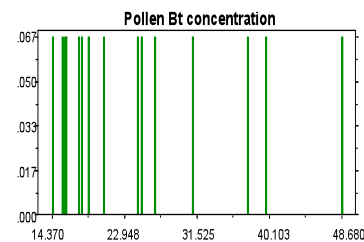
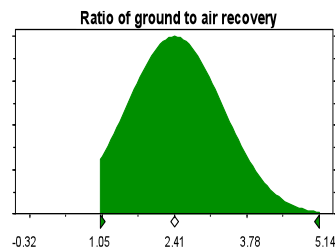
^a Volume = $(4/3)\pi(\text{Relative spherical diameter}/2)^3$.

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

tier I/II QRA

5 develop distributions for model inputs

exposure characterization



tier I/II QRA

5 develop distributions for model inputs

effects characterization

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

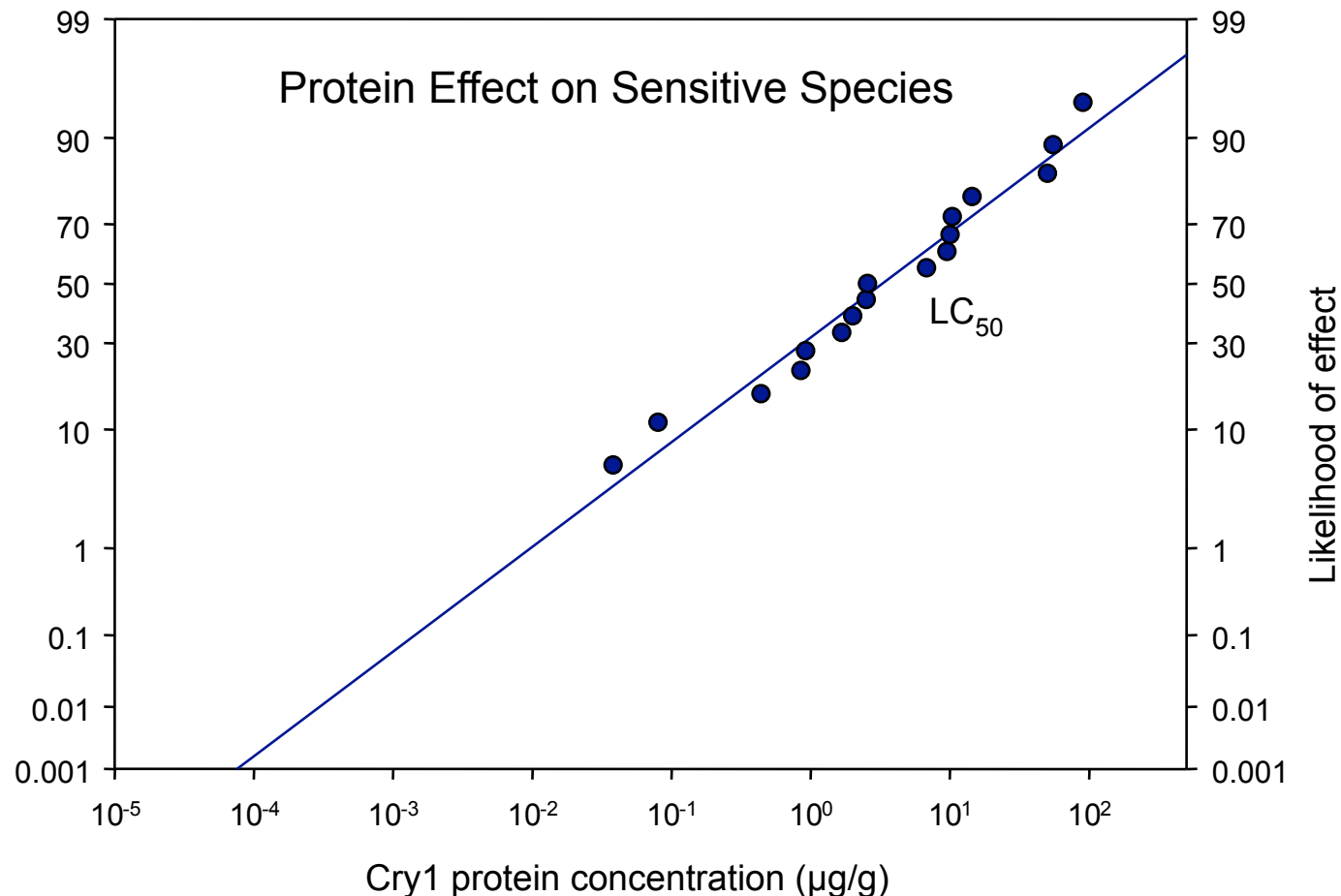
Species (Common name)	LC ₅₀ (μ g/g)	Reference
<i>Manduca sexta</i> (L.) (tobacco hornworm)	0.04	MacIntosh et al. 1990
<i>Diatraea grandiosella</i> Dyar (southwestern corn borer)	0.08-0.15	Song et al. 2000 ^a
<i>Trichoplusia ni</i> (Hübner) (cabbage looper)	0.19	MacIntosh et al. 1990
<i>Heliothis virescens</i> (F.) (tobacco budworm)	0.2	Luttrell et al. 1999
<i>Pseudoplusia includens</i> (Walker) (soybean looper)	0.67	Luttrell et al. 1999
<i>Helicoverpa armigera</i> (Hübner) (old world bollworm)	1.55	Chakrabarti et al. 1990
<i>Spodoptera exigua</i> (Hübner) (beet armyworm)	3.18	Luttrell et al. 1999
<i>Helicoverpa zea</i> (Boddie) (corn earworm)	3.45	Luttrell et al. 1999
<i>Ostrinia nubilalis</i> (Hübner) (European corn borer)	3.6	MacIntosh et al. 1990
<i>Agrotis ipsilon</i> (Hufnagel) (black cutworm)	>80	MacIntosh et al. 1990
<i>Spodoptera frugiperda</i> (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 CryIAb. Unpublished study submitted to EPA (part of Monsanto's 2000 IRM report). MRID # 453205-02.

tier I/II QRA

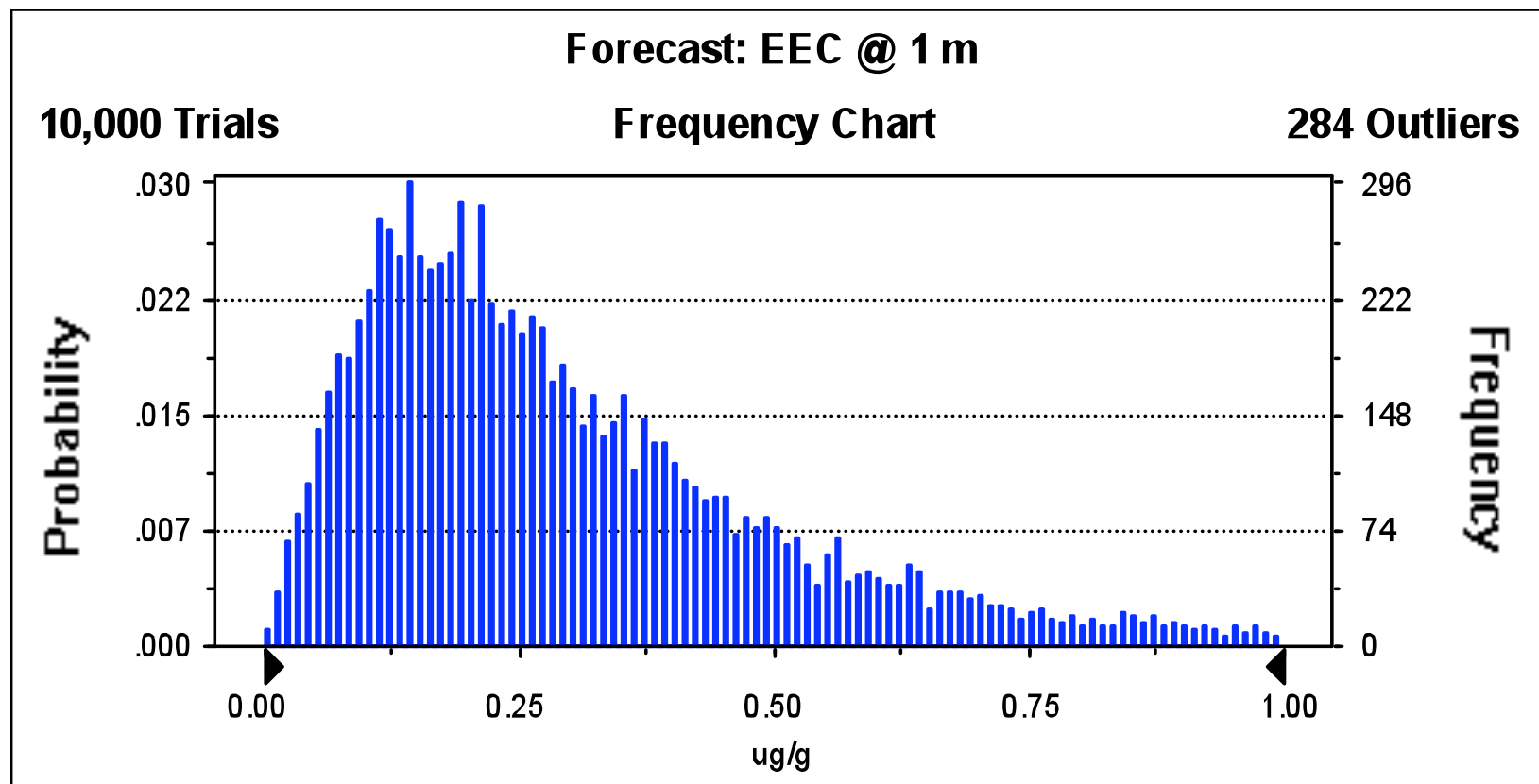
5 develop distributions for model inputs

effects characterization



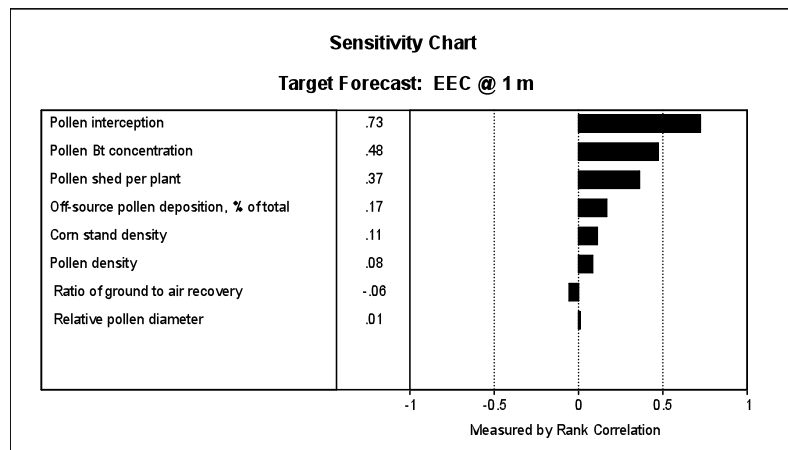
tier I/II QRA

6 propagate variance and generate output distribution



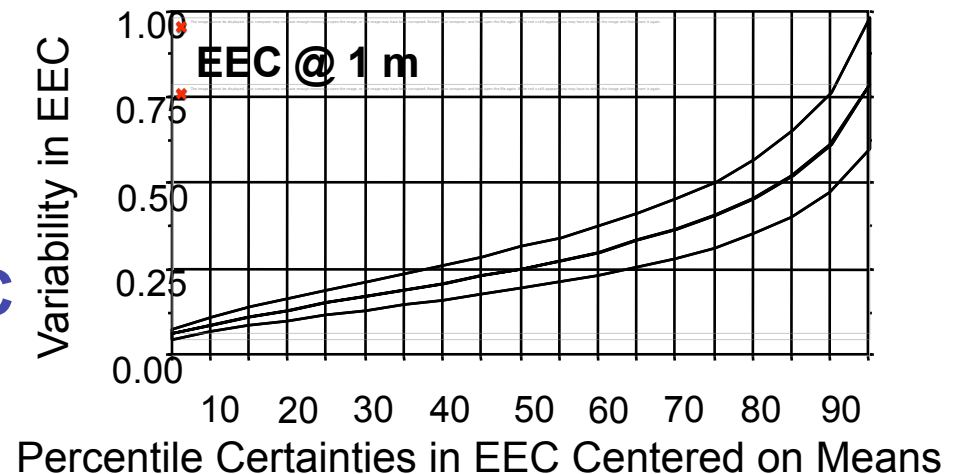
tier I/II QRA

7 assess significant contributors to variance



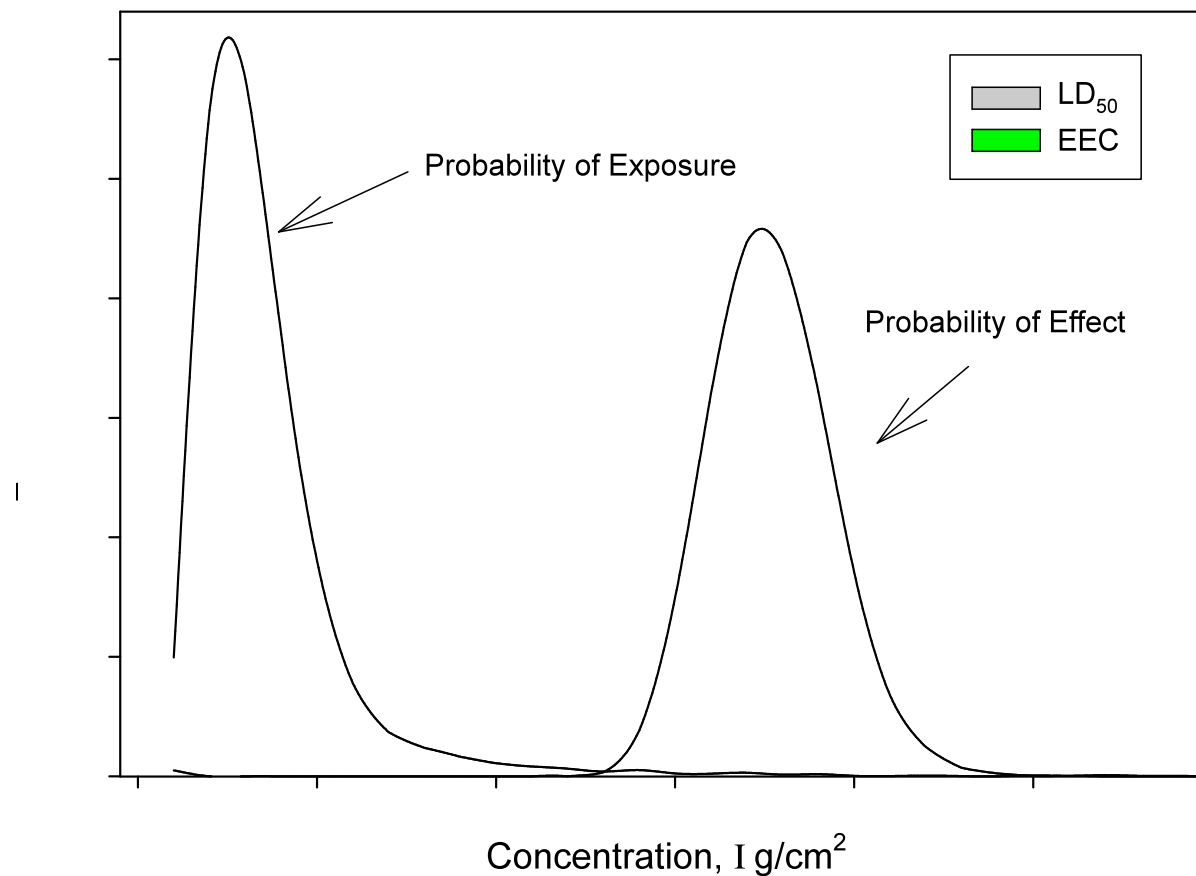
Sensitive components of EEC

Trend in variability and uncertainty for EEC



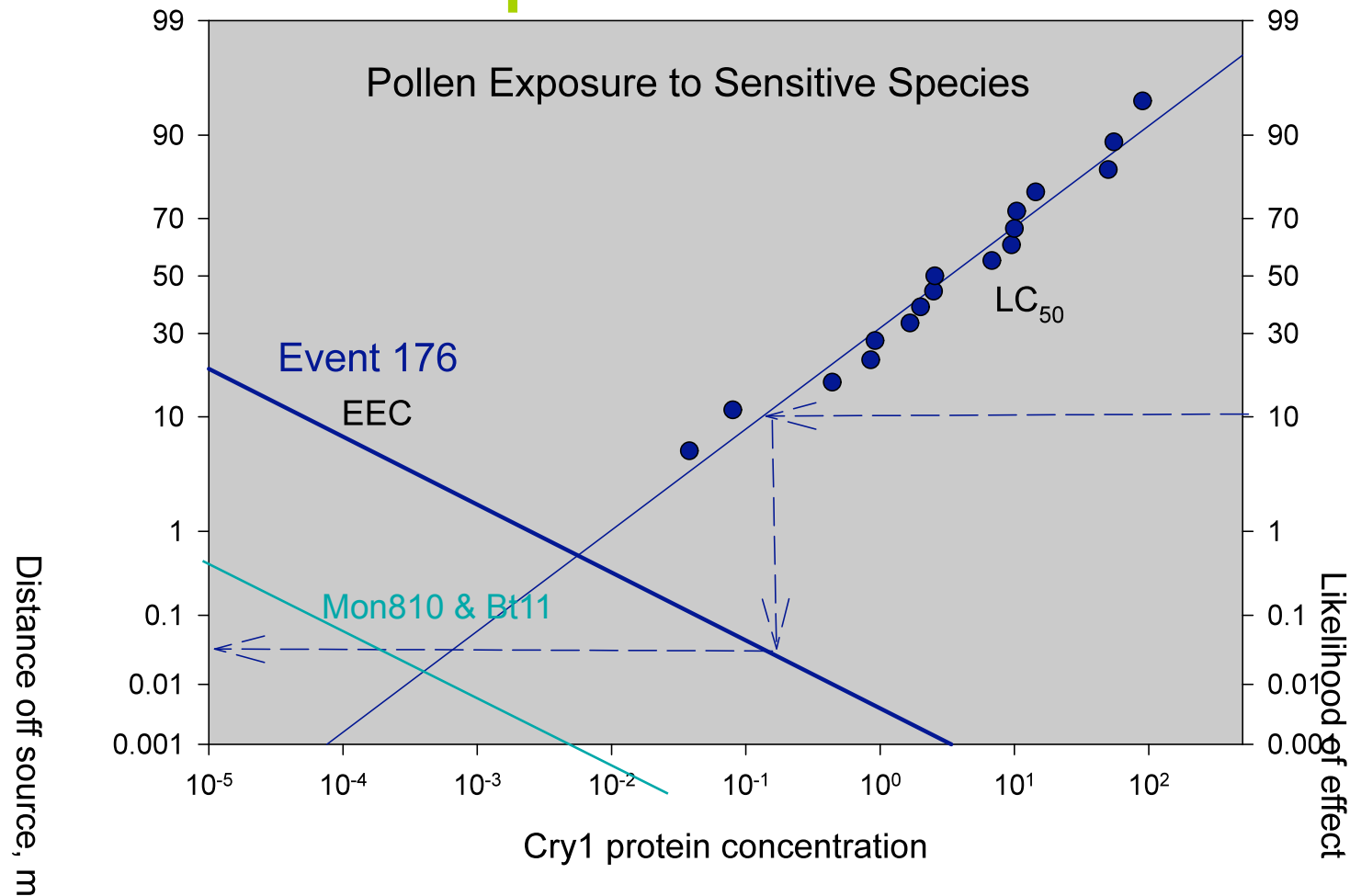
tier I/II QRA

8 characterize probability of occurrence and associated uncertainty



8

tier I/II QRA

risk as the joint likelihood of
exposure and effect

tier III QRA

■ Tier III

Sears et al. 2001. *PNAS*, 98:11947-11942

- **problem** considered impact to populations over regions
- **hazard** described as a short-term (acute) effect
- physical model for **exposure** timing and duration
- **risk** described as probability harm accruing to populations

– risk formulation

$$R = P_e \times P_t$$

where,

P_e is the probability of larval occurrence in a Bt cornfield

P_t is the probability of toxicity given exposure

tier III QRA

1 problem formulation

- level of concern compels data generation to address lack-of-knowledge

- analysis plan

—effects characterization

- dose-response

Hellmich et al. 2001. *PNAS*, 98:11925-11930

- semi-field verification

Stanley-Horn et al. 2001. *PNAS*, 98:11931-11936

—exposure characterization

- define spatial-temporal relationship of stressor to entity of concern

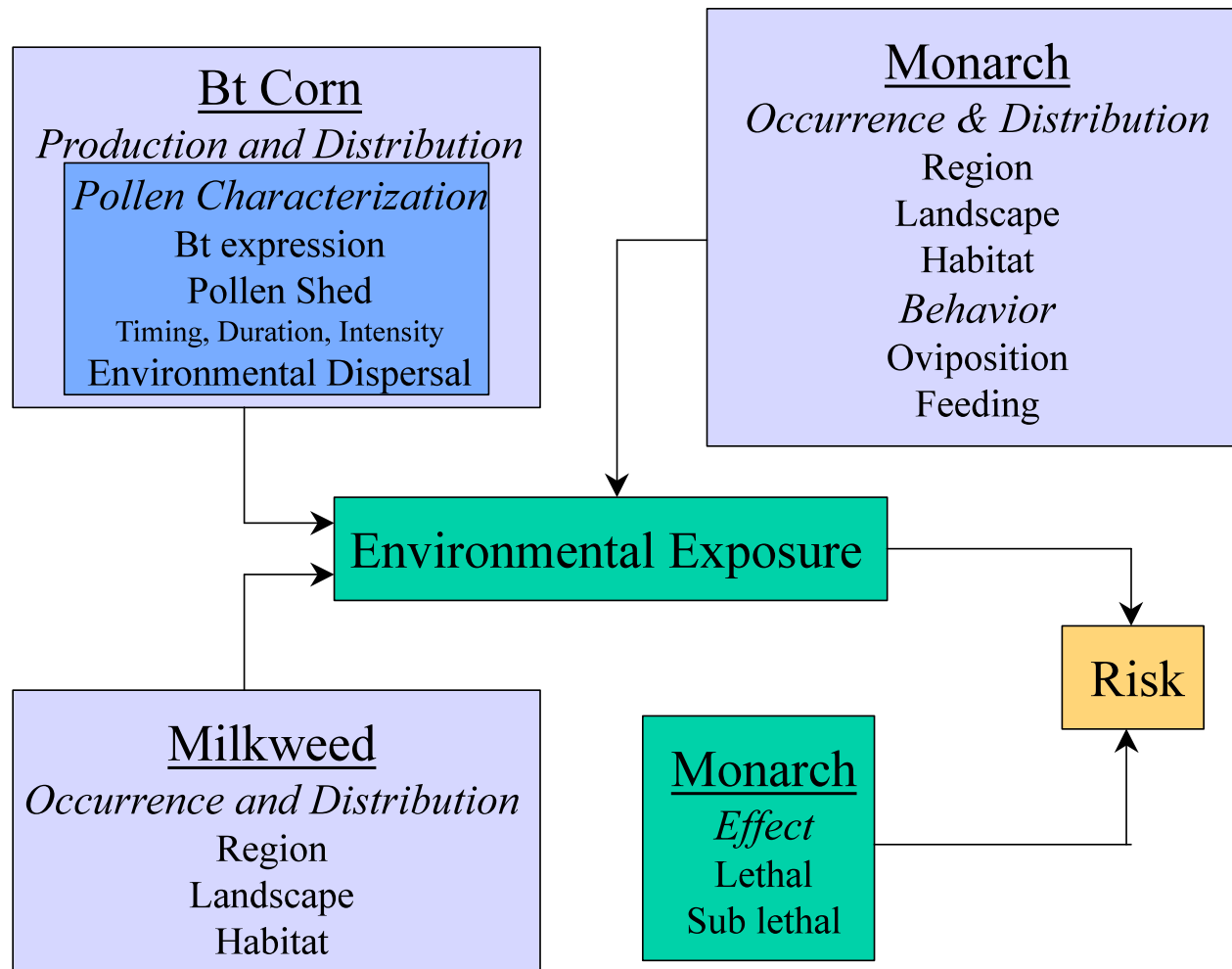
Oberhauser et al. 2001. *PNAS*, 98:11913-11918

- quantitation of exposure duration and intensity

Pleasants et al. 2001. *PNAS*, 98:11919-11924

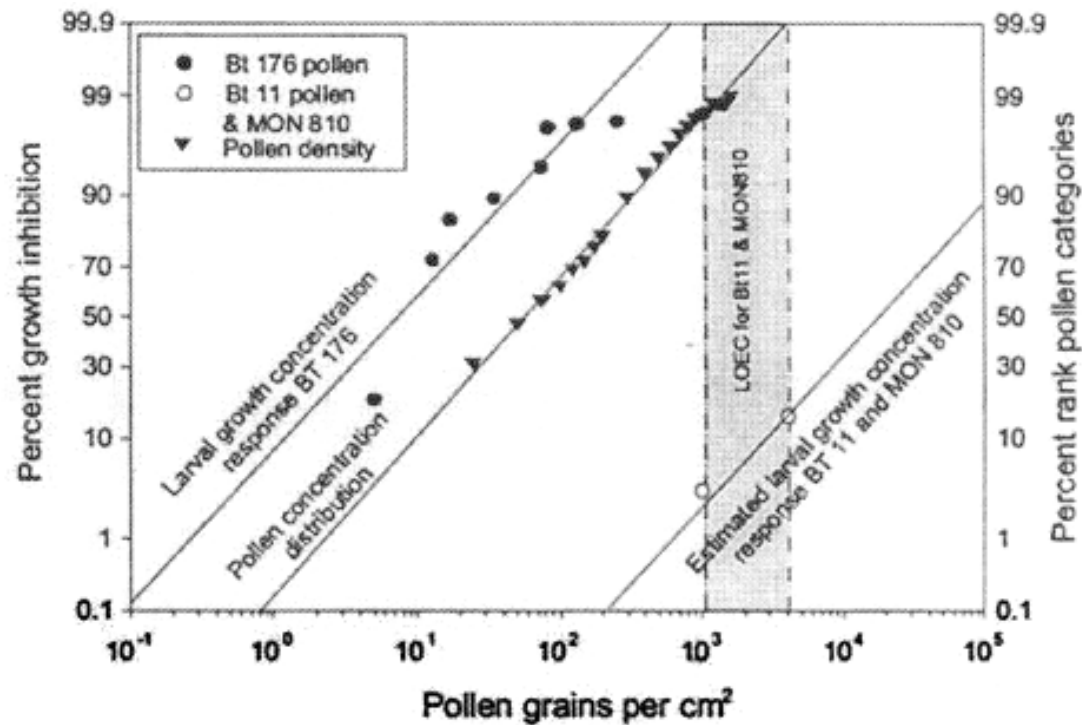
tier III QRA

2 conceptual model



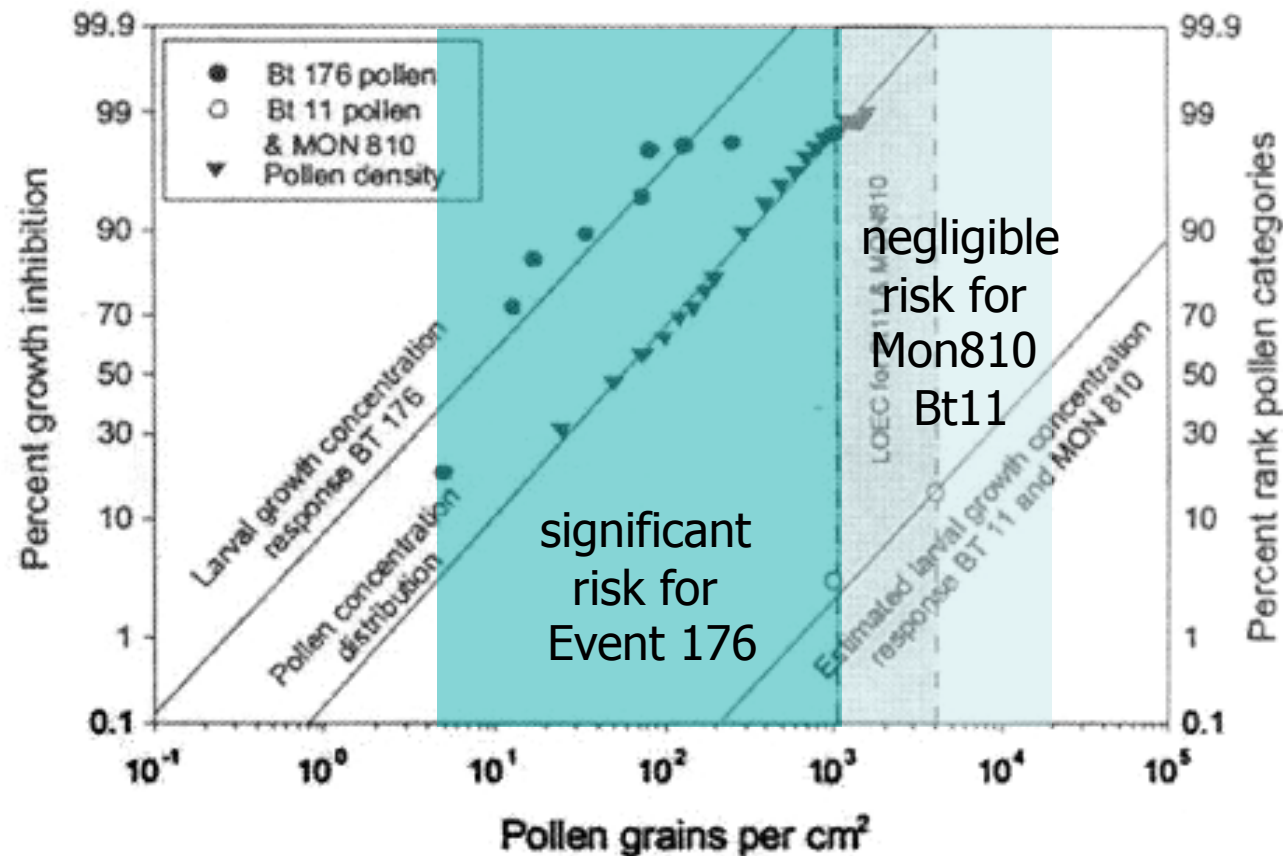
tier III QRA

risk as the joint likelihood of
exposure and effect



tier III QRA

risk as the joint likelihood of exposure
and effect for Cry1Ab events



tier III QRA

population level effect

$$P_e = l \times o \times a \times m$$

probability of effect is the fractional contribution Bt cornfields to breeding habitat (1.6%)

$$P_t$$

probability of toxicity is the fraction of milkweed plants w/ in cornfields where pollen density is > LOEC (10% for Mon810 & Bt11)

$$R = P_e \times P_t$$

0.16% of the breeding population of monarchs may be affected

tier III/IV QRA

■ Tier III/IV

Dively et al. 2004. *Environ Ent*, 33:1116-1125

- **problem** considered impact to populations over regions
- **hazard** described as a long-term (chronic) effect
- physical model for **exposure** timing and duration
- **risk** described as probability harm accruing to populations

—risk formulation

$$R = P_e \times P_t$$

where,

P_e is the probability of larval occurrence in a Bt cornfield

P_t is the probability of toxicity given exposure

tier III/IV QRA

1 problem formulation

- address residual uncertainties regarding long-term exposure
- analysis plan
 - effects characterization
 - long-term effects *Dively et al. 2004. Environ Ent, 33:1116-1125*
 - anthers as route of exposure
 - Anderson et al. 2004. Environ Ent, 33:1109-1115*
 - exposure characterization
 - define spatial-temporal relationship of stressor to entity of concern
 - quantitation of exposure duration and intensity

tier III/IV QRA

8 characterize probability of occurrence and associated uncertainty

August 2004

DIVELY ET AL.: EFFECTS OF Bt CORN ON MONARCH BUTTERFLY

1123

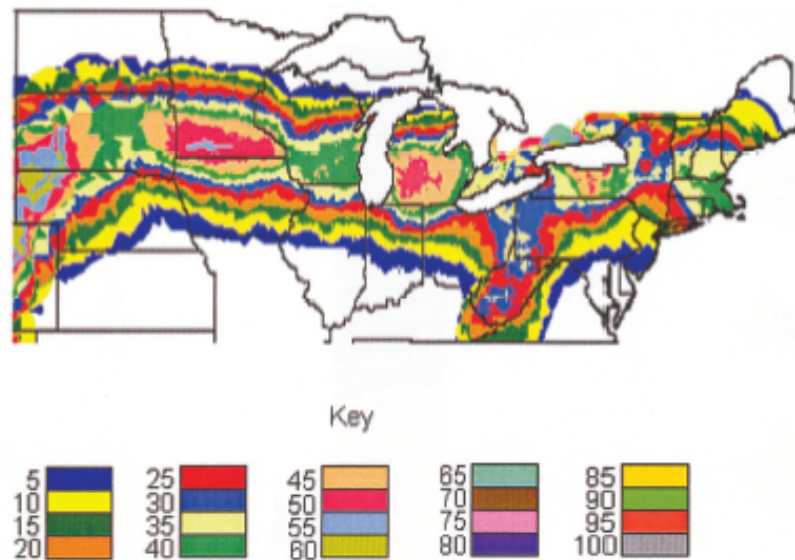


Fig. 3. Model predictions of the percentage of the second generation of monarch butterfly exposed as first, second, and third instars to the first 6 d of corn anthesis.

Tiered refinement of assessment to address uncertainty

- **Tier I/II:**
 - identified 2 most sensitive components as pollen dispersal and interception by milkweed
- **Tier III:**
 - addressed through direct measurement of pollen on milkweed
- **Tier III/IV:**
 - chronic effects inclusive of anthers
- **Tier I/II**
 - identified Bt concentration in pollen as a significant uncertainty
- **Tier III**
 - measured effect directly on pollen
 - identified potential for anther exposure
- **Tier III/IV**
 - long-term exposure
 - co-effect of pollen and anthers

Comparison of risk findings

Tier I/II

- the lethal affect of Cry1Ab corn pollen on neonate monarch larvae is negligible beyond the edge of Bt cornfields (1999)

Tier III

- Cry1Ab corn pollen is acutely toxic to 0.16% of the monarch breeding population (2001)

Tier III/IV

- Cry1Ab corn pollen is chronically toxic to 0.6% of the monarch breeding population (2004)

ERA Summary

The process described for environmental safety assessment follows a formal ERA framework that is intended to be transparent and sufficiently flexible to meet the case-by-case considerations of GE plants, which vary widely in the types of traits they may express.

This process is tiered, recursive, and matches the complexity of the analysis to the nature of concerns and degree of uncertainty that is being addressed.

The science-based assessment of risk through the ERA process bridges scientific knowledge to the risk management process where scientific understanding and mitigation of uncertainties is integrated into the decision-making process.

BIGMAP

Biosafety Institute for Genetically
Modified Agricultural Products

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