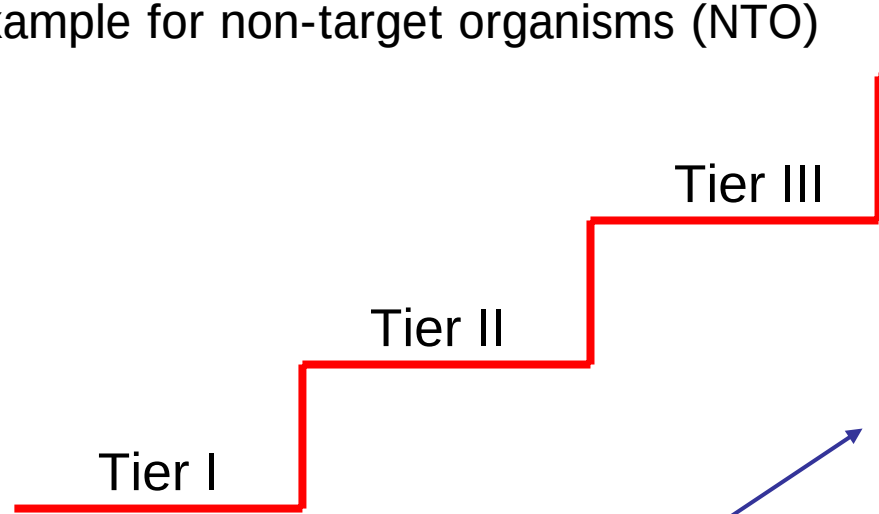
Monarch Butterfly and Bt Maize

Example of Tiers
in Non Target Organism Risk Assessment

risk assessment
recursive and proceeds in tiers

example for non-target organisms (NTO)



Extremely conservative assumptions to screen out negligible risks
deterministic
single scenario (generalized)
data poor
high uncertainty

Most refined assessment
stochastic (probabilistic)
multiple scenario (regional)
data rich
uncertainty and variability defined
highly refined exposure
assumptions verified by monitoring

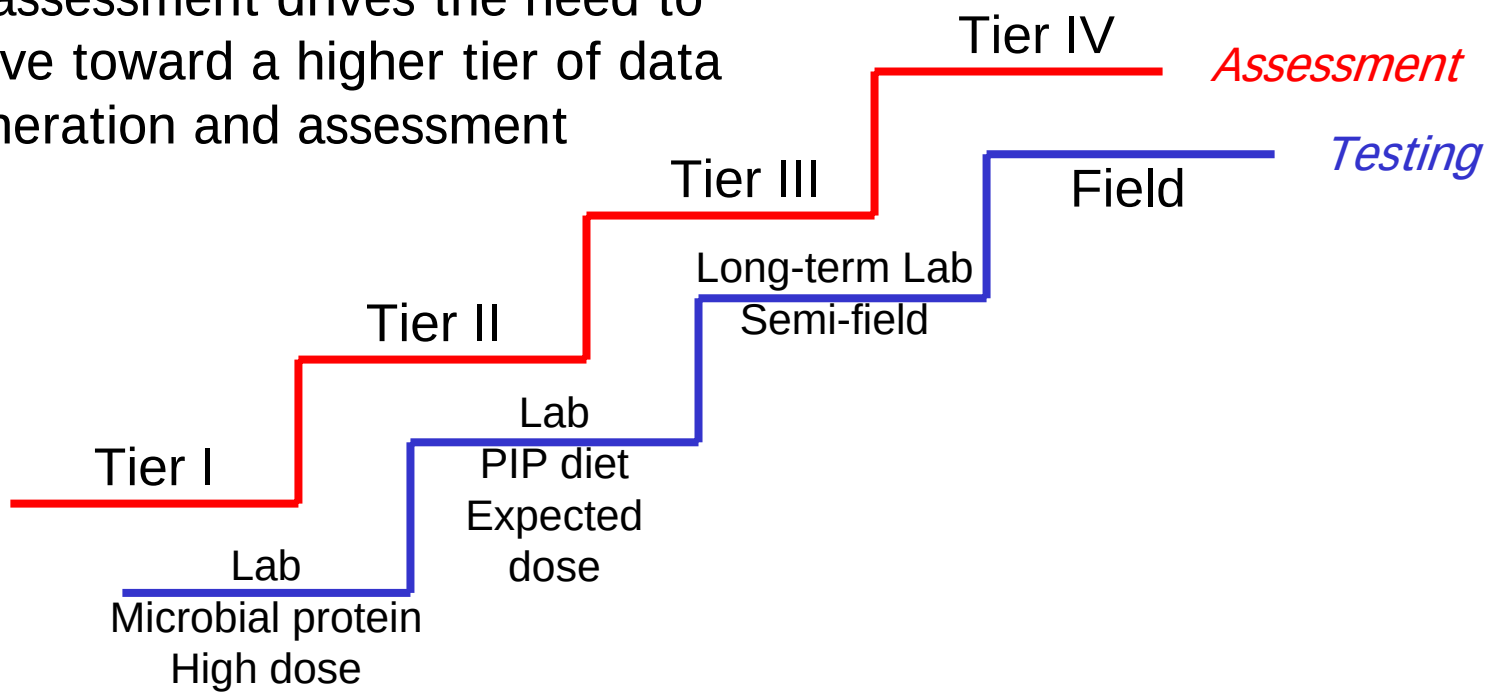
risk assessment
recursive and proceeds in tiers

Tier	Description	Scenario(s)
I	*Screening case.* Deterministic. Uses metamodels or empirical information	Worst case assumptions: most vulnerable environment and inputs representing the upper 95-98th percentile of exposure and effect
II	*Partially stochastic.* May use metamodel or physical model	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	*Fully stochastic.* May incorporate the physical model into a patch model or a GIS.	Multiple scenarios representing the breadth of anticipated uses. Geographic information reflects the use landscape or use region.
IV	*Field monitoring*. May occur in conjuction with refined tier III model.	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



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
  - ∴ 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

non-target QRA for PIPs ... monarch butterfly

- 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

non-target QRA for PIPs ... monarch butterfly

- 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

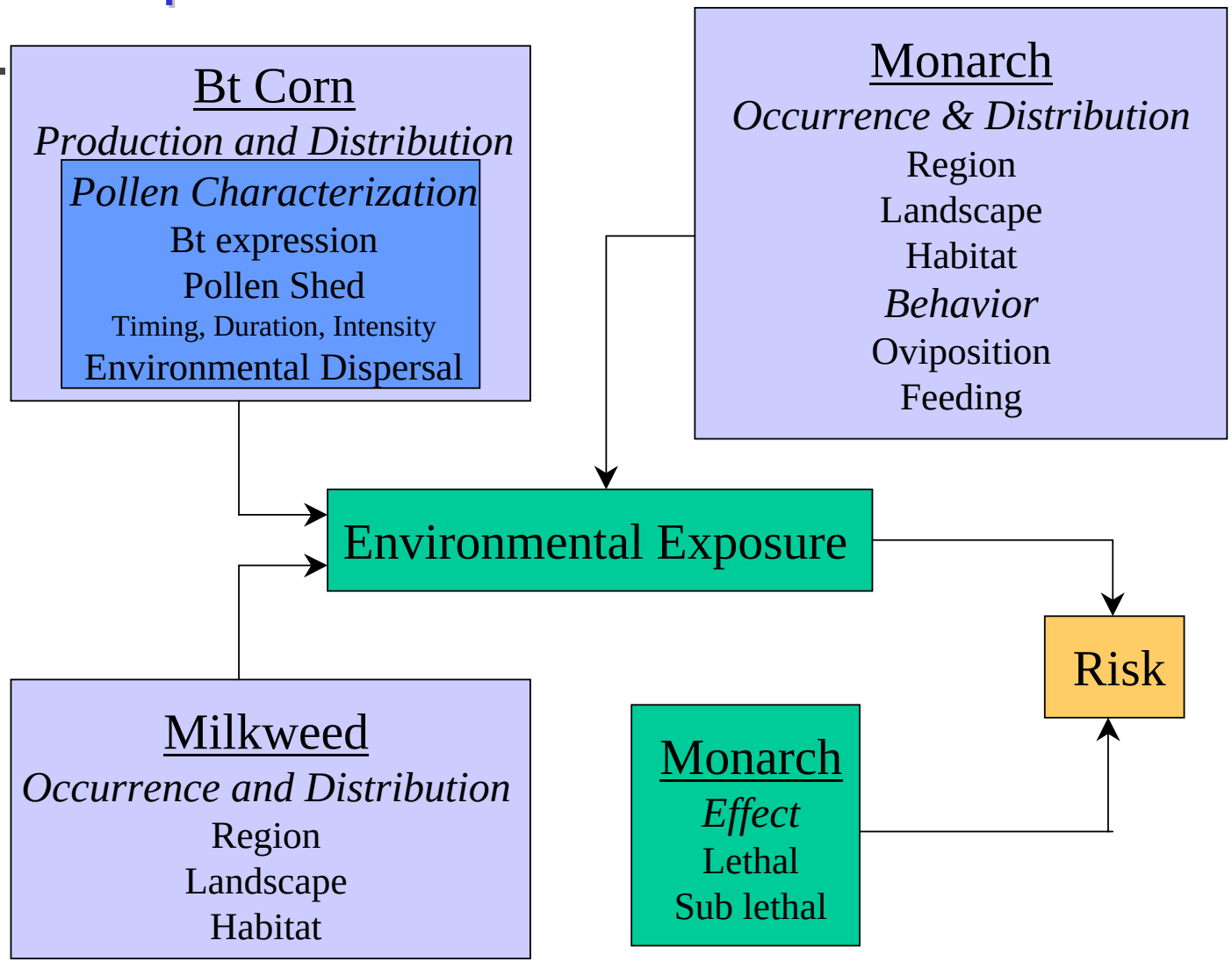
tier I/II QRA

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
4

determine modeling approach
identify sources of information

Use conventional approach for non target risk

Risk = *f*(exposure, effect) where RQ = EEC/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
4

determine modeling approach
identify sources of information

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	8.9×10^9	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

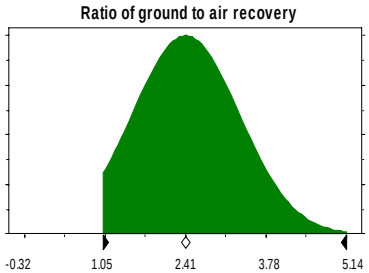
Equation	Unit
Protein concentration (Expression) (Density) (Volume)[a]	μg/grain
Pollen deposition (with distance) (ΔMass flux) (Off-plot movement) (Total pollen) (Scaling factors)[b]	Grains/m^2
Estimated Environmental Concentration (EEC) (Protein concentration) (Pollen deposition) / (Leaf weight) (Milkweed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

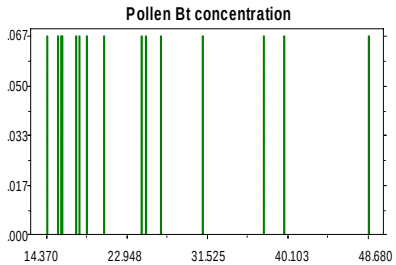
[a] Volume = $(4/3)\pi$(Relative spherical diameter/2)^3.
[b] Scaling factors = (SF1) (SF2) (SF3) (SF4) = 9.6.

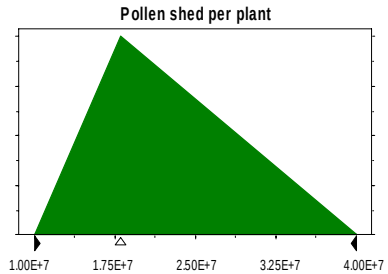
tier I/II QRA

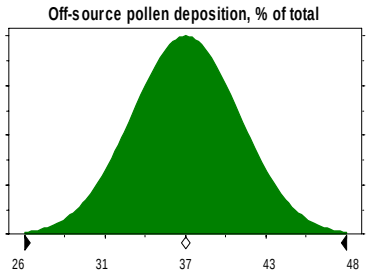
5 develop distributions for model inputs

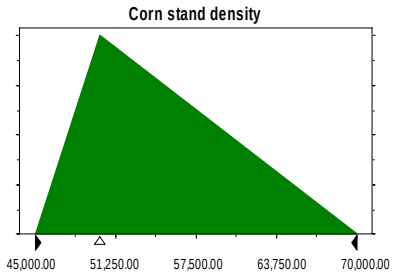
exposure characterization

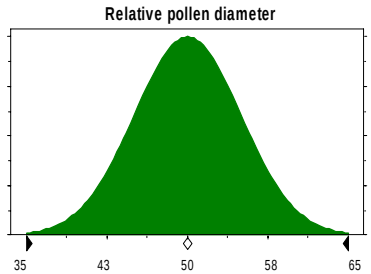


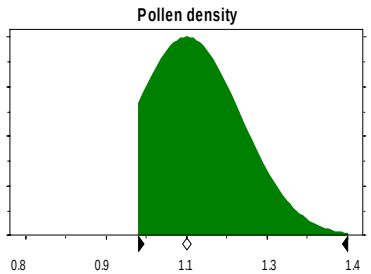


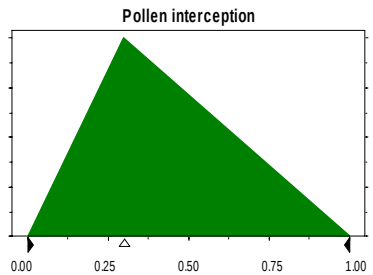












tier I/II QRA

5 develop distributions for model inputs

effects characterization

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

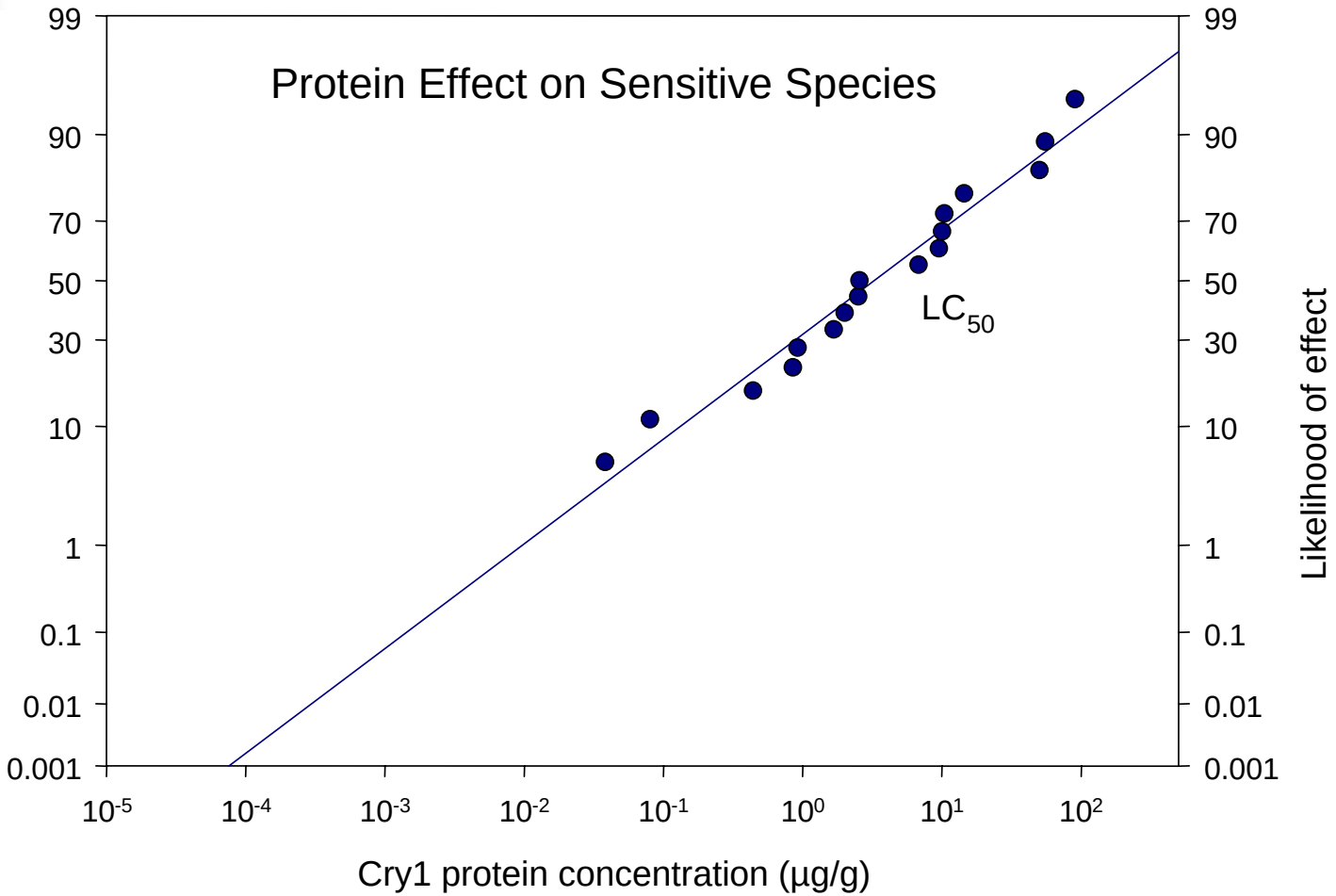
Species (Common name)	LC_{50} (μg/g)	Reference
Manduca sexta (L.) (tobacco hornworm)	0.04	MacIntosh et al. 1990
Diatraea grandiosella Dyar (southwestern corn borer)	0.08–0.15	Song et al. 2000[a]
Trichoplusia ni (Hübner) (cabbage looper)	0.19	MacIntosh et al. 1990
Heliothis virescens (F.) (tobacco budworm)	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.

tier I/II QRA

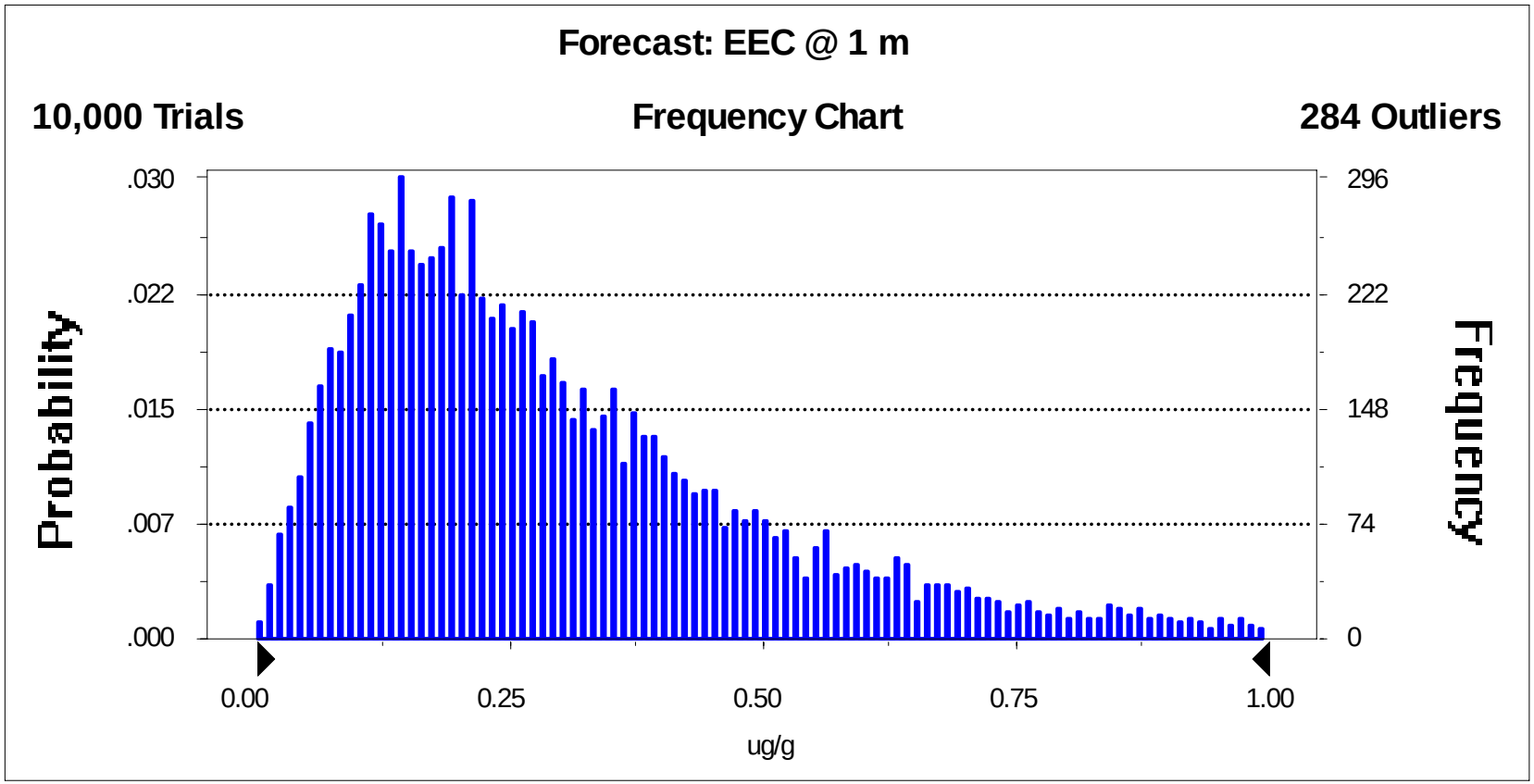
5 develop distributions for model inputs

effects characterization



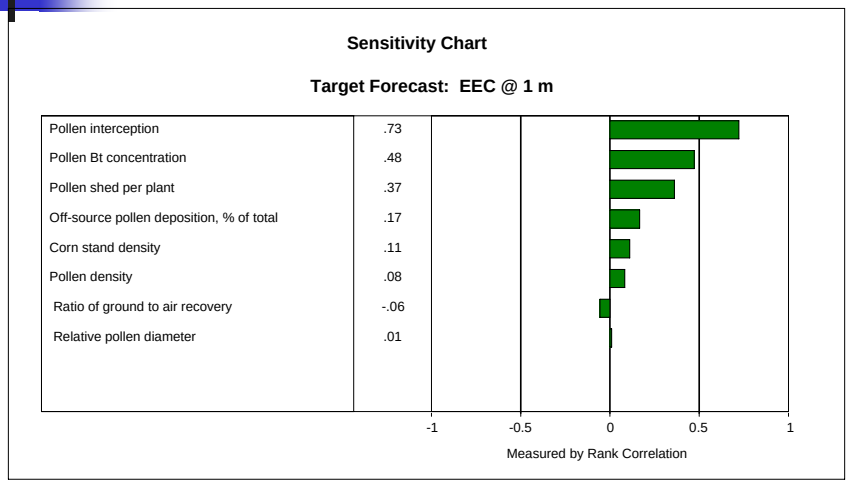
tier I/II QRA

6 propagate variance and generate output distribution



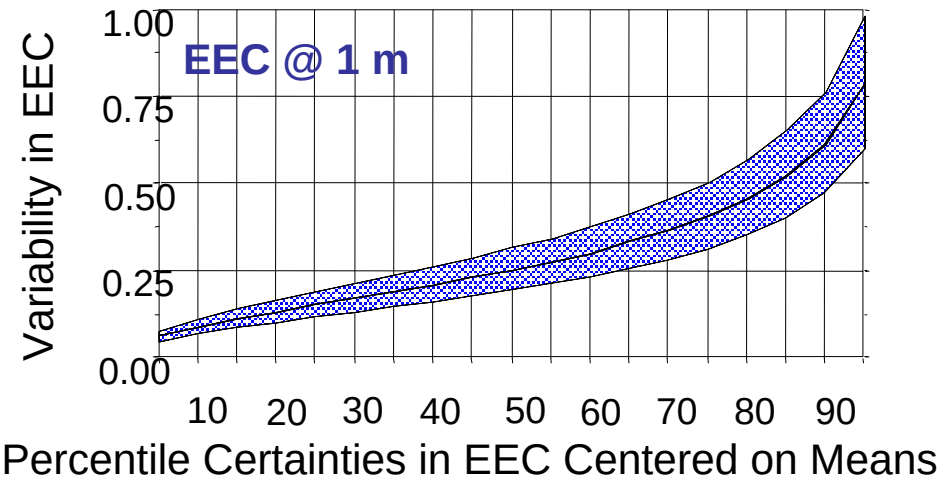
tier I/II QRA

7 assess significant contributors to variance



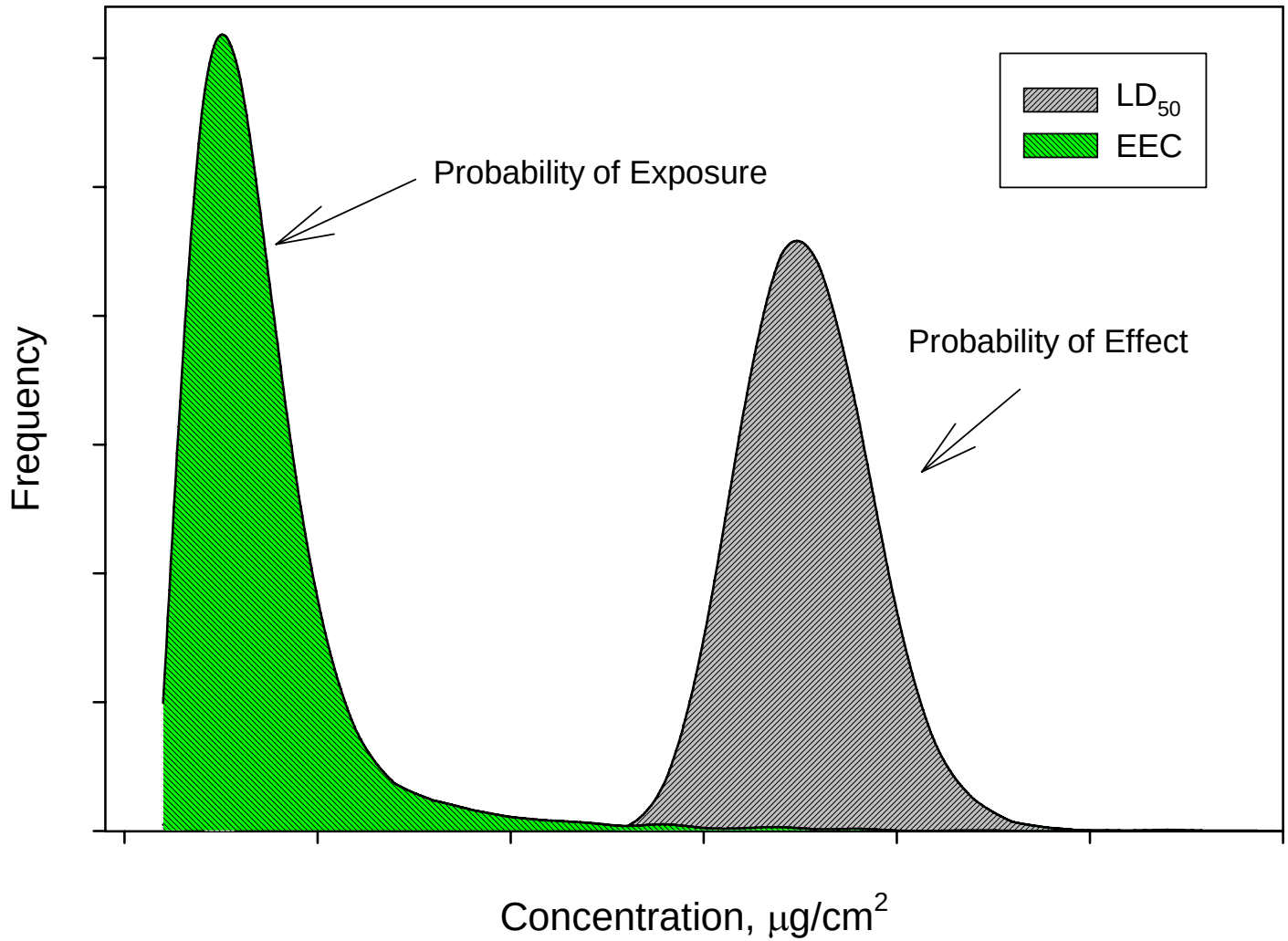
Sensitive components of EEC

Trend in variability and uncertainty for EEC



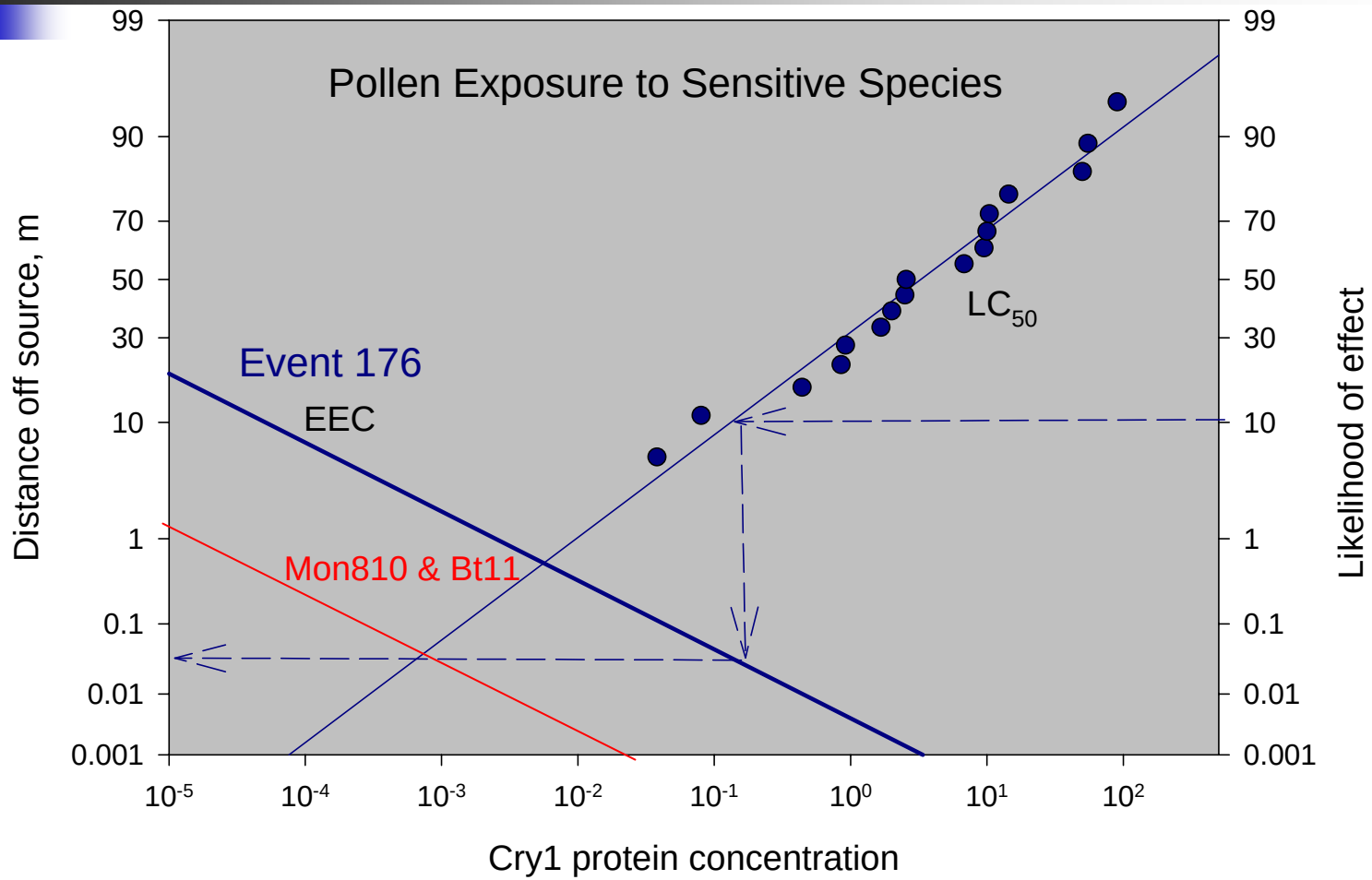
tier I/II QRA

characterize probability of occurrence and associated uncertainty



tier I/II QRA

risk as the joint likelihood of exposure and effect



non-target QRA for PIPs ... monarch butterfly

- Tier III

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

- *problem* considered impact to populations over regions
- *hazard* described as explicit measurement of 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

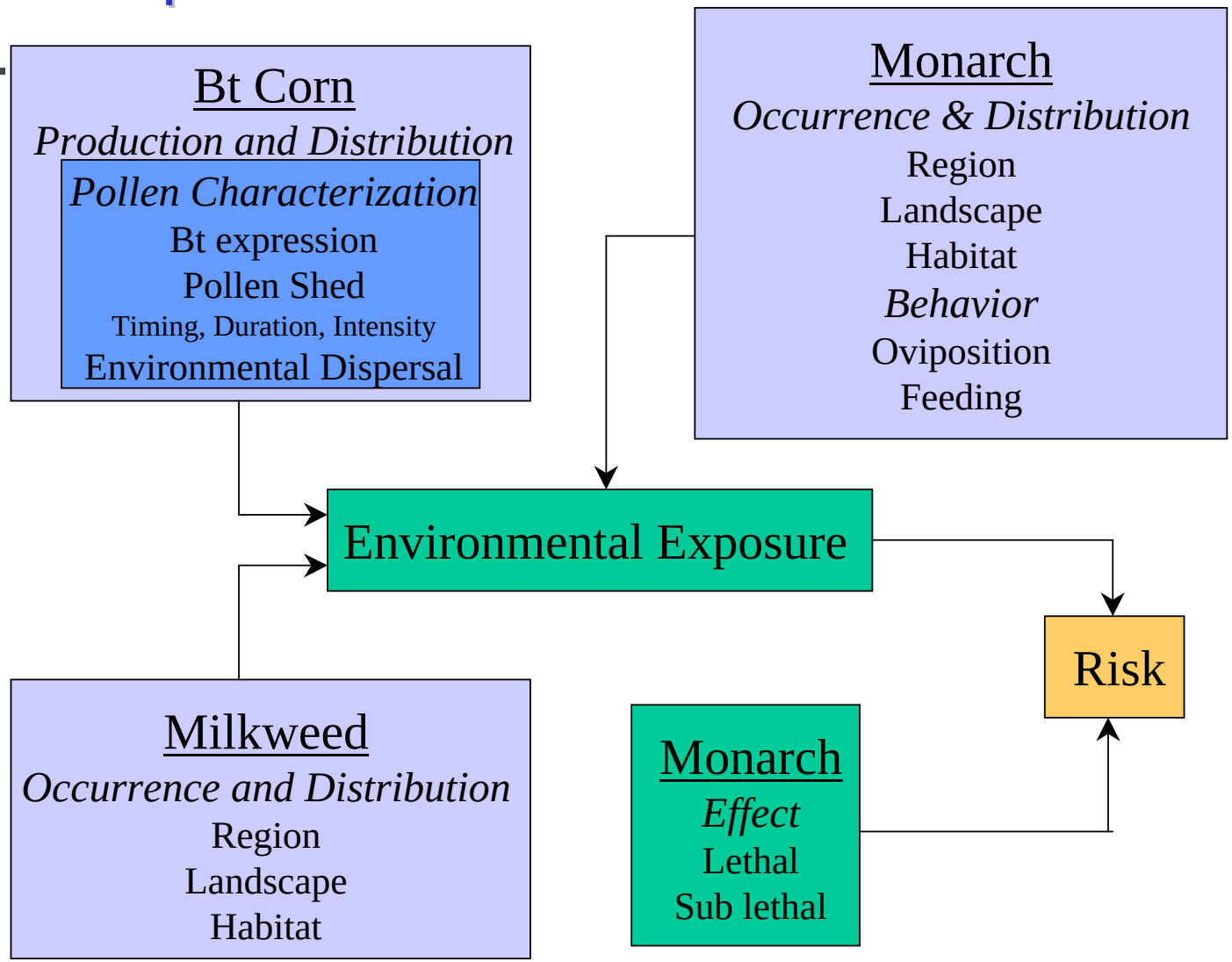
tier III QRA

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

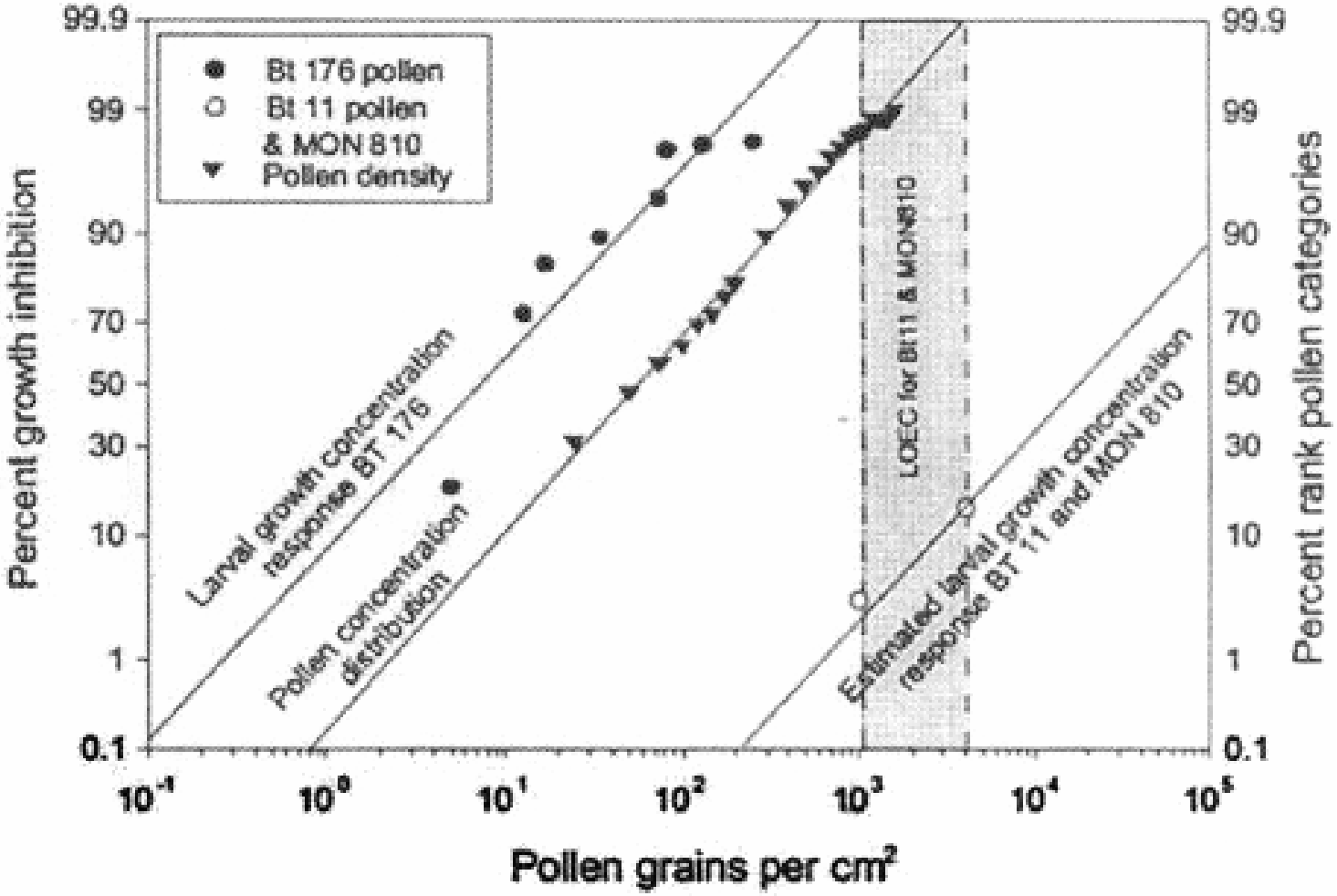
tier III QRA

2 conceptual model



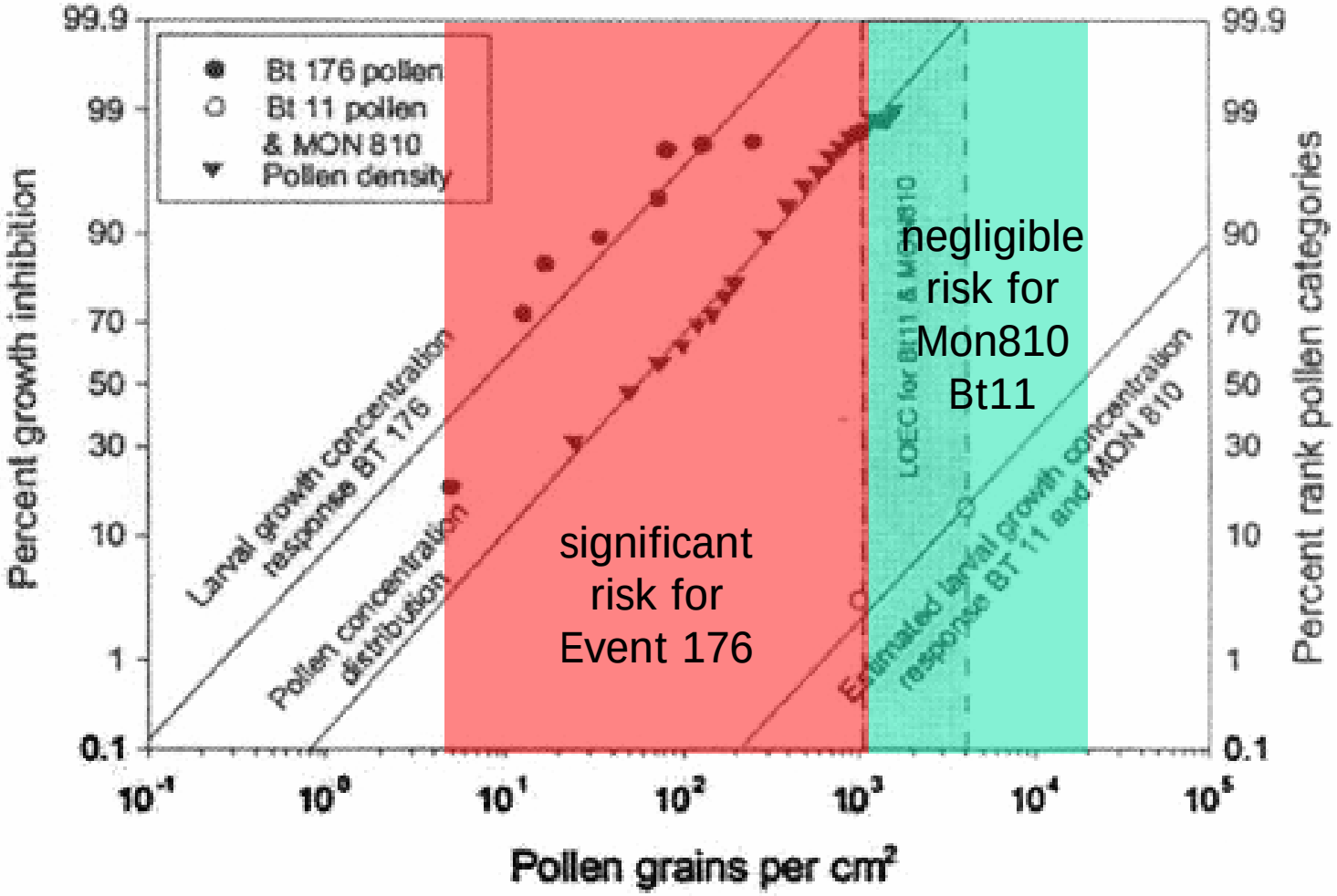
tier III QRA

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

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

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

non-target QRA for PIPs ... monarch butterfly

- 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
    - anthers as route of exposure

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

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

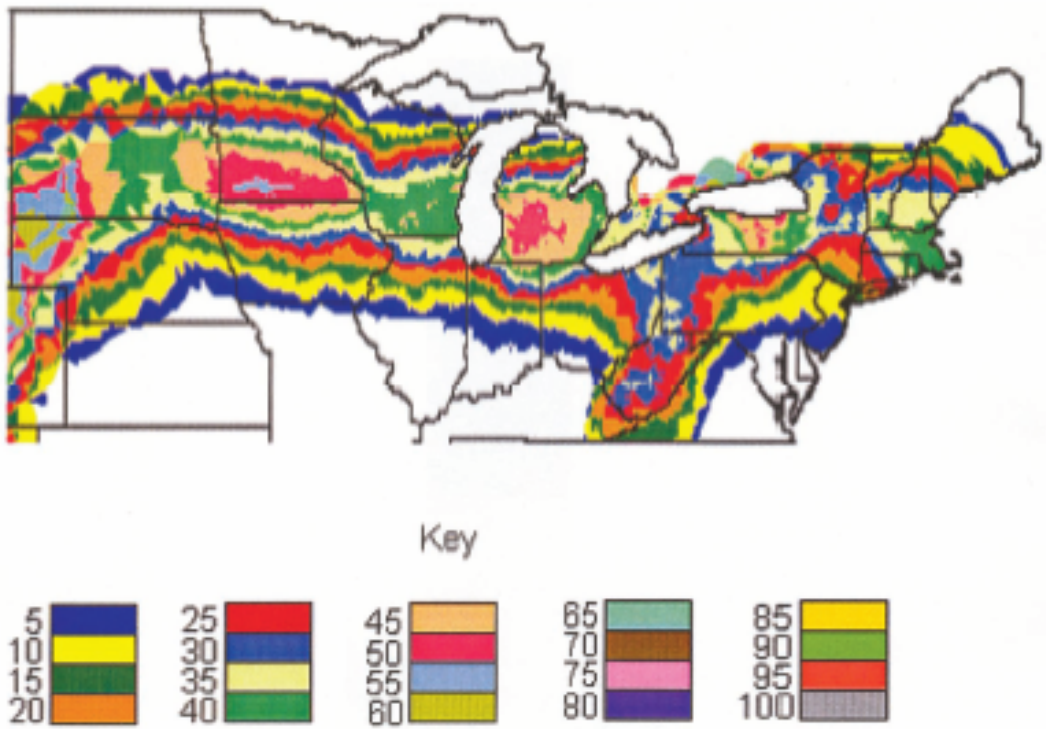


Fig. 3. Model predictions of the percentage of the second generation of monarch butterfly exposed as first, second, and third instars to the first 6 d of corn anthesis.

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)

flow path for a QRA