

So Many Strains, So Few Products! Opportunities and Constraints to Commercial Development of New Bt Products: The Regulatory Challenge

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Health
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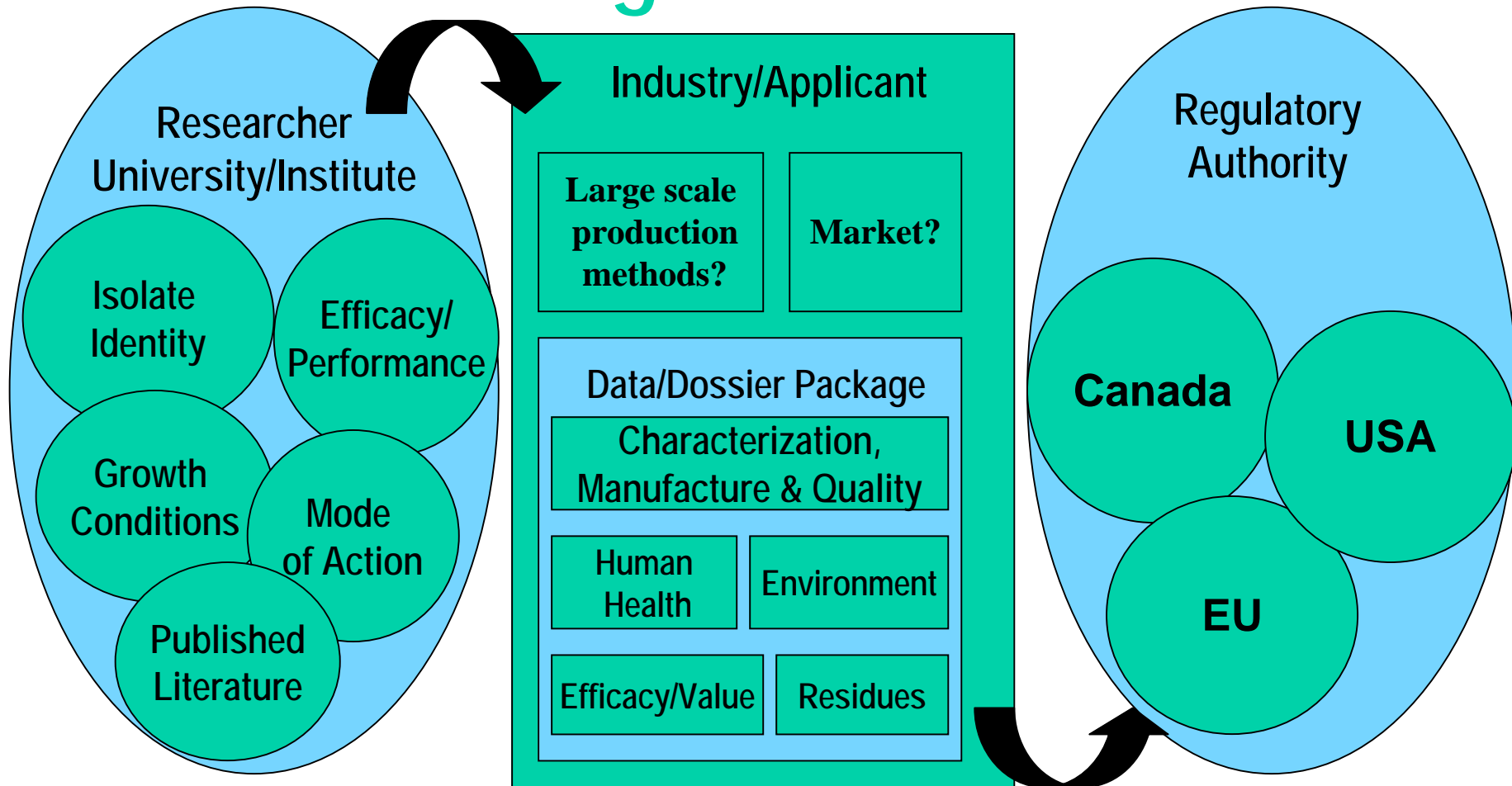
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Scope of Presentation

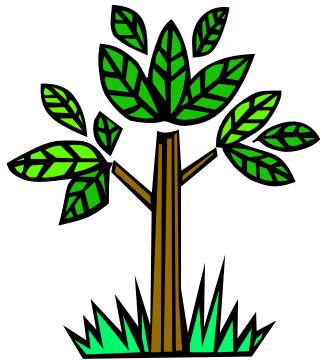
- ▶ Regulatory mandate and the regulator's legislative responsibilities
- ▶ Bt-based pesticides – update on ongoing re-evaluations among key OECD countries
- ▶ Regulatory issues and future for new strains and subspecies
- ▶ The importance of maintaining strong communication ties with regulators



Steps on the Road to Registration



The Regulator's Responsibility



Pesticides are only registered if:

there is reasonable certainty that no harm to human health, future generations or the environment will result from exposure to, or use of, the product when used as directed

the product has value and is efficacious when used as directed

Some Recent Bt Re-evaluation Regulatory Activities

- ▶ U.S. EPA published “Reregistration Eligibility Decision (RED) *Bacillus thuringiensis*” (EPA738-R-98-004) in March 1998
- ▶ Canada published Proposed Acceptability for Continuing Registration (PACR2006-09), “Re-evaluation of *Bacillus thuringiensis*” in November 2006
- ▶ EU RMS’s initiated re-evaluation in 2006. Draft Assessment Reports to be completed for peer review by MS’s in 2007
- ▶ All jurisdictions currently regulating Bt to the **strain** level

General Conclusions/Assumptions

- ▶ Human health and environmental risks of **existing** registered strains of Bt, (including Btk, Bti, Bta, Btt) are largely acceptable
 - ◆ supporting human health and environment data bases largely complete
 - ◆ “long history of safe use” also a strong consideration in allowing continuing registration of existing strains

Unresolved Issues and Implications on New Strains and Subspecies

- ▶ Bt's reputation as a safe biocontrol agent is being challenged by recent discoveries that are drawing attention to its potential as an opportunistic human pathogen
- ▶ Bt is a member of the *Bacillus cereus* group that have human pathogenicity profiles, including *B. cereus* (Bc), the causative agent of gastroenteritis and *B. anthracis* (Ba), the etiologic agent of anthrax disease

Unresolved Issues and Implications on New Strains and Subspecies

- ▶ Bt expresses many of the same soluble exoproteins as *B. cereus*, including gastroenteric enterotoxins; no demonstrated reason why any Bc toxin could not be produced by Bt
- ▶ Bt has been implicated in sporadic human infections (none definitively proven)

B. cereus-type Diarrheal Enterotoxins

- ▶ Hemolysin BL (HBL)
 - ◆ vegetative growth metabolite
 - ◆ hemolytic, dermonecrotic and vascular permeability properties
 - ◆ 3 genes coding for HBL components found in majority of Bt strains tested
 - ◆ commercial RPLA detection kit (Oxoid) is of questionable value (semi-quantitative);
 - ◆ other detection methods include Vero cell or Chinese hamster ovary cytotoxicity cell assays, HBL component antibodies, multiplex PCR, Southern hybridization, partial gene sequencing

B. cereus-type Diarrheal Enterotoxins

- ▶ Non-hemolytic enterotoxin (NHE) / Enterotoxin FM (EntFM)
 - ◆ 3 genes coding for NHE components found in majority of Bt strains
 - ◆ commercial VIA test kit (TECRA) only partially useful since detects only one of three gene components and a reaction may not always reflect a toxigenic isolate

B. cereus-type Diarrheal Enterotoxins

- ▶ Cytotoxin K (CytK/EntK)
 - ◆ gene coding for CytK found in Bc detected in Bt by PCR, including commercial strains
 - ◆ member of the β -barrel pore-forming family of toxins
 - ◆ necrotic and hemolytic activity and highly toxic to epithelial cells

Other Bc-group Toxins

- ▶ Anthracis tripartate toxin
- ▶ Cereulide (emetic toxin)
- ▶ β -exotoxin
- ▶ Cyt endotoxins
- ▶ Phospholipases (especially PCPLC)
- ▶ VIPs
- ▶ Enterotoxin T /BceT (probable artifact)

What should we do?

- Stop using Bt as an insecticide?
- Accept the risk?

- Understand the risk
 - Delete specific virulence traits
 - Select/design new strains
 - Establish in vitro bioassays or animal models
 - Understand pathogenicity mechanism/
diversity, etc.

Data Needs for New Bt products

- ▶ Detailed information on characterization significantly influences the nature and extent of data required to assess risks to human health and the environment



Characterization and Analysis

- ▶ Information related to origin, derivation and identification of the Bt to strain level using std. phenotypic/biochemical methods, H-antigen serotyping (?) or multi-locus sequence typing, *cry* gene profiles, CRY protein analysis, PIB morphology, plasmid profile, DNA fingerprinting (?)
- ▶ Biological properties, including details on insect host range
- ▶ Demonstrated absence of β -exotoxin (fly larvae test) and emetic toxin
- ▶ Other biological properties, including potential for *B. cereus*-type enterotoxin production; genomic mapping to distinguish benign from pathogenic strains (?)
- ▶ Details of manufacturing methods and quality assurance programme, disclosure of ingredients (including contaminant analysis and mouse injection to confirm absence of Ba), storage stability testing, physical-chemical properties of product



Human Health and Safety: Tier I Studies

Study Type	Dose	Test Material
Acute oral	single high dose (10^8 MPCA units/animal)	TGAI
Acute pulmonary	intratracheal instillation of single high dose (10^8 MPCA units/animal)	TGAI
Intravenous infectivity	single high dose (10^7 MPCA units/animal) injected intravenously	MPCA
Acute dermal toxicity	single high dose (2g/kg bw) applied to ~ 10% of body surface area for 24 h	EP
Dermal irritation study	single dose (0.5 mL or 0.5 g/animal) applied to small area (6 cm ²) for 4 h	EP
Hypersensitivity incidents	all MPCAs are considered potential sensitizing agents	MPCA or EP

Environmental Fate and Effects: Non-target Organism Testing (Tier I)

Test	Test Substance	Type of test
Avian oral	TGAI or EP	Maximum Challenge Concentration
Avian pulmonary, inhalation or injection		
Wild mammals		
Fish: Freshwater		
Fish: Estuarine or marine		
Arthropods: Terrestrial		
Arthropods: Aquatic		
Non-arthropod invertebrates: Terrestrial		
Non-arthropod invertebrates: Aquatic		
Plants: Terrestrial		
Plants: Aquatic		

Addressing Health and Environment Requirements

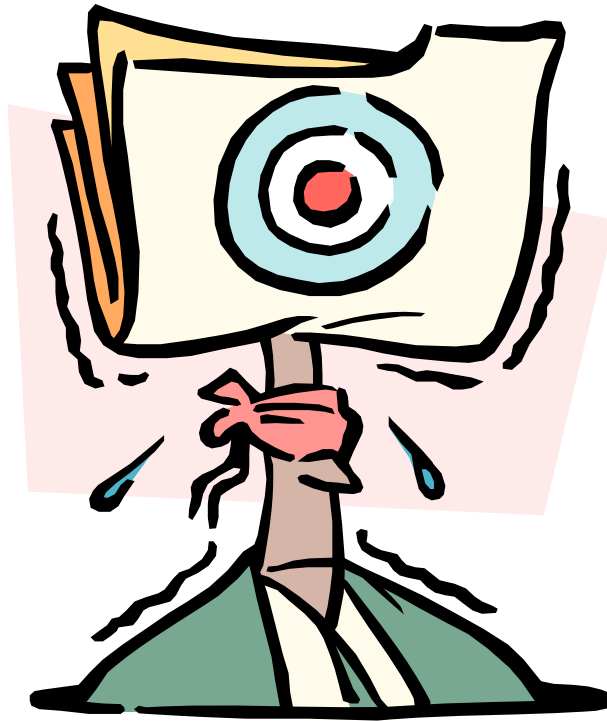
- ▶ Registration requirements can be addressed by submitting:
 - ◆ actual test data on the organism
 - ◆ published scientific literature
 - ◆ surrogate information or bridging data to another strain/species, if both belong to a well-known (familiar) taxon
 - ◆ a rationale to waive the requirement based on sound scientific reasoning

Parting Advice!

- ▶ Seek guidance from regulatory authorities during product development phase and **BEFORE** you undertake studies intended to support registration/authorization (we really are here to help!)
- ▶ Pay careful attention when choosing formulants (inerts) – stick to the U.S. EPA and PMRA List 4A and 4B ingredients as much as possible (you are after all developing SAFER alternatives to conventional pesticides)



Questions?



Data Waivers

- ▶ Data requirements for registration of MPCAs may not always be applicable for every microorganism
- ▶ Regulatory authorities may waive data requirements on a case-by case basis in response to written requests

Waiver Requests

- ▶ Waiver requests must:
 - ◆ explain why the data requirement should be waived
 - ◆ suggest alternative means of obtaining data to address the underlying concern(s) of the data requirement
 - ◆ furnish any other information that may support the request

Possible Reasons To Waive Testing

- ◆ A study is not practical or is not applicable for a particular organism
- ◆ A study is already available in published scientific literature
- ◆ The data are not useful in risk evaluation

Underlying Concerns

- ▶ Infectivity of MPCA
- ▶ Toxicity of MPCA:
 - ◆ Allergens
 - ◆ Microbial toxins and other toxic metabolites
 - ◆ Extraneous host residues from MPCAs produced in cell cultures, whole animals or other living forms

Underlying Concerns

- ▶ Potential effect(s) of manufacturing impurities or formulation ingredients
- ▶ Exposure

Supporting Information

► Biological properties

- known host range
- growing temperatures
- natural occurrence
- ecological niche



Supporting Information

- ▶ Surrogate data
 - data on a related organism or strain
 - relationship of tested strain to the MPCA must be well described

- ▶ Information on the effects of formulation ingredients/impurities
 - Material Safety Data Sheets (MSDS)
 - published literature

Supporting Information

- ▶ Literature search results in relevant databases
 - BIOSIS, PubMed, Biological Abstracts, AGRICOLA, TOXLINE, etc.
 - include key search words

- ▶ Published scientific literature
 - legible copies must be submitted

Notes on Published Literature

- ▶ Mini literature review(s) summarizing all relevant studies
- ▶ Explain how the literature addresses the underlying concern
- ▶ Make sure all the necessary information is available in published study

Waivers for Human Health and Safety

- ▶ avoid requesting waivers for all health studies
- ▶ some studies may be easier to waive than others
 - e.g., combined dermal toxicity & irritation
 - infectivity requirement may be waived for a particular route of exposure



Waivers for Human Health and Safety

- ▶ In waivers, include a discussion on:
 - ◆ toxicity of metabolites/toxins
 - ◆ toxicity of formulation ingredients for EP studies
- * additional personal protective equipment may be required

Environmental Fate

- ▶ not required at Tier 1
- ▶ fate data may be useful in supporting waivers for environmental toxicology



Waivers for Environmental Toxicology

▶ Waivers can be based on:

◆ Hazard:

- literature search for adverse effects
- toxicity of metabolites/toxins
- toxicity of formulation ingredients

◆ Exposure:

- natural occurrence and/or fate data



Estimated Timelines for R&D

