Health Canada / Santé Canada
Pest Management Regulatory Agency / Agence de réglementation de la lutte antiparasitaire

Date Received - Date de réception :

Office Use Only - à usage interne seulement

Notice of Objection to a Registration Decision under Subsection 35(1) of the *Pest Control Products Act*

Avis d'opposition à une décision d'homologation en vertu du paragraphe 35(1) de la *Loi sur les produits antiparasitaires*

1. Objector information - Information sur l'opposant

Name - Nom / Corporation - société / Organization - organisation: David Davies, Forest Protection Limited

Postal Address - Adresse postale: Fredericton International Airport, 2502 Route 102

City/Town - Ville	Province/State - Province/État	Country - Pays	Postal Code/ZIP - Code postal/Zip
Lincoln	NB	Canada	E3B 7E6

Phone - Téléphone	Fax - Télécopieur	E-mail - Adresse électronique
506 446-6530	506 446-6534	ddavies@forestprotectionlimited.com

2. Product information - Information sur le produit

Name of active ingredient to which the decision relates:
Nom de la matière active à laquelle la décision se rapporte : Bac. Nus thuringiensis

Name of end-use product to which the decision relates:
Nom de la préparation commerciale à laquelle la décision se rapporte : Foray

3. Registration decision to which the objection relates - Décision d'homologation pour laquelle vous déposez un avis d'opposition

Decisions on application - Décision concernant la demande

- ☐ Granting registration - Homologation accordée
- ☐ Denying registration - Homologation rejetée
- ☐ Granting an amendment of a registration - Modification à l'homologation accordée
- ☐ Denying an amendment of a registration - Modification à l'homologation rejetée

Decisions on re-evaluation or special review - Décision concernant la réévaluation ou l'examen spécial

- ☐ Confirming registration - Homologation confirmée
- ☐ Cancelling registration - Homologation annulée
- ☑ Amending registration - Modification à une homologation

4. Date the decision statement was made public: Date de la publication de l'énoncé de décision :

6 May 2008

5. Area of scientific evaluation to which the objection relates - Volet de l'évaluation scientifique touché par l'avis d'opposition

- ☑ Health risk assessment (toxicology, food residue, occupational exposure) - Évaluation des risques pour la santé (toxicologie, résidus dans les aliments, exposition professionnelle)
- ☑ Environmental risk assessment (environmental fate, environmental toxicology) - Évaluation des risques pour l'environnement (devenir dans l'environnement, écotoxicologie)
- ☑ Value and efficacy assessments (crop tolerance, value) - Évaluation de la valeur et de l'efficacité (tolérance des cultures, valeur)

6. Scientific basis for the objection / Fondement scientifique de l'opposition

Attachment included: ☑ Yes ☐ No
Pièce jointe incluse : ☐ Oui ☐ Non

7. Signature of objector or representative - Signature de l'opposant ou de son représentant

David Davies

Printed Name - Nom en lettres moulées

Objectors who submit confidential information (i.e., confidential business information, confidential test data) are responsible for identifying this information which is part of their submission.

Information required to process the notice of objection may include some personal information as defined in the *Privacy Act*. In accordance with that Act, such personal information may be made public as authorized by the *Pest Control Products Act* and its regulations. Under the *Privacy Act*, individuals have the right to look at their personal information. For more information on how PMRA manages personal information, contact the PMRA Information Services at 1-800-267-6315 within Canada and 1-613-736-3799 outside of Canada or via e-mail at pmra_infoserv@hc-sc.gc.ca.

Les opposants qui soumettent des renseignements confidentiels (c.-à-d. des renseignements commerciaux confidentiels, des données d'essai confidentielles) sont responsables de les désigner comme tels dans leur envoi.

L'information requise pour traiter cet avis d'opposition peut comprendre certains renseignements personnels tels que définis dans la *Loi sur la protection des renseignements personnels*. Conformément à cette Loi, ces renseignements peuvent être rendus publics, ce qui est permis par la *Loi sur les produits antiparasitaires* et son Règlement. En vertu de la *Loi sur la protection des renseignements personnels*, tous les individus ont le droit de consulter leurs renseignements personnels. On peut obtenir des précisions sur la gestion des renseignements personnels auprès de l'Agence de réglementation de la lutte antiparasitaire (ARLA) en communiquant avec le Service de renseignements au 1-800-267-6315 au Canada, ou au 1-613-736-3799 de l'extérieur du Canada, ou par courrier électronique à pmra_infoserv@hc-sc.gc.ca.

PLP

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Latitude N45°52.62765'
Longitude W66°32.24505'

VIA COURIER

June 30, 2008

To: Health Canada Pest Management Regulatory Agency
Address Location: 6606E
2720 Riverside Drive
Ottawa, Ontario K1A 0K9

RE: NOTICE OF OBJECTION, RE-EVALUATION DECISION BACILLUS THURINGIENSIS, 6 MAY, 2008

This letter and attachments are to serve as a notice of objection from Forest Protection Limited (owned by the Province of New Brunswick, Weyerhaeuser Company Limited, J.D. Irving, Limited, AV Nackawic Inc., Fraser Papers Inc., UPM-Kymmene Miramichi Inc., Acadian Timber) to the above noted decision.

It is very disappointing if not outright frustrating to review the PMRA Responses in this document and Revised Label Amendments which on several issues have nothing to do with science based decisions. I will note our specific objections in point form.

1. Labels should remain silent on aquatic issues.

2. Storage period of six months is unreasonable especially if based on possible declining potency of the product. It is a very common practice in forestry operations to carry over product from one year to the next (or several years) especially if one considers the order time to obtain required product and areas requiring possible treatment may be deleted at the very last moment for biological reasons.

3. All programs that we have been involved with in the last several decades involve "dosed' loading systems. The use of goggles and NIOSH - approved respirators by loading staff working around aircraft increases the risk of an accident for unnecessary reasons. The requirement to decontaminate aircraft cockpits and vehicle cabs is ludicrous at best.

4. Resistance Management Recommendations are not required for forestry applications. Please note attached document prepared by Dr. Kees Van Frankenhuzen.

5. "Appropriate marking devices" in lieu of GPS and data logging systems is a step back into 20 year old technology. Note attached presentation by myself given on April 28, 2008 in Ottawa at the PMRA sponsored Sensitive Habitat Workshop. This makes me wonder why I took three days out of my life to educate regulators on state-of-the-art application technology. FPL definitely can be considered an "expert" in this field yet our opinions are continually ignored. Using terminology on labels such as historic 1:50000 topo maps or "more up-to-date information" in GPS systems (which literally would state Global Positioning Systems systems) and not noting GIS maps indicates a total misunderstanding by PMRA staff of how a forestry program is planned and carried out. You should start stepping into twenty-first century technology.

If you require and further detail re my comments please contact me.

Yours truly,

David C. Davies
Managing Director

DCD/mp

Enclosure

Valent BioSciences Corporation (VBC) Notice of Objection to the PMRA Decision on Label Requirements for Bacillus thuringiensis

Application to Aquatic Habitats

Valent BioSciences Corp. is concerned about the need of label language specific to Btk under **DIRECTIONS FOR USE** - "Do not apply directly to aquatic habitats ..... "

This language as written will preclude the primary intended uses for some of the Btk products. Standard label language can prevent a product from being used for an approved use because it makes practical application of the product impossible. Bts do not pose actual aquatic concerns (please see some of the scientific indications of this below). Hence additional label language needs to be developed which clearly indicates that forestry uses of Bt, do not present a direct application to aquatic habitats.

Labels already clearly indicate the applications permitted. There are no aquatic uses on the labels for Btk. The concern comes on possible precautionary statement interpretation/liability when doing aerial Btk applications on, for example, large forestry tracks that may contain streams, prairie potholes, creeks, etc. While the application is not 'directed' at aquatic habitats overspray cannot be strictly controlled as aquatic habitats intermingled in forests are overflown.

Btk sprays have been undertaken for years in Canada. Projects undertaken by Environment Canada continue to show the innocuousness of Bt to the environment. For example the study: **PROJECT, Ecotoxicology of Biotechnology Products: The case of *Bacillus thuringiensis*** (www qc.ec.gc.ca/csl/pro/pro032fg_e.html)

Gives the following conclusion:

"Main Results to Date

Results have shown that although Bt crystals appear rather stable, the toxin is found in very low concentrations in the aquatic environment and degrades more quickly in water than in soil. Work is continuing in order to determine the presence and persistence of the gene and of the Bt corn toxin (Cry1Ab) in the aquatic environment."

During nearly 45 years of registration and about 30 years of wide scale aerial and terrestrial application around the world, Btk applications have not resulted in any significant aquatic effects when applied in accordance with the label. The proposed wording is not supported by fact. The science clearly shows the little risk Bts poses to aquatic habitats in all their complexity.

The paper: Functional Effects of the Bacterial Insecticide *Bacillus thuringiensis* var. *kurstaki* on Aquatic Microbial Communities. David P. Kreutzweiser[a], J. Lawrence Gringorten[a], David R. Thomas[a] and Jason T. Butcher

[a] Canadian Forest Service, 1219 Queen St. East, Sault Ste. Marie, Ontario, Canada, P6A 5M7

Ecotoxicology and Environmental Safety Volume 33, Issue 3, April 1996, Pages 271-280

Indicates: "These results from laboratory and controlled field experiments indicate that contamination of watercourses with Btk is unlikely to result in significant adverse effects on microbial community function in terms of detrital decomposition."

In addition, an up-to-date book by two New Zealand scientists covers the potential environmental impacts of Bt in great detail.

- Glare, T.R. and O'Callaghan, M. 2000. *Bacillus thuringiensis*; Biology, Ecology and Safety. John Wiley and Sons, Chichester, UK. 350 pp.

The World Health Organisation have also recently reviewed the environmental impacts of Bt (Anonymous 1999. Microbial Pest Control Agent *Bacillus thuringiensis*. Environmental Health Criteria 217. World Health Organisation, Geneva).

Both publications offer no significant effects on aquatic systems by Bts.

Water quality should not be directly affected by Btk as it is not likely to affect most aquatic organisms. Some North American laboratory studies have shown decreases in detritus decomposition rates at high doses of Btk. However, these effects are unlikely in the environment because of the lower doses of Btk used and the purification processes in natural systems (USDA 1995).

VBC Proposal: As indicated in the Bt Re-evaluation Document, there are no aquatic impacts associated with the use of Bt. This proposed label language should be modified/present an addendum to prevent the liability questions raised by the language in its current state. The control of pests that are present in vegetation over waters should not be considered a direct application of pesticide to water. Standard label language which is appropriate and feasible for agricultural uses, is not workable for forestry applications. Labels should be reflective of actual concerns and safety precautions that are in fact called for.

Precautionary Statements:

Valent BioSciences Corp. believes precautionary wording should be specific to the product being labeled. Thus we agree with the indication that "CAUTION EYE IRRITANT" may be waived if data is submitted that indicates that eye irritation would not be an issue with a particular Bt product.

Additionally, the Re-evaluation Decision Document would require that Bt labels must contain precautionary statements indicating that the product is a POTENTIAL SENSITIZER. VBC believes that data should also be considered for determining language on sensitization. While bacterial products, due to their protein content may have the potential to be sensitizers, if data is sufficient to indicate that sensitization would not be an issue for a specific strain and/or formulation, then the requirement for a sensitization statement should also have the possibility to be waived.

In its PACR for Bts, PMRA has evaluated a large number of publications and numerous surveillance reports of large scale human exposure. None of the epidemiological studies identified sensitization from, at least, specific Btk formulations. The potential for sensitization should be able to be waived when there is a large volume of work which would indicate that a specific product does not in fact cause sensitization.

A science based risk evaluation should take into consideration all studies available, for example, where sensitization studies with standard protocols indicate a lack of sensitization there should be no requirement for cautionary statements indicating the product is a potential sensitizer.

Additional scientific evidence as to the different potential of different microbial species to elicit sensitization is attached as Appendix I.

VBC Proposal:
Label language should be left open to being modified by specific data.

Personal Protective Equipment:

The precautionary statements include a statement to 'Avoid breathing dust/spray mist' and a mandatory requirement for a NIOSH-approved '–95 'respirator face-mask when handling, applying, or performing cleanups associated with the product. This requirement will provoke a negative response amongst applicators and the public alike.

Given that, as indicated in the PACR document:

"The risk from occupational exposure, however, was determined to be low given the lack of mammalian toxicity." and that "Occupational and non-occupational exposure in Canada is expected to be lower in than in the United States, as the Canadian use rates are generally lower."

And that

"This decision was based on the sum total of all toxicology data submitted to the USEPA along with the lack of any report of significant human health hazards of the various B. thuringiensis strains."

Therefore, the proposed label language is excessively negative and not supported by the available data. The precautionary language and mitigation required is inconsistent with the potential risk due to exposure. The requirement to have workers exercising this level of precaution sends a message to workers and the public that the material being applied is relatively dangerous since similar precaution is not required of many chemical alternatives. There should be no requirement for a dust mask when friability and other applicable studies indicate negligible potential for exposure to dust and inhalation studies indicate minimal toxicological concern from exposure. While harmonization of Canadian labels with the U. S. for similar products is a worthwhile objective in principle, the policy of requiring dust masks for microbials when testing indicates no apparent risk and no guidelines exist for studies to refute the perceived risk is bad policy on either side of the border.

In most forestry and agricultural application scenarios, liquid Bt formulations are handled via a closed-loop pesticide mixing and loading system; mixers and loaders may not be exposed to the product; consequently the need for a NIOSH –95 filter is not required.

The new label language requiring the use of PPE for all handlers of Bti formulations is unnecessary and poses undue requirements upon registrants and end-users.

It has been stated that based on all available data, current labeled applications pose no risk to applicators, handlers or bystanders; therefore the use of a NIOSH-95 filter is an unwarranted restriction not supported by fact.

Not only does this drive up the cost of public health programs, (a significant concern for many large scale municipal programs) it absolutely provides the wrong message to residents of the treated areas. A dust mask for hygiene purposes is adequate protection for Bti handlers. Residents will not easily accept the safety of Bti based products when they are confronted with NIOSH-95 equipped applicators working in their neighborhood.

VBC Proposal: PMRA should propose label language that is supported by data. PMRA recognizes that there are already several strain sources and therefore PMRA should also recognize the variability within these sources for numerous factors, including unique characteristics and usage patterns. The cautionary notes re Sensitization

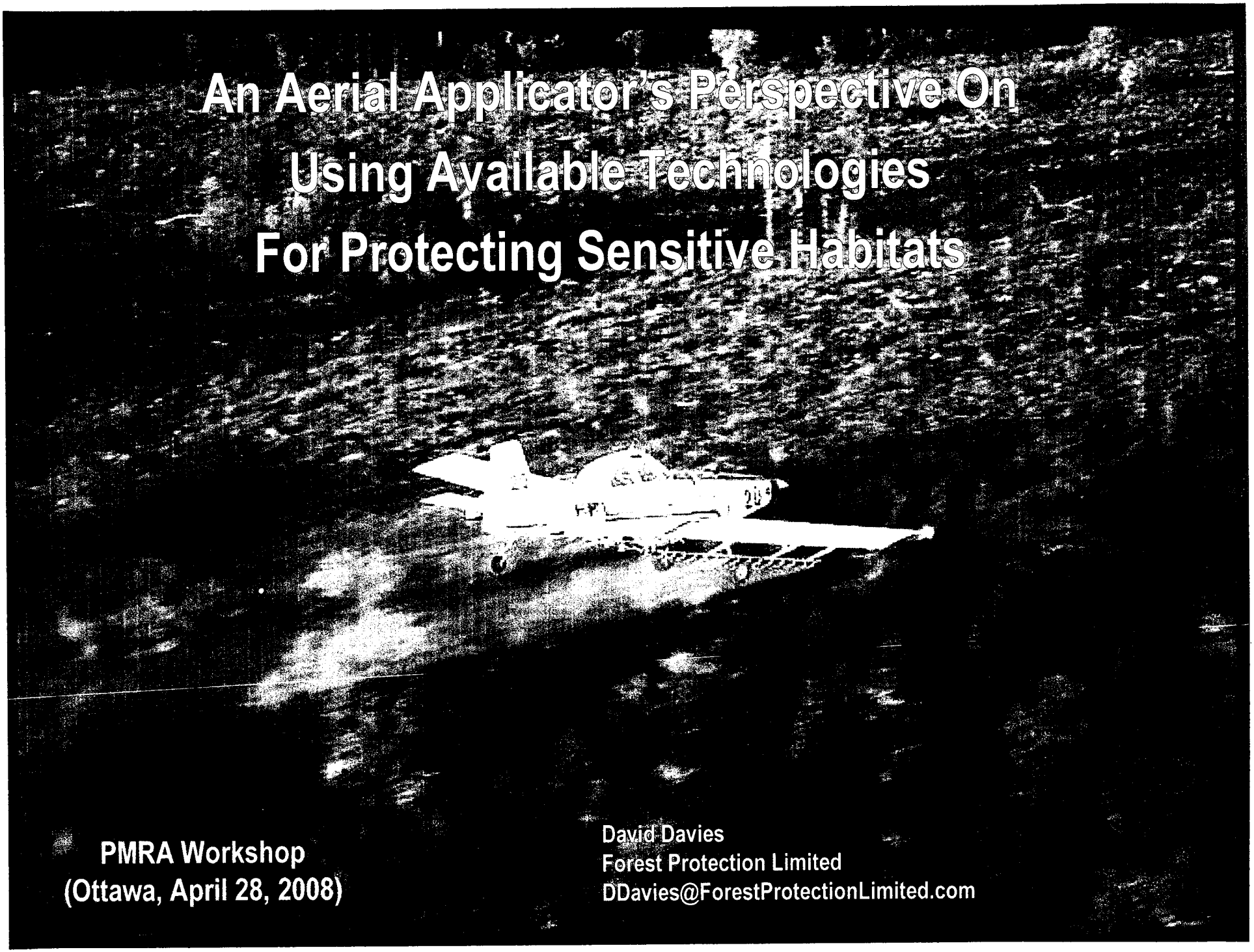
and the requirement for '–95' NIOSH respirators should be left open to being ameliorated based on scientific data.

Label Language:

End-use products:

VBC would question the requirement to have all end-use labels include a "Do not apply by any type of irrigation system." For certain agricultural Btk uses, chemigation may be an effective way of applying the product. No scientific rational for the statement has been proposed by PMRA.

VBC Proposal: The possibility for this application method should be open to a risk assessment.



An Aerial Applicator's Perspective On Using Available Technologies For Protecting Sensitive Habitats

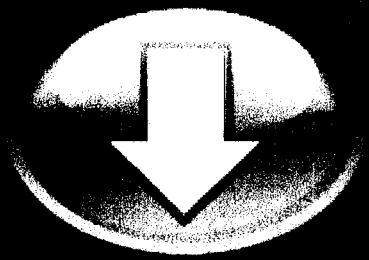
PMRA Workshop
(Ottawa, April 28, 2008)

David Davies
Forest Protection Limited
DDavies@ForestProtectionLimited.com

Presentation

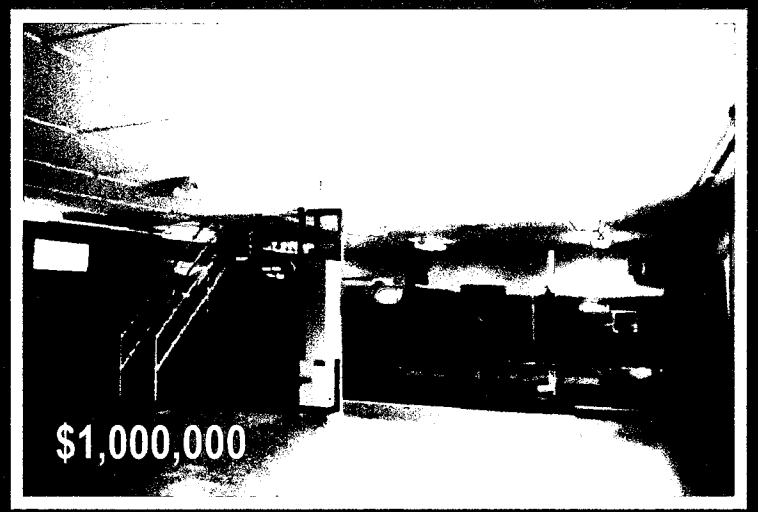
- Aerial Application Technologies
- Habitat Protection Requirements

Aerial Application Technologies Available



Operational Aerial Application Objectives

Aerial Application Technologies Available (Current & Under Development)



Operational

- Meteorological measuring systems
  - real time (ground & air)
- Wind tunnel tests
- Droplet spectrums
- Spray drift models (AGDISP)
- Geographic information systems – GIS (Forestry)
- Spray Aircraft:
  - guidance systems
  - data logging systems
  - aircraft "auto flow" systems
  - aircraft "auto boom" systems
  - aircraft real time tracking
  - radar / laser altimeters
- DSS (PROPS, Spray Advisor, Casper-Forestry)

Under Development / VALIDATION

- Spray aircraft "optimization" systems/real time aerial management system

Operational Aerial Application Objectives

1) Address Environmental Concerns . . .
   - long & short-term, buffers, application rates, acceptable NOELs
   - accountability, accurate records.

2) Maximize target deposit (leaf / needle / ground / block) to . . .

   Maximize pest control (spruce budworm, etc.) and . . .

   Minimize off-target deposit (anything but above) to . . .

   Minimize Environmental Concerns.

Operational Aerial Application Objectives

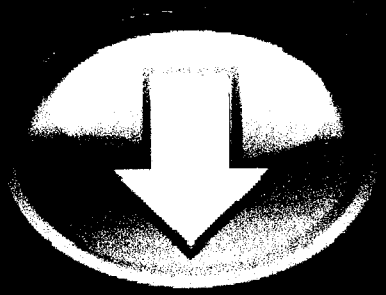
3) Optimize Costs & Benefits – "biggest bang for the buck"
   – rough rule of thumb 50:50 application:pesticide costs
   – reduce application costs & pesticide use – conservative estimate 10-20% savings.

4) Make life simpler, save time.

Suggestions for continuing to enhance & develop Aerial Application Technologies – Need Funding / Sales / $

- Regulators – some operational well-proven enhanced technologies should be "mandatory" or acceptable alternatives noted, label changes required, certification (ISO, CSA)?
- Insurers – lower rates for enhanced / protective operational technologies
- Customers – require / specify enhanced technologies and / or at least "pay" for their operational use
- Public / Stakeholders – must be better informed and lobby for use of technology that is available for operational use – no longer "crop dusters"
- Operators / Developers / Suppliers – increase tech transfer to all of the above re costs and benefits of new technologies

Habitat Protection Requirements



Must be practical to address and mitigate "legitimate" environmental concerns for various pesticides

Predominant pesticides used in forestry

- Glyphosate (Vision, Forza, Vantage)
- Btk (Foray)
- Tebufenozide (Mimic)
- Viruses (Abietiv)
- Pheromones (Disrupt)

Practical Considerations

- Spray aircraft can travel up to 75 m/sec
- Pilot reaction times at low levels (just above crop)
- Aircraft system reaction times (autobooms, auto flow)
- Safety of some pesticides being used (Pheromones, Viruses, Bt, Carbaryl)
- Defining sensitive habitats accurately on GIS maps that "require" buffers
- Buffer requirements based on science, not public perception
- Number of upwind spray lines, modeled / actual deposit

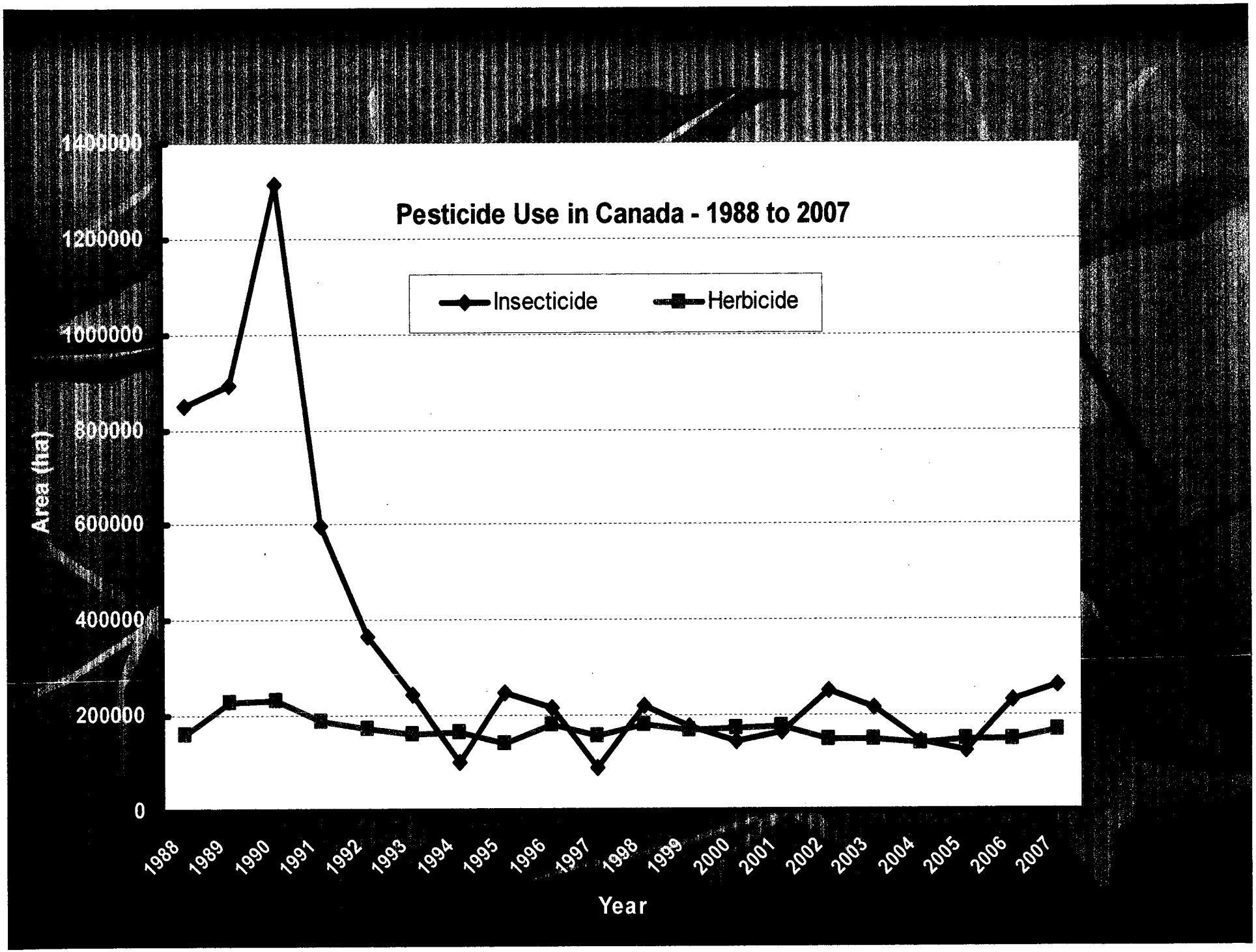
Practical Considerations (Continued)

- Contentious label language ("do not deposit in water", "use appropriate marking devices" for guidance, Etc.)
- Required label language ("wind tunnel instruments must be used to adjust equipment to produce acceptable droplet size spectra", Etc.)
- Optimized applications – booms on outside of block/over water
- Location of maximum stipulated wind speed (required?) – aircraft height VS ground
- Other??

20 Year (1988-2007) Canadian Forestry Pesticide Use (excludes Territories)

- Total Forest Land 345 330 000 ha
- Average Annual Area Treated With Herbicide 170 057 ha (0.05%)
- Average Annual Area Treated With Insecticides 342 802 ha (0.10%)
- Average Annual Area Treated With Pesticides 512 859 ha (0.15%)

Note: the same area is rarely treated more than once or twice.


Pesticide Use in Canada - 1988 to 2007
Insecticide
Herbicide
Area (ha)
1400000
1200000
1000000
800000
600000
400000
200000
0
1988
1989
1990
1991
1992
1993
1994
1995
1996
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2004
2005
2006
2007
Year

Daves's Simple Requests (to make life easier)

- 1) New Btk labels remain silent on water deposit
- 2) National aerial applicators licenses (just like cars & pilots)
- 3)"Fast track" system for research permits for new safe biological pesticides


Thank You
Questions / Comments / Discussion
David Davies
Forest Protection Limited
DDavies@ForestProtectionLimited.com
www.ForestProtectionLimited.com

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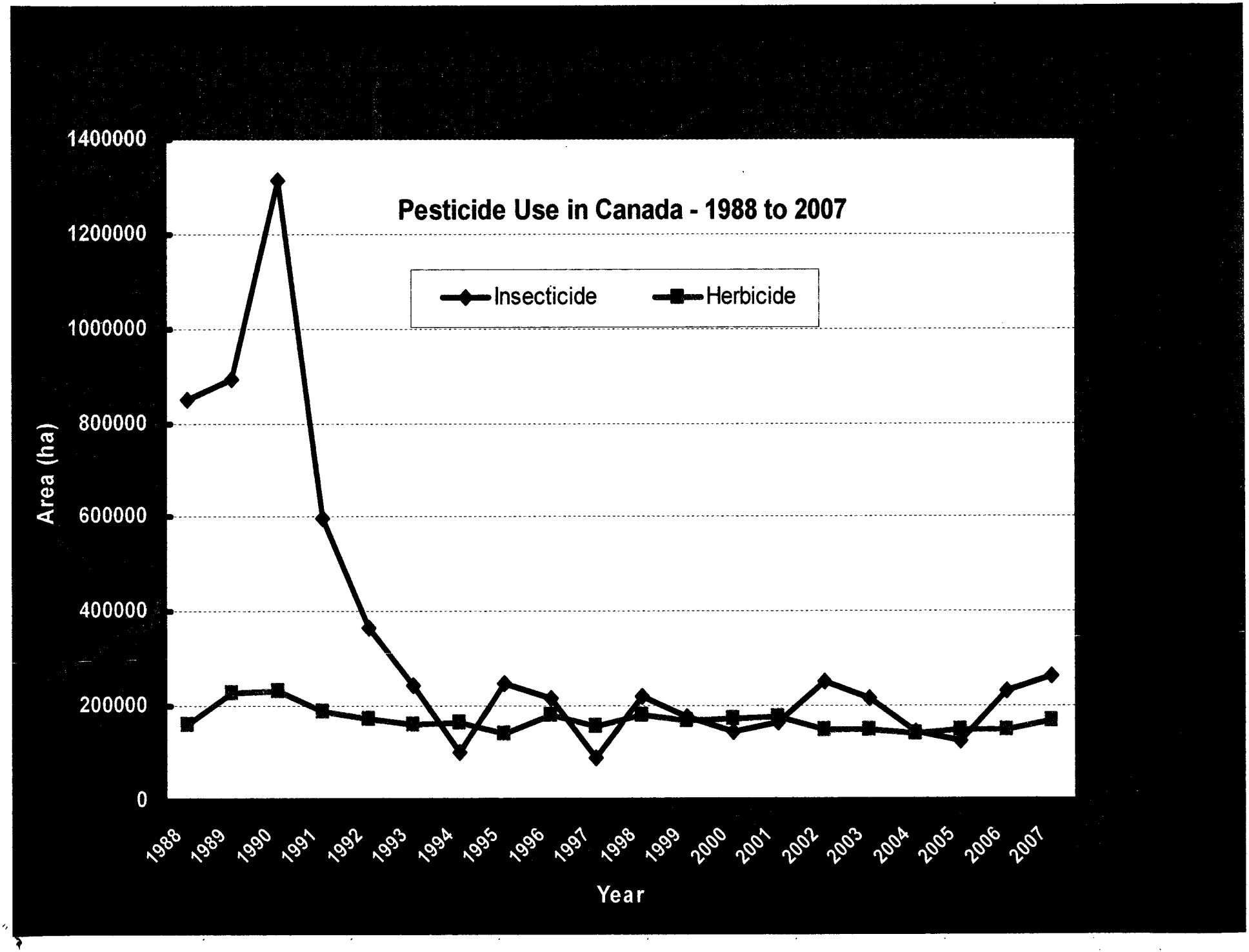
Pesticide Use in Canada - 1988 to 2007
Insecticide
Herbicide
Area (ha)
1400000
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800000
600000
400000
200000
0
1988
1989
1990
1991
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Daves's Simple Requests (to make life easier)

- 1) New Btk labels remain silent on water deposit
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- 3)"Fast track" system for research permits for new safe biological pesticides


Thank You
estions / Comments / Discussion
David Davies
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DDavies@ForestProtectionLimited.com
www.ForestProtectionLimited.com

Comments regarding Proposed Acceptability for Continuing Registration, Re-evaluation of *Bacillus thuringiensis*, document PACR2006-09

Submitted by Dr. K. van Frankenhuyzen,

Team leader Microbial Control Products
Great Lakes Forestry Centre
Canadian Forest Service
Natural Resources Canada
kvanfran@nrcan.gc.ca
phone: 705-541-5671
fax: 705-541-5700

January 12 2007

The main conclusion of this document (PACR2006-09) is that "Based on the USEPA RED document on *B. thuringiensis*, and in consideration of Canadian use patterns, the PMRA has determined that *B. thuringiensis* is acceptable for continued registration provided that the mitigation measures specified in section 8.1 are adopted and the data requirements specified in Section 9.0 are addressed".

I would like to make some comments regarding the proposed label modifications, and the proposed data requirements.

Proposed label modifications

PMRA examined continued acceptability of *B. thuringiensis*-based pesticides using current scientific approaches. The decision for continued acceptability is based on a synthesis of current scientific knowledge, as reflected in Section 2, and a thorough review of current data regarding human health, environmental fate and toxicology (sections 5 and 6). The review shows that extensive data collected to date overwhelmingly support the public and environmental safety of Bts in their current registered uses.

The label changes proposed in Section 8.1 do not always logically follow from or are not always supported by the review's conclusions, and therefore undermine the very premise that the re-evaluation is based on scientific procedure. Some indicative examples are highlighted below.

1. The label must include an extensive resistance management statement (p. 24)
The review in sections 2-6 does not address resistance problems associated with the use of Bt, so how did PMRA conclude the need for such a statement? An assessment of resistance risks within the context of Canadian use patterns would have shown that inclusion of such a statement is not justified. A review would have concluded that Bt resistance in field populations is very rare and has occurred in a few instances under rather exceptional circumstances that are not relevant to Canadian applications. A basic risk assessment would have concluded that the selective pressures needed to give rise to

resistant biotypes are not likely to occur under any Canadian use pattern, not even under the repeated application scenarios aimed at pest eradication.

2. The label must include a statement requiring the use of an NIOSH-approved respirator when handling the end-use product (p. 22).
After concluding that health risks associated with Bt are negligible, what is the justification and scientific rationale for increasing the use of protective gear to include the use of respirators?

3. The label must include the statement: Environmental hazard – "Do not apply directly to aquatic habitats" (p. 25).
After concluding that health risks associated with drinking water exposure are negligible, and that aquatic ecosystem impacts are minimal or non-existent, what is the justification and scientific basis for re-instating the directive to avoid application to aquatic habitats?

Inclusion of the latter two statements conveys the message to end-users and the Canadian public that PMRA says Bt is safe but does not really believe it. Such ambiguity could damage the value and credibility of the re-evaluation process, and could result in unnecessary restrictions on operational pest management activities with severe economic consequences.

Proposed data requirements

In its review, PMRA noted a problem with product identification and strain equivalency among products (Section 2.5), largely based on the premise that serotyping is no longer an appropriate method for strain characterization. PMRA proposes to require registrants to submit complete characterization data, as company-specific strain designations will be required on product labels. A few comments are needed:

1. More detailed strain characterization is highly desirable and will be an extremely useful addition to current requirements. Although serotyping by itself is not sufficient for strain characterization, it is a widely used and scientifically accepted tool, and should still be required as a component of the strain characterization data package.

2. The document is ambiguous as to why more detailed strain characterization is desirable. Is it to better assess strain equivalency among products, or to ensure batch to batch consistency? It is important to differentiate between these two different objectives, as they require different techniques.

3. To ensure consistency and comparability among submissions, it might be desirable to be more specific in defining the data requirements. For example, RAPD produces different profiles with different decamer primers, and won't be useful for between-strain comparisons unless every company uses the same primer set.

4. Why does the document not include the proposed abolition of *T. ni* bioassays as a means of standardizing product potency?

Response to Potential Sensitizer precautionary statement for ***Bacillus thuringiensis*** **subsp. *kurstaki* strain ABTS-351**

Sensitisation/allergenicity observations

Bernstein et al. (1999) observed farm workers (vegetable harvesters) before and after exposure to *Bacillus thuringiensis* subsp. *kurstaki* (strain SA-11). There was no evidence of occupationally-related respiratory syndromes. Positive skin test responses to spore extracts of *B. thuringiensis* subsp. *kurstaki* were observed, and specific IgE and IgG antibodies were present. However, following repeat exposure in 579 Danish greenhouse workers exposed to DiPel® (strain ABTS-351), there was no evidence of sensitisation based on a measurement of total IgE (Larsen & Bælum, 2002).

Doekes et al. (2004), studying more than 300 greenhouse workers, reported that exposure to *B. thuringiensis* biopesticides (Bactimos® and Vectobac®; *B. thuringiensis* subsp. *israelensis*) confers a risk of IgE-mediated sensitization. No increase in respiratory health symptoms was observed.

Laferrière et al. (1987, as cited in WHO, 1999) demonstrated antibody titres against vegetative cells of *B. thuringiensis* in workers exposed to *B. thuringiensis* subsp. *kurstaki*. Little or no formation of antibodies against spores or crystals was detected. No adverse health effects were reported.

Two incidents of possible allergic reaction to *B. thuringiensis* have been reported to US EPA (McClintock et al., 1995). However, *B. thuringiensis* was not considered to be the causative agent in any of the cases.

Comment from Registrant:

All papers cited indicate no adverse health effects.

What is the Significance of Elevated IgE levels in People Exposed to Btk?

Allergic sensitization is a multi-step process. Additionally, the absence of symptoms in exposed individuals, who present measurable allerginicity parameters that may be indicative of sensitization, needs to be explained. No single parameter is currently predictive of allergic potential. It may be that asymptomatic responses represent a normal distribution of responsiveness in the general population without clinical consequences.

Microbial caused sensitization:

Sensitization potential with microbial products appears to be better documented with certain microbes than for others. Gram-negative bacteria with their bacterial liposaccharide (LPS) endotoxin have been proposed as a major candidate for pulmonary inflammation reaction due to their presence in organic dusts. Rylander (2002) reviewed endotoxins in the environment and a possible relationship among exposed persons. Rylander concludes there is a relationship between exposure and disease, yet at certain exposure levels and /or at certain periods of life, exposure may be beneficial and may even reduce the risk for disease. A review of the literature also indicates there is "still a need to investigate other potentially active agents in environments with endotoxin exposure."

Even within the well documented gram-negative endotoxin story, additional studies are now questioning the difference between purified LPS and the true effects of environmentally exposed bacterial systems. There appears to be a differentiation in the Th1 or Th2 cascade. Th1 immune deviation has been shown to decrease airway hyper-reactivity, AHR, and therefore has been suggested that LPS induced immune deviation toward a Th1 response inhibits the Th2 response that leads to asthma (Zuany-Amorim et al. (2002); Erb et al. 1998; Herz et al. 1998). Growing evidence is indicating that bacterial exposure could drive the atopic response into a TH1 direction and away from allergicity (Douwes and Pearce, 2002).

Tulić et al. (2000) looked at the potential role of exposure to bacterial lipopolysaccharides on the development of sensitization to allergen and the response to allergen challenge *in vivo*. They found that exposure to LPS after allergen challenge in sensitized animals abolished the hyper-responsiveness and modified the inflamatory cell influx of late-phase response to allergens.

George et al. (2006) reared C3HeB/FeJ mice (a strain particularly sensitive to endotoxin) in corn dust considered to have a high endotoxin content and microbial product or in low-endotoxin environment. The influence of the corn dust was accessed by BAL cell analysis and immuno-staining of lung tissue. The corn dust was not associated with an inflammatory response in pulmonary alveoli at any time point. There was a high concentration of lung eosinophils early in life which were proposed to possibly be associated with the TH2 bias (or reduced Th1 presence) previously described in neonatal mice and human infants. The conclusion of the study showed that exposure to a nonhygienic environment did not induce significant airway neutrophilia, yet it altered the population of immunologically active cells in the lung and reduced subsequent inflammation.

Gram-positive bacteria have little documented allerginicity potential, especially with any non-pathogenic species. Rather, several species have been proposed to reduce immune responses to antigens. For example, United States Patent 20070190076 (2006) by Institut Pasteur and Institut National de la Santé et de la Recherche Médicale is defined as a bacterial preparation that contains killed Gram positive bacteria, obtainable by a process which does not denature the structure of the molecules from the bacteria cells, and it is able to induce, *in vivo*, a modulation of the immune response against an antigen.

Lactobacillus casei, a nonpathogenic gram-positive bacterium widely used in dairy products has been shown to enhance the cellular immunity of the host. To examine the inhibitory effect of *L. casei* on IgE production, Shida et al. (1998) used splenocytes obtained from ovalbumin (OVA)-primed BALB/c mice that were restimulated *in vitro* with the same antigen in the presence of heat-killed *L. casei*. *L. casei* induced IFN-γ, but inhibited IL-4 and IL-5 secretion, and markedly suppressed total and antigen-specific IgE secretion by OVA-stimulated splenocytes. The Th cell development assay showed the ability of *L. casei* to induce Th1 development preferentially.

Ciprandi et al. (2005) used *Bacillus claussi* spores administered in oral suspension to study potential effects on nasal symptoms in children presenting nasal allerginicity symptoms. *B. clausii* restored physiological Th1 polarization and reduced nasal aesoniphils. B. claussi has also been shown by Ciprandi et al. (2004)to exert modulary activity in allergic children with recurrent respiratory infections.

Von Hertzen and Haahtela (2006) present an overview indicating that settings associated with exposure to microbes in soil and vegetation might be beneficial, even necessary, for the normal maturation of the immune system. High-level exposures to microorganisms in soil have been associated with reduced risk for asthma and atopy.

Are IgE levels/skin reactivity always indicative of clinical allerginicity?

Human response to microbial antigens may induce IgE or IgG antibodies that connote prior exposure, but not necessarily a symptomatic state. Vojdani et al. (2003) indicated: "Furthermore, presentation of clinical symptomatologies did not correlate with the levels of mold antibodies in all patients. Therefore, it seems that antibody levels do not correlate with disease severity, but are indicative of exposure. Therefore, in some individuals, detected mold IgG, IgM, and IgA antibodies may be protective--but not pathogenic."

Two Italian studies with populations selected according to standarized procedures were investigated and followed over time (Baldacci et al., 1997). The populations lived in two areas, namely a rural area in northern Italy and an urban one in Central Italy. The prevalence of respiratory symptoms was higher in the urban area compared to the rural area. On the other hand, no difference between the rural and urban areas was evident when comparing skin reactivity prevalence as a marker of atopy.

A growing body of knowledge indicates the promotion of clinical tolerance to allergens by certain microbial exposures. For example, the July 16, 2007 issue of Thorax contains two articles which add to the observations of an inverse link between mycobacterial exposure and atopic disorder. The relationship between atopy, asthma and mucobacteria are reviewed by JM Hopkins in the same issue.

Georges et. al (2006) showed that both early life and later life sub-chronic exposure to corn dust (high endotoxin-containing) reduced atopic airway inflammation in mice in

response to a known sensitizer (ovalbumin). Yet this exposure did not alter the Ig-E levels in serum in response to the sensitizer.

There is a large range in normal IgE levels (Merrett, 1997) indicated that circulating levels <20 kUL^{-1} render a diagnosis of atopy unlikely, while concentrations > 180 kUL^{-1} indicate a likely diagnosis of atopy. Jansen et al. (1998) showed that analyzing for symptomatic and asymptomatic subjects indicated a higher risk for bronchial hyper-responsiveness correlated with a positive skin test only in symptomatic subjects, independent of high serum total IgE levels.

IgE may actually help defend against certain infections and not be indicative of allerginicity at all. Duarte et al. (2007) studied the increase of specific IgE antibodies to *Plasmodium falciparum* in infected patients. The *P. falciparum* specific IgE response did not correlate with anti-inflamatory cytokin patter bias during malaria but rather seemed to contribute to the control of parasites, since functional activity was higher in asymptomatic and uncomplicated malaria patients than in severe or cerebral malaria groups. Bereczky et al. (2004) found the same sort of response where elevated *P. falciparum* specific IgE levels reduced the change of presenting clinical malaria.

IgE induction by Bt

Laferrire et al. (1987) reported that forest spray workers who used Btk had a significant elevation in antibody titers and levels were higher in workers who were exposed for 2 years. Antibody titers reduced rapidly after exposure ceased and thus the probability that this would result in clinically defined allerginicity in these workers is low. The class of Btk antibodies is not reported so allerginicity is also not clear. Only 5/112 workers were reported to be positive for the antibodies. The study did not report any exposure-related clinical manifestations in the workers.

A longitudinal, follow-up investigation of 48 workers who were involved in picking Bt (Javelin®) sprayed crops (celery, parsley, cabbage, kale, spinach, strawberries) was conducted by Bernstein et al. (1999). Bernstein presented three exposure groups: "Low" (handled onions not Bt sprayed 3 miles away), "Medium" (packaged Bt treated crops) and "High" (picked Bt treated crops). There was no evidence of occupationally related respiratory symptoms. Ocular and dermal symptoms occurred across the three groups and appear to be related to crop exposure reactions and not Btk. Positive skin tests to several spore extracts were seen, chiefly with exposed workers (35%). Yet the "Low" exposure group presented at 25% for an atopic response. Positive skin test were seen with water and mercaptoethanol-sodium dodecyl sulfate extracts of Javelin, yet did not increase for Javelin extracted pro-Delta-endotoxin and protenaise K spore extracts. Specific IgE and IgC antibodies to vegetative cells were present in all groups, which could bring up the question of whether these antibodies were possibly reacting to some cross reactivity.

Doekes et al. (2004) tested sera from the BIOGART project, a longitudinal respiratory health study on >300 Danish greenhouse workers. This study had a 2 and 3 year follow up. While many sera had detectable IgE to Bt (23-29%), all positive reactions were relatively weak IgE anti-Bt positive sera showed, with one exemption, only OD492 values <0.2. These highly exposed workers presented no evidence for work related respiratory symptoms, even after numerous years of exposure. The authors conclude that even while the IgE binding components may be genuine Bt components, they could also be shared with some or many commonly found bacterial species, and in that case the IgE levels may be found in the general population.

What sensitization would be expected from airborne microbials: Asthma?

Even Burrows et al. (1998), who showed a relationship between IgE levels and asthma, pointed out that "although pneumococcal-specific IgE can sometimes be demonstrated, there is no firm evidence that allergy to bacteria in the airway is of importance in the pathogenicity of asthma." Asthma is a heterogeneous condition with multiple biological unique etiologies and is considered to involve allergic and non-allergic mechanisms.

As recommended by Burrows et al., clusters of asthma within occupational groups appear to be a good way to determine allergic stimuli. This is how swine dust, cotton dust, etc. have been identified as allergens. Yet, in the 30+ years that Bts have been extensively used, these asthma clusters have not been seen.

Several studies have in fact shown that exposure to bacterial lipopolysaccharide can either prevent or inhibit asthma in humans or laboratory rodents. While the mechanism of the effect is open to further research and several hypothesis have been put forward, the actual down modulation to antigen induced asthma is well documented (Lundy et al.2003). It is therefore doubtful that at the exposure levels experienced in the field upon spray application that Bts would lead to clinical symptoms of asthma.

Epidemiological Review:

Several epidemiological studies have shown an inverse relationship between human asthma and environmental exposure to LPS (von Mutius et al. 2000; Braun –Fahrlander et al. 1999 and 2002).

The lack of Bt clinical allergenicity development is strengthened by epidemiological data because no increase in respiratory problems has been demonstrated, even when asthmatic individuals are closely monitored.

In a 2-year study, Green et al., 1990, conducted a surveillance program with the four largest medical laboratories in a Btk spray area (80,000 people in 1985 and 40,000 people in 1986). A non-sprayed community 100 km away served as a control population. There

was no increase in the number of telephone complaints nor was there a change in the pattern of complaints.

Another study was conducted (Nobel et al. 1992) in an area with a population of 1,400,000. There was no difference in emergency room visits between spray days and non spray days, nor a significant change in the pattern of telephone calls. There was no evidence that Bt was associated with illness or infection and, in fact, there was a lower incidence of diarrhea in the spray zone than in the control area.

Similar conclusions, of no health effects, can be drawn from the epidemiological studies carried out during eradication spray programs conducted in New Zealand.

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