

IPM-4-Citrus, Citrus disease Integrated Pest Management: from Research to Market

STRATEGY FOR BIOSAFETY ASSESSMENT OF NEW STRAIN-BASED BIOPESTS USING LABORATORY ANIMAL TOXICITY MODELS

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Background and aim: Even *Bacillus thuringiensis* (Bt) Kurstaki group, is generally recognized to be safe for environmental, human and animals, new formulations of biopesticides, had to be tested using toxicity assays for assessing the biosafety, according to European, American and/or international regulations. Nowadays, advantages of alternative methods are well recognized. However, until now most regulations believe that in vitro complementary methods could not be considered as equivalent to animal testing incites for evaluating the toxicity. For these reasons, the human risk assessment of any new formulated biopesticide must be carried out on laboratory animals. The aim of our research is the modeling of a comparative strategy for assessing the biosafety of two new Bt-based biopesticides, belonging to Bt Kurstaki strains: Bt Kurstaki-BLB1 and LIP from Tunisia and Lebanon, respectively.

Keywords: *Bacillus Thuringiensis*, toxicity assessment, biocontrol, Laboratory animals, welfare.



The Biopesticides and Pollution Prevention Division (BPPD), falls under the Environment Protection Agency (US EPA), responsible for all regulatory activities associated with biopesticides, consider Biopesticides as "reduced-risk pesticides" category, with lower risk than conventional pesticides.



Product classification For European Authorities

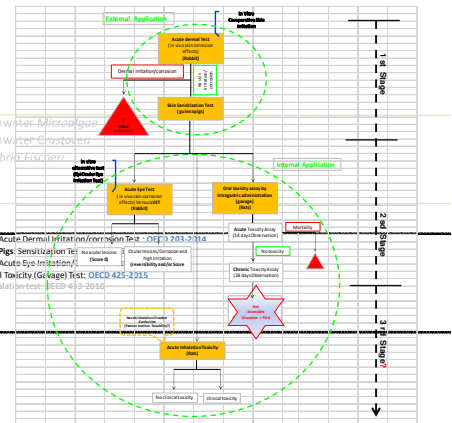
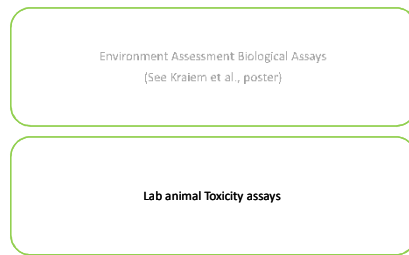
In Annex V of the BPR, the Biocidal products are classified into 22 biocidal product-types and grouped in four main areas

Number	Product-type	Description
Main group 3: Pest control		
PT 18	Insecticides, acaricides and products to control other arthropods	Used for the control of arthropods (e.g. insects, arachnids and crustaceans), by means other than repulsion or attraction.

Methods: Strategy & Action Plan

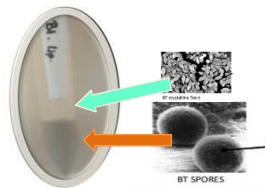
OECD Guidelines & feasibility: Among the OECD guidelines, 5 the requested acute tests has been listed. Only the inhalation test is not possible to complete because it requires a device/equipment that is currently unavailable. NB: the inhalation test is required for soluble products in organic solvents (with air-suspended droplets)

Risk assessment of B.t.k based biopests: Selected tests



Product to use:

Unformulated Btk-LIP = crude mix of Btk spores & endotoxins



Animals: NZW rabbits, albinos guinea pigs & outbred rats from IPT lab animal facilities

Risk Assessment for the new B.t.k based biopests:
General Action Plan

Results & Discussion

1- pH value determination 2- First Acute Dermal Test 3- First Acute Ocular Test

pH value of 8.28, compatible with dermal contact test (Annex B-ISO 10993-10: 2010): (2.0 < pH < 11.5)



Conclusions

- 1- Ethical Approval obtained on December 8th, 2019, registered under the number : 2019/23/LR16IPT
 - 2- General action plan and preliminary tests have been carried out
 - 3- Result related to first carried out acute dermal test : absence of dermal irritation as recorded 24 hours and the following days
→ The product is non-irritant over the observation time period.
 - 4- Result related to first carried out acute ocular test : absence of Eye Irritation as recorded 24 hours and the following day
→ The product is non-irritating to the eyes over the observation time period.
- In conclusion:** These preliminary results are promising and the observation must be continued over 14 days. The BPR (reference product) and the formulated LIP and BLB1 products should be tested according to the guidelines for risk assessment.



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