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SPEAKING LEAF

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A glimpse on whats inside

Microbial Preparations(*) in Cropcare and the Regulatory Expectations

BACKGROUND

The microbial pesticide (*Bacillus popilliae*, a naturally occurring bacterium) was registered in 1948. Since the late 1960s and early 1970s, interest in the application of live microbes and viruses for control of pests and diseases has been slowly gathering speed. There was a time, when these products were expected to largely replace chemical agents. The realization that they wouldn't replace the chemical cousins, surfaced after a few years of their trials, and the current thought process is driven IPM (Integrated Pest Management). The Microbial products are registered for use in agriculture, forestry, mosquito control, lawns and home-gardens. Both EPA and EFSA have considered risk from such products to health and safety as low, especially for *Bacillus subtilis* and B. *amyloliquifaciens* (EFSA DAR 2012).

Synthetic chemical agents have been traditionally used for crop protection as well as healthy, rich and bountiful produce. However, a strong movement has gathered momentum over the last decade to enter into "IPM" using natural, biological as well as chemical agents for control of pests and diseases.

Generally the Microbial Products consist of preparations of living microorganisms. They provide several distinct benefits, in that, being natural life forms, they are expected to be inherently safe as compared to their chemical cousins, since they generally only impact the target organism. This eliminates unwanted adverse effects on the ecosystem as the products and their degradants are natural, non-accumulative and non-toxic.

Biopesticides fall into 3 Major classes

01 Microbial pesticides

02 Biochemical pesticides or herbal pesticides

03 Plant-incorporated protectants (PIPs)

Biopesticides have usually no known function in photosynthesis, growth or other basic aspects of plant physiology.

Definition: Microbial Pesticides / Biopesticides.

The Microbial preparations are "certain types of products derived from natural organisms such as bacteria, fungi, viruses and the other naturally occurring microbes".

EU, defines biopesticides as;

> "a form of pesticide based on micro-organisms or natural products".

The US EPA definition covers several products and, as per their definition given below.

> "The MPCA (Microbial Pest Control Agents) are naturally occurring substances that control pests (biochemical pesticides), micro-organisms that control pests (microbial pesticides), and pesticidal substances produced by plants containing added genetic material (plant-incorporated protectants) or PIPs".

The manufacture involves cultivation and processing of the selected strains of specific bioactive microbes. The live microbial mass is suitably formulated under sterile conditions.

(*) *Synonyms: Microbial pesticides, bio pesticides, microbial biocontrol agents etc.*

Registration requirements:

Generally, the global registration authorities tend to follow the lead established by the US EPA in determination of the relevant guidelines (OCPSS guidelines series 885)[1]. These are designed to ensure no potential pathogenicity implications in the mammals, when these enter via alimentary canal or respiratory route for the safety of the farm operator,

JRF's expertise in regulatory testing of Microbial preparations for crop care

JRF has developed deep expertise in handling MPCAs. JRF is capable of performing different types of *in vivo* studies for bacteria and fungi carriers such as:

- Acute Oral Toxicity / Pathogenicity
- Acute Pulmonary Toxicity / Pathogenicity
- Acute Intraperitoneal Toxicity/ Pathogenicity

The basic principle of testing is to evaluate toxic effects of the preparation after potential oral ingestion or respiratory exposure. The product is tested for development of significant clinical signs, morbidity and mortality as well as survival in the organs of the test system (generally wistar / SD rats). The testing also evaluates potential effects of the microbial secondary metabolites

as well as possible invasion / colonization within the organs exposed to the product.

Acute Oral toxicity test:
The testing differs from conventional testing in terms of number of animals used followed by periodically testing (at least four times) within the stipulated incubation period of typically minimum 21 days. Evaluation is based on the viable microbial counts, to establish if the microbes are cleared through faeces. The organ counts establish, if the microbe could grow and multiply within the alimentary canal.

The presence is also evaluated in the major tissues as detailed below in"Clearance of MPCA".

Acute Pulmonary toxicity test:
This test again uses more animals, for an extended incubation period of minimum 21 days. As in the case of the acute test, there are several interim evaluations for microbial counts in lungs as well as in selected tissues. In case of oral testing, this test also evaluates viable microbial counts in several tissues as detailed below in "Clearance of MPCA".

Acute Intraperitoneal / Intravenous toxicity.
This test is undertaken to establish the potential risks if the microbes enter the circulatory system and colonize in critical organs. The test system is incubated for a minimum of 21 days with interim tissue collection and evaluation of microbial counts, as detailed below in "Clearance of MPCA".

Further testing
In the event that the product hints at toxicity and pathogenicity, the testing moves to the higher tier, involving extended duration, repeat exposure and much deeper biomarker testing.

Genotoxicity testing hasn't been mandated by OCPSS. However, some of the EU DARs do show that genotoxicity testing has been undertaken voluntarily by some of the manufacturers.

Clinical Observations
All the tests above undertake evaluation of the test systems for signs of toxicity and mortality at 1, 3 and 5 hours post-administration on the day of dosing. Subsequently, the rats are observed twice a day for morbidity and mortality for a period of minimum 21 days. The clinical signs are recorded once a day. Individual body weight is recorded prior to dosing on day 0 and at interim and terminal sacrifices on days 3, 7, 14 and 21.

Necropsy
Interim sacrifices are performed on days 3, 7 and 14 for Acute Oral and Intraperitoneal whereas Day 0, 3, 7 and 14 interim sacrifice are performed for Acute Pulmonary. On each of the three days 3 to 5 rats/sex of treated group are sacrificed. Animals, if found dead during the course of the study and animals at interim sacrifice or final sacrifice, are subjected to gross pathological examination as early as possible.

Clearance of MPCA

- Faeces will be examined for the presence of MPCA for Acute Oral
- Lungs will be examined for the presence of MPCA for Acute Pulmonary
- Blood will be examined for the presence of MPCA for Acute Intraperitoneal

Interim Sacrifice/at final Sacrifice
The presence / absence of the test microbes is evaluated in the tissues like blood, brain, lungs, liver, kidneys, stomach, whole intestine, caecum, mesenteric lymph nodes, and spleen.
JRF has undertaken several studies for the major European manufactures and are happy to extend this service to our valued customers across the globe.

Meet Our Team....

[1] *Reference: http://www.epa.gov/ocspp/pubs/frs/publications/Test_Guidelines/series885.htm*

For Business Inquiries:
HJCL: k.shimizu@hodogaya-mb.jp
JRF India: bd@jrfonline.com
JRF America: jrfa@jrfamerica.com
JRFI & GCL: bd@gclabs.com

For further information on microbial, please contact us at bd@jrfonline.com

Visit our website: www.jrfglobal.com