Regulatory Review of *Burkholderia cepacia* Under FIFRA

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The Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) defines pesticides broadly to include agents, chemical and biological, which are intended to prevent, destroy, repel, or otherwise mitigate a pest. This definition includes bacteria which target other microbes and nematodes to preclude or antagonize the plant pathological properties of these infective agents. Hence, strains of the naturally occurring biological control organism, *Burkholderia* (*Pseudomonas*) *cepacia* and its related genomovars, were considered under the authority of FIFRA as part of the regulatory approval process for Section 3 pesticide registrations in 1992 and 1996.

During the course of the review process conducted by the Environmental Protection Agency (EPA), the taxonomic designation of the bacterium initially referred to as *Pseudomonas cepacia* was altered to reflect a greater understanding of its underlying phylogeny and an appreciation for the plasticity of the genome. *Ps. cepacia* was moved in 1992 into the newly established genus *Burkholderia* as *B. cepacia*, with recognition of its original isolation by Cornell University scientist Dr. Walter H. Burkholder in 1949 from diseased onions. As molecular techniques were refined, it became clear that the genome of *B. cepacia* consisted of 2 to 4 self replicating chromosomes and varied in size from roughly a size comparable to *E. coli* (4.5 Mb) to more than twice that. The taxonomic distinctions between species within this group were less than clear at the time the EPA was considering individual strains of this complex for registration as biopesticidal agents. The designation of *B. cepacia* for the purpose of this discussion will consider the species *B. cepacia* and related genetic species (genomovars; *e.g.*, *B. multivorans*, *B. vietnamiensis*, *B. ambifaria*, etc…), most of which have now received specific epithets as named species, as the '*B. cepacia* complex' or simply '*B. cepacia* '.

The guideline studies which must be addressed as part of the FIFRA Section 3 registration process at EPA include those concerned with human health issues and a second set of standardized tests aimed at uncovering any environmental concerns. The human health tests include intravenous, oral, pulmonary, and ocular challenges, using a rodent model and a dermal toxicity / pathogenicity test using a rabbit model. The results of these tests with the *B. cepacia* isolates submitted for review in 1990 by Stine Microbial Products were largely unremarkable (no experimental animal deaths resulted). Similarly, the environmental / non-target organism guideline pathology / toxicity tests, including avian oral challenge, freshwater fish, beneficial insects, honeybees, earthworm, and non-target plant studies were without evidence of significant toxicity or pathogenicity for the test organisms.

A FIFRA mandated Science Advisory Panel (SAP) was convened July 20 – 23, 1999, to discuss the fate of the *B. cepacia*-based fungicidal products (*i.e.*, Blue Circle, Deny) as registered in 1996 by Stine Microbial Products. A growing concern in the medical community indicated that *B. cepacia* (the complex) was capable of colonizing patients afflicted with cystic fibrosis and chronic granulomatous disease. A significant decrease in lifespan of individuals colonized with *B. cepacia* was noted in some instances, although of the total number of Cystic Fibrosis (CF) patients in the United States, approximately 3.5 % were known to be colonized with *B. cepacia*. The colonization and changes in health status attributed to this bacterium became known as 'cepacia syndrome' and had serious health and practical implications for CF patients who were no longer allowed to join Cystic Fibrosis Foundation (CFF) functions due to fear of patient to patient spread.

While it is clear from the FIFRA SAP report (1999) that taxonomic distinction of the various genomovars of *B. cepacia* would not serve adequately to allow for 'safe' biopesticidal strains to be distinguished from those with clinical or nosocomial potential, the question of possible human exposure to *B. cepacia* applied as a seed treatment, soil drench or side dress treatment was less than certain. The SAP report detailed what was known about the levels of *B. cepacia* occurring naturally in agricultural fields with various crop plants and compared this with what levels would be anticipated given application of the highest label rates of Deny® Fungicide. This rate would result in the approximate addition of 3.7×10^{11} cfu/ha, while the available measurements of naturally occurring *B. cepacia* suggest a background population 7.5×10^{12} cfu/ha. The net result is an anticipated transient increase in *B. cepacia* population size of approximately 5 %.

It is notable that the CFF at the time of the SAP and also currently on their website through associated videos does not request that CF patients refrain from gardening, contact with soil, plants, raw vegetables or salad bars. If the environment is seen as a harbor for *B. cepacia* strains with potential to develop into clinical pathogens or as a source of virulence genes which may be exchanged with human-associated isolates, then exposure to these sources should be considered as significant. Further, it is noteworthy that the primary use of the Deny® Fungicide product containing *B. cepacia* was as a seed treatment in which seeds of peas, maize, soybeans and other crops would be treated prior to planting and placed into the soil using mechanical seed drills and covered. The route of increased exposure to humans, other than applicators, from this delivery system remains nebulous and even doubtful, however, it was not fully explored in the SAP analysis due to a variety of factors. As a result of a subsequent 'data call-in', Stine Microbials was requested to conduct several field tests to determine the potential for aerial transport and survival of their *B. cepacia* strains in the environment, as well as development of more detailed genetic information. In 2000, the company decided to voluntarily cancel their registration and limit distribution of remaining stocks.

The risk assessment of *B. cepacia* and, more importantly, the political ramifications of the species' unfortunate association with a serious human disease condition, dictated that the EPA must act to resolve the contentious issue of *B. cepacia* and human health risk. Unfortunately, the emphasis on the 'hazard' side of the risk equation (*i.e.*, clinical infection) overwhelmed the 'exposure' (*i.e.*, increased human exposure in agricultural

settings) side of the equation during the SAP process. With a two thirds panel membership consisting of medical / clinical personnel, it is noteworthy in the panel report that the 'majority' opinion of the SAP appears to dismiss the lack of increased environmental exposure from biopesticidal applications. Given the lack of demonstrable clinical infection of CF patients from environmental *B. cepacia* isolates and the decrease in numbers of CF patients infected with *B. cepacia* since institution of measures to reduce patient contact with cepacia positive individuals (*e.g.*, elimination of CF summer camps, segregated clinics), it would appear that patient-to-patient spread of this organism is certainly worthy of attention, but perhaps environmental exposure and outcome are weighted too heavily in the absence of precise data.

The take home message from this retrospective look at the history of *B. cepacia* as a biopesticidal agent is that meeting early on with the regulators who will be assessing the agent in question may save some significant time and expense. Even then, however, as new scientific information related to the organism comes to light, the risk assessment process may take on renewed life with different resultant conclusions.

[http://www.epa.gov/scipoly/sap/meetings/1999/index.htm#072099] - FIFRA authorized Science Advisory Panel

[http://www.epa.gov/pesticides/biopesticides/regtools/guidelines/40cfr158_740c.htm] – EPA Human Health Testing Guidelines

[http://www.epa.gov/pesticides/biopesticides/regtools/guidelines/40cfr158_740d.htm] – EPA Non-target Organism Testing Guidelines