Information on *Bacillus thuringiensis* subspecies *kurstaki (Btk)*

Excerpts from a Forestry Technical Manual produced by Valent BioSciences, manufacturers of Foray® and DiPel®, two formulations of commercially produced *Bacillus thuringiensis var. kurstaki* (Btk)

www.valentbiosciences.com/docs/pdfs/forestry_and_public_health/manual.pdf

7.0 COMMONLY ASKED QUESTIONS CONCERNING FORAY & DIPEL, THE ENVIRONMENT AND THE SAFETY OF BTK

7.1 What are Foray and DiPel?

Foray and DiPel are microbial or biorational insecticides produced by Valent BioSciences. They contain the spores and unique crystalline proteins produced by a naturally occurring bacterium, *Bacillus thuringiensis* subspecies *kurstaki* (Btk). These biological components are combined with approved ingredients and water to make the final product.

7.2 What is *Bacillus thuringiensis* or Bt?

Bacillus thuringiensis or Bt is a naturally occurring rod-shaped, spore-forming, aerobic, gram-positive micro-organism (bacterium) that is found throughout most areas of the world. It can be found in soils and on leaves/needles and in other common environmental situations. When the bacteria produces spores, it also produces unique crystalline proteins. When eaten, these natural proteins are toxic to certain insects, but not to human beings, birds, or other animals.

7.3 How many other Bt's are There?

There are many varieties or subspecies of B.t., and they do not all share the same properties. *Bacillus thuringiensis* subspecies *kurstaki* (Btk) is the B.t. most widely used commercially to protect agricultural crops, fruit trees and rural and urban forests from defoliating lepidopteran larvae. This particular type of B.t. has been used for over 30 years. Other subspecies of B.t. developed commercially by Valent BioSciences are subspecies *aizawai,* active against lepidopteran pests; *israelensis*, active against mosquito and blackfly larvae; *sphaericus*, active against mosquito larvae, and *tenebrionis*, which is active against some beetle larvae.

7.4 How Does Btk Work?

Btk must be eaten by the target pest larvae in order to cause mortality. The ingested bacterium is not what kills the larvae, but rather a protein crystal produced by the bacterium. The crystal carries a toxin that is lethal to some lepidopteran larvae. To release the toxin, the crystals require the alkaline environment found in the gut of lepidopteran larvae. When Btk is eaten by a susceptible larva, the toxin is released, the midgut wall is destroyed, the gut becomes paralyzed, and the larva stops feeding within a few minutes. Destruction of the midgut wall allows the bacteria to enter the blood of the target insect, causing full-scale infection and death of the insect. This process may take 3-5 days so, unlike the situation with some chemical insecticides, there is not an immediate knockdown of insects following treatment.

7.5 Does Btk Occur Naturally?

Soil is the natural environment for Btk. Varieties of Bt were isolated from urban, forest, and agricultural soils long before the material was used in insect control programs. Bt has been detected in soils and other substrates around the world including Canada, the United States, Japan, Germany, France, and Israel. Bt can also be found on the leaves of deciduous and coniferous trees. Varieties of Bt have also been found in grain elevators and grain dust.

7.6 How are Foray and DiPel Made?
Foray and DiPel are produced from Btk grown in large quantities in enclosed fermentation tanks, in ways very similar to those used for the production of antibiotics and alcoholic beverages. The fermentation broth containing spores and the crystalline proteins is formulated with approved ingredients and water to make the final formulation.

7.7 How are Foray and DiPel Different From Chemical Insecticides?
Btk is not a chemical. Chemical pesticides kill a wider range of insects, including many beneficial ones. The active ingredient of Foray and DiPel is a natural bacterium, *Bacillus thuringiensis.* It has been shown to kill certain caterpillars such as the destructive gypsy moth. Additionally, Foray and DiPel are quickly biodegraded in nature, unlike a number of chemical pesticides that form by-products and residues of environmental concern.

7.8 Why is Btk used for Forest Spraying?
Btk was developed in response to the growing concern among the scientific community and the public in the 1960's and 1970's over the use of chemical pesticides in the forest environment. At that time, forest managers realized that an alternative to broad spectrum chemical insecticides would be needed if forest protection was to remain a component of future forest management efforts. The new insecticide would have to be: effective when applied in small amounts, more host-specific than chemicals, more quickly broken down in the environment than chemicals, and harmless to non-target organisms such as bees, birds, fish and mammals. As well, the cost 26 of the new insecticide would have to be comparable to the cost of chemicals. Btk was not an immediate success in terms of effectiveness and cost, but intensive research and development produced a product that now meets all of these criteria. Btk is now the material of choice in the majority of forest protection programs in North America. This product has gained a level of public acceptance that was unheard of even 10 years ago and, as a result, Btk is widely used to protect trees from insect infestations in both rural and urban settings. The major reasons that Btk is used today is because it is considered ecologically friendly and effective.

7.9 How Effective is Btk?
Btk effectiveness is comparable to chemical applications in controlling many pest insects when pest population densities are low to moderate. Btk is less likely to be as effective as chemicals when pest populations are extremely high unless multiple applications are conducted. However, a control strategy does not have to kill all the target insects in order to be successful. In fact, studies indicate that there are benefits to maintaining some pest insects in an area to support the population of natural enemies. Because it can take several days for Btk to kill larvae, there is not an immediate reduction in the pest population as is the case when some chemical insecticides are used. This has created the erroneous perception that Btk does not work. Btk does work but it takes a little longer to see the results. Appropriate conditions are essential for Btk to be effective. Btk is sensitive to sunlight and heat and will only persist on foliage for 3-7 days. Since Btk has to be eaten to kill target insects, sprays are most successful when medium-sized caterpillars are actively feeding. Depending on the life cycle of the pest and climatic conditions, more than one application of Btk may be necessary to achieve the desired level of control. When eradication is the goal of a control program, a single application of Btk may be somewhat less effective than some chemical insecticides in reducing the population to zero. However, because of its low impact on non-target organisms, Btk is the product of choice for most forest pest control programs (including eradications) conducted in North America and around the world.

7.10 Are Foray and DiPel Harmful to Humans and Animals?
As required by the United States Environmental Protection Agency and Health and Welfare Canada, extensive, oral and intravenous animal studies have been conducted with Foray and DiPel. No evidence of any poisonous, infectious or disease-causing effects were found. In inhalation tests with Btk, there were no mortalities and the Btk was shown to have a low pathogenic potential. Feeding, skin, breathing, and eye irritation animal studies were also carried out with Foray and DiPel. No toxic effects were seen when significant quantities of Foray and

DiPel were fed or inhaled. Very mild, temporary skin irritation and moderate, temporary eye irritation was observed in the tests when Foray and DiPel were applied directly to the skin and into the eyes. These effects were totally reversible. In addition, the Environmental Protection Agency and Agriculture Canada have determined that Foray and DiPel are exempt from the requirement of tolerance on all labeled crops. Due to this exemption, there is no required interval before re-entering a sprayed area. This exemption is based on extensive testing of Btk to determine both short-term and long-term effects on humans and warm-blooded animals. Finally, Btk has been used extensively in commercial urban and rural forest pest management for over 30 years. A solid record of safety and health has been amassed over this time.

7.11 What Effect Will Bt Have On People, Especially Those with Immunodeficiency, Asthma or Allergies?

Bt is a common bacterium found in soils throughout the world. People are exposed to Bt and many other microbes everyday. Many of the microbes we encounter, including Btk, do not produce any toxins which affect humans. Btk and other common microbes are frequently found in blood, urine and other samples from healthy people. It has been shown that the presence of Btk in patient specimen samples is not indicative of pathological or toxic effects. As with many other microbes naturally present in the environment, it can be detected as an insignificant contaminating organism among infectioncausing organisms isolated from patient samples. Individuals with an immuno-deficient condition are somewhat more likely to be affected by microbes that are normally controlled by a healthy immune system. Such 27 microbes are referred to as opportunistic pathogens. Bt is not considered an opportunistic pathogen. Exposure to a Btk spray program is not likely to result in the development of new allergies, asthma or other hypersensitive reactions. Individuals with pre-existing allergies, asthma or hypersensitive individuals, especially those sensitive to normal exposure to soil or smoke and pollutants, could feel some temporary effect. The exposure level to Btk from an aerial spray program is very low in comparison to the levels applied in safety and health related testing. Even at higher levels used in tests, Btk has been shown to be safe. That safety has been confirmed in over 30 years of use in urban and rural applications. Individuals with any of the particular medical conditions described above should consider seeking the advice of their physician.

7.12 Will Foray or DiPel injure plants?

Foray and DiPel have been sprayed on millions of acres of trees and other plants. There have been no reports of any plant damage. Foray and DiPel and other Bt products produced by VBC are commonly used on market gardens and in greenhouses.

7.13 Are Foray and DiPel harmful to non-target animals, birds and beneficial insects?

The Btk in Foray and DiPel has been tested against mammals, birds and other insects. In all cases, when Foray and DiPel were tested at doses far in excess of the levels to which these organisms would be exposed during a routine forestry or urban tree spray program, no harmful effects were observed.

7.14 Are Foray and DiPel Harmful to Aquatic Organisms?

Foray and DiPel have shown no adverse effects in aquatic environments. Btk has been tested against freshwater fish and aquatic invertebrate. After extended exposure tests, there were no adverse effects observed.

7.15 Can Btk Grow and Replicate in the Environment?

Btk is a naturally occurring bacterium but it requires alkaline conditions to complete its life cycle. The vegetative form of Btk is generally not well adapted to soil, and it requires the specialized habitat of vulnerable insects to persist. However, Btk endospores can survive in some soils for at least four months. Foliage, water, and acidic soils are not suitable environments for Btk growth and replication. In these environments, Btk will degrade quite rapidly.

7.16 Won't Target Insects Build Up a Resistance to Btk?

It is very unlikely that forest pests will build up a resistance to Btk. It appears that in order for an insect species to develop resistance to a pesticide, it must have several generations per year, and it must be exposed to multiple applications of the pesticide over a relatively short period of time. In forestry, only a very small area of the total forest is sprayed, and that area will likely not receive more than 2 or 3 treatments over the entire lifespan of the trees. The pest population exposure to Btk is, therefore, extremely low. The chances of a pest developing resistance to Btk in the forest is almost zero. More intensive spray programs are used against agricultural pests. After repetitive applications of Btk to control the diamondback moth in watercress fields in Hawaii, the insect developed a resistance to Btk. Over 5 years, the watercress fields had been treated an average of 10-20 times/year. This is a very high level of exposure. New techniques of implanting Btk genes into cotton and food crops may lead to the development of resistance in the species that feed on the plants. However, these insects never cause infestations on tree species. Resistance to Btk has been documented for the Indian meal moth and the almond moth, both storedproduct insect pests. Storage-product insects are found in confined environments where a Btk treatment would not be subjected to the conditions that would inactivate it in the forest, such as rain or sunlight. In this closed environment, the probability of developing resistance to Btk is significantly greater than in the field.

7.17 What else is in Foray and DiPel besides Btk? Will These Other Ingredients Harm the Environment?

Foray and DiPel are biological insecticides which contain spores and crystal-shaped proteins produced by the naturally occurring bacterium *Bacillus thuringiensis* variety *kurstaki*, or Btk. Foray and DiPel are very selective insecticides and are not designed to control a wide variety of insect species. All Bt products, including Foray and DiPel, are produced in a similar fashion. The Btk is grown in large enclosed fermentation tanks. Foray and DiPel are produced using ingredients and a technology which are similar to those used to make beer or spirits. During fermentation, the bacteria (Btk) reproduce in a presterilized growth medium containing basic food sources, such as corn, potatoes, grains, etc. After the fermentation is complete and the bacteria are grown, the fermentation material, including the Btk, is collected. This material becomes the basic ingredient of Foray and DiPel. This basic ingredient is composed of the Btk, which is the active ingredient, and the residual fermentation growth material and water. The water and residual fermentation growth material are referred to as "inerts" or inactive, because they are not "active" against insects. Several other inerts are added to this fermentation material, Btk and water, to make up the final formulations of Foray and DiPel. These other ingredients comprise a small proportion of the total formulation. In fact, nearly 90% of Foray 48B for example is composed of water, the residual fermentation growth material, and the Btk, (and one other inert which is a food-approved carbohydrate). The other inactive or inert ingredients are added to maintain the quality of the Btk formulation, to make it easier to handle, and to protect the activity of the Btk. Some of these ingredients help ensure the microbial quality of Foray and DiPel by acting to control the level of possible contaminating natural microorganisms. These ingredients, added in very minor amounts to control contaminating bacteria and molds, are also used in many foods in Canada and the U.S. for the same purpose. All inert ingredients in Foray and DiPel formulations are included in 40 CFR 180.1001. This list has been designated by the EPA as "exempt from the requirements of a residue tolerance on raw agricultural commodities". VBC verifies that none of its Btk formulations contain toxic inert ingredients, such as benzene, xylene, or formaldehyde. The Foray and DiPel toxicology profiles are outlined in Appendix VI *(note: appendices may be viewed online)*. Additionally, and of considerable importance, not just the Btk powder itself, but our final end-use formulations are tested toxicologically. In this process the safety of both the active ingredient and inerts are assessed and quantified.

7.18 How Can We Prove That Btk is Not a Harmful Product?

We can never prove that a product is absolutely safe. We can only demonstrate that when Btk is applied following the label instructions, that the risk to non-target organisms, whether they are birds or humans, is acceptably low. There are many drugs on the market today that, when properly taken, will effectively relieve pain or even save lives. Those same drugs come with the warning that if used improperly, they can be harmful or even cause death. As a society we must set standards and we do not permit the sale of commercial products until they have met those standards. Btk does meet the safety standards set in the USA, Canada and in all other countries. It is also acknowledged that Canada has some of the toughest regulatory standards in the world. In the United States and Canada, commercially available products are reviewed and certified for use by federal agencies including the Environmental Protection Agency in the United States, and in Canada, several agencies including the Pesticide Management Regulatory Agency (Health Canada), Agriculture Canada, Natural Resource Canada, and Environment Canada. All pesticide applications must comply with local, state/provincial, and federal regulations. In addition, researchers continue to monitor programs for potential impacts.

7.19 Will Foray or DiPel Cause Damage To Car Finishes?

There is nothing in Foray or DiPel that will cause damage to automobile finishes. These products are formulated to stick to the surface of leaves when they dry. Therefore, it is easiest to remove from any surface while it is still wet. To remove dried Foray and DiPel from any surface, simply soak the dried droplets with water and then sponge or wipe with a soft cloth. A cleaning product normally labeled for car washing may be needed if the dried spray has been on the surface for awhile. The sooner the surface is cleaned, the easier it will be to remove the spray droplets. If the automobile's paint is old, oxidized, and/or severely weathered, Foray and DiPel will adhere to this porous surface; it will be more difficult to remove. A large bath towel may be soaked and placed upon the painted surfaces for several minutes to allow the Foray and DiPel deposits to become rehydrated. This will make the spray deposit easier to remove. In extreme cases, several soakings with a wet towel may be required. DiPel 8L will form oily rings on the car finish - these are especially apparent on weathered paint. Regular car cleaning detergents will completely remove these temporary blemishes.