Bacillus thuringiensis (*Bt*) fact sheet

NATIONAL PESTICIDE INFORMATION CENTER

This fact sheet describes *Bt* used as a pesticide in sprays, granules, and other products. *Bt* crops are different. They're called "genetically engineered" or "transgenic" crops because they use DNA from *Bt* bacteria. The plants make *Bt* proteins (toxins) that only target specific insects.[1] The *Bt* toxin made by the plant is called a "plant-incorporated protectant."

What is *Bacillus thuringiensis (Bt)*?

Bt is a bacterium that is found naturally in soils throughout the world. To reproduce, *Bt* makes spores that grow into new bacteria. *Bt* spores have proteins that are toxic to insect larvae when eaten.[2] Because *Bt* comes from a natural source, it is called a biopesticide. In general, biopesticides tend to pose fewer risks than typical human-made pesticides.

photo credit: pam carter, pixabay

There are many types of *Bt*. Each type or strain affects different insect groups. Target insects include the beetle family, the fly family including mosquitoes, and the butterfly family. It is used to kill beetles and the larvae of mosquitoes, black flies, and moths.[2,3,4] *Bt* is also toxic to nematodes.[5]

Bt has been registered for use in pesticides by the U.S. Environmental Protection Agency (U.S. EPA) since 1961. The U.S. EPA requires routine testing to ensure that unwanted toxins and bacteria are not present.[2] Some of these unwanted toxins may be harmful to non-target animals and people.[6]

Technical Grade *Bt*: This fact sheet refers to the spores of *Bt* and the toxins the spores release when insects eat them. Products you buy from the store include other ingredients in addition to *Bt*. While many of the other ingredients may not pose health or environmental risks, some of them can be toxic. The other ingredients can pose greater risks than the *Bt* itself.

What are some products that contain *Bt*?

Currently, *Bt* is found in over 130 registered pesticide products.[7] *Bt* products are used on crops and ornamental plants. They are used in greenhouses, non-residential buildings, in aquatic settings, and in aerial applications. Common products include sprays, dusts, granules, concentrates, and pellets.[8] Some of these products are approved for use in organic agriculture.[9]

IMPORTANT: Always ***follow label instructions*** and take steps to ***avoid exposure***. If any exposures occur, be sure to follow the First Aid instructions on the product label carefully. For additional treatment advice, contact the Poison Control Center at 800-222-1222. If you wish to discuss a ***pesticide problem***, please call 800-858-7378.

How does *Bt* work?

Spores made by *Bt* damage the gut of insect larvae after the larvae eat them.[2] The insect gut must have a pH of 9.0 to 10.5 (high pH) in order to activate the toxin.[2,10] This is different from the human gut, which has a low pH and is more acidic. The activated toxin breaks down the insect's gut lining. The insect larva

dies of infection and starvation.[10,11,12] Death occurs within 1-5 days.[10,12] Young insect larvae are most affected.[1]

Each type of *Bt* toxin is specific to the target insect family.[2,3] Some strains of *Bt* toxins are also toxic to nematodes.[1]

Common types of Bt strains:

- *Bt israelensis* controls immature mosquitos, flies, and gnats.[2]
- *Bt aizawai* and *Bt kurstaki* controls caterpillars of moths and butterflies.[2]
- *Bt tenebrionis* and *Bt japonensis* control beetle larvae.[2,3]
- *Bt san diego* controls beetle larvae.[3]

Bt toxins are not activated when the spores are eaten by people, and no harm occurs.[2] The toxins are destroyed by acidic conditions such as those in the human stomach.[13] People and other mammals do not have the specific enzymes that break down the spore proteins to release the toxins. Mammals also do not have the necessary receptors that insect guts have.[1,2] In human stomachs, the toxin proteins are easily digested.[14]

How might I be exposed to *Bt*?

You can be exposed to *Bt* if you breathe it in or get it on your skin or eyes. For example, this can occur while applying sprays or dusts during windy conditions. You may also be exposed after using a product if you don't wash your hands before eating, drinking, or smoking. Because *Bt* is commonly found in soils, exposures not related to pesticides are also possible.[3] *Bt* has also been found in drinking water. The source may have been from mosquito spraying, use of other pesticide products containing *Bt*, or from naturally occurring *Bt*.[15]

What are pesticide tolerances?

The EPA sets legal limits for how much pesticide is allowed in food and drinking water. In food, those limits are called "*tolerances.*" Most pesticides have their own tolerance for each crop it can be used on. Some pesticides that are very low in toxicity do not require a tolerance and are "exempt." In water, those limits may be called Maximum Contaminant Levels (MCLs), health advisories (HA), or other names. The amount allowed in water is specifically regulated for some pesticides. Health advisories are issued for others.

Pets and people might be exposed to *Bt* from treated birdbaths or water fountains. You can limit exposure and reduce risk by carefully following the label instructions.

Bt is used on many crops, and you may be exposed to very low levels of *Bt* through your diet.[2] The U.S. EPA sets maximum limits for pesticide residues on food. Unlike most other pesticides, *Bt* is exempt from this requirement because of its low toxicity. **See the text box about pesticide tolerances.**

What are some signs and symptoms from a brief exposure to *Bt*?

Effects in laboratory animals:

Bt is very low in toxicity to people and other mammals when inhaled.[2,8]

- Rats breathed in high concentrations of *Bt* spores for a short period of time. Their exposures ranged from 26 million spores of *Bt kurstaki* per liter of air to 80 million spores of *Bt israelensis* per rat. The rats had no signs of infection or illness.[6]

Bt is low in toxicity to people and other mammals when eaten.[2,8]

- Scientists fed rats and rabbits high concentrations of spores for short periods of time. The rabbits ate 2 billion spores of *Bt israelensis* per rabbit and the rats ate 120 billion spores of *Bt israelensis* per kg of body weight. Neither the rats nor the rabbits showed signs of infection or illness.[6]
- Scientists fed pregnant rats moderate doses of a commercial pesticide product containing the *Bt* toxin and other ingredients. One group of rats ate 100 mg/kg of the *Bt aizawai* toxin and the other group got 200 mg/kg of the toxin. Both groups were treated until the seventh day of pregnancy. The low-dose group had some liver damage. The higher-dose group had both liver and kidney damage. These doses also affected the lungs.[16]

What is a mg/kg?

"mg/kg" is a way to measure a chemical dose. This can tell us how toxic a chemical is. "mg" means milligrams of a chemical. "kg" means one kilogram of an animal's body weight. Something that is highly toxic may kill a person with a very small amount of chemical. If something is very low in toxicity, it may take much more for that same person to become very sick or die.

- Researchers gave the same doses to other pregnant rats daily for their entire pregnancies. The treated mothers had fewer pups, but the pups appeared normal. None of these doses made the rats visibly sick.[16]

Effects in people:

There is little evidence of sickness or infection in people as a result of exposure to *Bt*.[6,14,17] However, some pesticide products with *Bt* in them have caused eye and skin irritation.[2]

- Eighteen human volunteers ate 1,000 mg of a pesticide product containing *Bt* daily for 5 days. Five of the volunteers also inhaled 100 mg of the product powder on alternate days. The volunteers did not have any negative effects.[18]

Pesticide Risk: **Any chemical, including pesticides, can pose risks to people, pets, or the environment. Understanding *pesticide risk* will help you take *steps to minimize it*.**

The risk of a pesticide depends on two things, exposure (how much?) and toxicity (how poisonous?). The exposure is the amount you get in or on your body, or the amount that is released into the environment. The toxicity of a pesticide is a measure of how poisonous it is to people or the environment. Even products that are low in toxicity can be hazardous if the exposure is high enough. Take steps to lower your chance of exposure to reduce your risk.

- Incident reports collected by the U.S. EPA include reports of skin irritation, burning, itchy eyes, and red skin rash following accidental exposure to products containing *Bt*. Other reports stated that people developed laryngitis, watery eyes, breathing problems, and headaches.[19]
- In another study, scientists surveyed local people before and after aerial applications of a pesticide product that contained *Bt*. Most people were not affected. However, some people with hay fever

reported symptoms. These included difficulty with sleep and concentration, stomach upset, and nose or throat irritation. Seasonal factors, such as pollen, may have contributed to some of the effects.[20]

- Scientists surveyed people before and after an aerial spraying of a pesticide product containing *Bt*. They found that there was no relationship between the spraying and any short-term health effects.[15] Other scientists found that children living in the area where there was aerial spraying of a *Bt* product for gypsy moths did not have increased asthma symptoms.[21]

Scientists also evaluated whether *Bt* can cause allergic reactions.

- Researchers found that farmworkers exposed for one to four months to a pesticide product containing *Bt* did not experience any problems related to their airways, nose, or skin. However, the scientists found evidence of an immune response in all of the farmworkers and the potential for skin allergies to develop at higher exposure levels.[22]
- Other scientists found that greenhouse workers with occupational exposure to sprays containing *Bt* had increased sensitization of the immune system to *Bt*.[23] A number of studies have suggested that immune responses may be triggered by exposure to *Bt* spore toxins.[24]

What happens to *Bt* when it enters the body?

When eaten, *Bt* is confined to the gut. *Bt* leaves the body in as little as 2 days.[6] Neither *Bt* nor the toxic proteins are stored in fat.[24]

The highlighted/shaded boxes in the table below reflect the toxicity classification (high, moderate, low, or very low) of exposure by the route indicated in the left-hand column.

TOXICITY CLASSIFICATION - PARAQUAT[1,2] (see the text box about **mg/kg**)

	High Toxicity	Moderate Toxicity	Low Toxicity	Very Low Toxicity
Acute Oral LD_{50}	≤ 50 mg/kg[2]	> 50 – 500 mg/kg	> 500 – 5000 mg/kg	> 5000 mg/kg
Inhalation LC_{50}	≤ 0.05 mg/L	> 0.05 – 0.5 mg/L	> 0.5 – 2.0 mg/L	> 2.0 mg/L
Dermal LD_{50}	≤ 200 mg/kg	> 200 - 2000 mg/kg	> 2000 – 5000 mg/kg	> 5000 mg/kg
Primary Eye Irritation	Corrosive (irreversible destruction of ocular tissue) or corneal involvement or irritation persisting for more than 21 days	Corneal involvement or other eye irritation clearing in 8 - 21 days	Corneal involvement or other eye irritation clearing in 7 days or less	Minimal effects clearing in less than 24 hours
Primary Skin Irritation	Corrosive (tissue destruction into the dermis and/or scarring)	Severe irritation at 72 hours (severe erythema or edema)	Moderate irritation at 72 hours (moderate erythema)	Mild or slight irritation at 72 hours (no irritation or erythema)

You may be wondering why the "High Toxicity" column has smaller numbers than the "Low Toxicity" column. This is because if a smaller amount of the pesticide caused a health effect, it's more toxic. If it takes a larger amount of the pesticide to cause a health effect, it's less toxic.

Modeled after the U.S. Environmental Protection Agency, Office of Pesticide Programs, Label Review Manual, Chapter 7: Precautionary Statements. epa.gov/sites/default/files/2018-04/documents/chap-07-mar-2018.pdf.

If breathed in, *Bt* can move to the lungs, blood, lymph, and kidneys. Levels of *Bt* decrease quickly one day after exposure.[6]

Rats that inhaled *Bt israelensis* spores for 30 minutes had spores present in their lungs afterwards for 3 days. The spores disappeared by seven days after exposure. Scientists concluded that the spores remained in the lungs.[25] The presence of spores does not mean that there is an infection.[14]

Is *Bt* likely to contribute to the development of cancer or birth defects?

No data were found on the carcinogenic (cancer causing) effects of *Bt* in humans or animals.

The toxins produced by *Bt* are proteins. Research has shown that eating *Bt* proteins has not led to cancer, genetic mutations, or birth defects.[26]

Has anyone studied non-cancer effects from long-term exposure to *Bt*?

Long-term feeding studies in rats showed few negative effects. Scientists used doses of 1.3 billion spores of *Bt kurstaki* per kg of body weight per day for 13 weeks, and 8,400 mg/kg of *Bt kurstaki* for 90 days. **See the text box about mg/kg.** The rats showed no signs of illness. Female rats fed 8,400 mg/kg *Bt kurstaki* for two years gained less weight than the rats who did not eat *Bt*, but none of the rats showed signs of toxicity or that they were infected.[6]

Are children more sensitive to *Bt* than adults?

Young children may act in ways that put them at greater risk of being exposed. For example, they may spend more time near or on the floor. They may also be more likely to place their hands in their mouths after touching treated surfaces or pets.

The U.S. EPA concluded that "risk is not expected" to children or infants from eating food treated with *Bt*.[2] Children living in an area sprayed with *Bt* for gypsy moths did not have more asthma or lower lung function compared to children outside the spray area. The researchers used peak expiratory flow rate to measure lung function.[21]

What happens to *Bt* in the environment?

Naturally occurring *Bt* spores have been found in soil, on plants, and in dead insects.[27] Spores may spread from insect droppings and from the bacteria growing in dead insects that were not killed by *Bt*.[28]

Soil

Bt spores in soil may break down more quickly or slowly depending on the conditions. *Bt* toxins generally break down much faster than the spores, with half-lives of less than 1 day to 46 days. However, some toxin may remain in soil for up to six months.[29]

Rain, plant growth, and presence of invertebrates in natural soil do not seem to affect naturally occurring *Bt*. However, *Bt* may pass through the digestive tracts of animals and multiply in their feces. This may help *Bt* spread in the environment.[30]

Additional details about how *Bt* breaks down in soil:

- When researchers mixed *Bt* spores in the top 5 cm (2 inches) of three types of soil, spores degraded over the first two weeks. After that, the number of spores remained constant for six weeks, when the experiment ended. Fewer *Bt* spores remained in the more acidic soil under pine trees. The pH of that soil was 4.3 compared to the soil under oak trees or in a field, whose pH was 4.9 and 6.5 respectively.[31]
- When scientists compared a formulated product containing *Bt* with pure spores in soil in the laboratory, there was no difference between how long the two groups of spores lasted.[31]
- Other researchers found that spores can last for months or years below the soil surface.[32]
- Other microbes in soil can break down the spores.[33]

Bt spores usually remain in the top several inches of soil.[32] *Bt* may remain dormant in most natural soil conditions.[34] Whether *Bt* spores survive and grow in soil depends on soil properties.[30]

- However, *Bt* spores did germinate and grow in sterile soils in the laboratory.[34]
- Other scientists found some growth in nutrient-rich soils.[33] Acidic soils with low pH reduced spore growth into new bacteria.[35]

Plants

On leaf surfaces, *Bt* is broken down by the sun's UV light, weather, and enzymes. These naturally occurring plant enzymes speed up the breakdown of proteins on the leaf. Spores may wash off the leaf surface as well.[1] Typical foliage half-lives are 1 to 4 days.[2]

In one study, the half-life of *Bt* spores on soybean leaves was less than one day. After one day, less than 9% of the spores were still able to grow into new *Bt* cells.[3] The spores break down rapidly on plant surfaces because of UV light.[2]

Bt from the soil can settle on plant leaves naturally.[28] It can be splashed by rainwater from soil to leaves, or can be deposited by animal feces, sprouting seeds, or infected insects.[30]

Researchers found that cotton and cabbage seedlings took up *Bt kurstaki* through their roots. The *Bt* was then found throughout the plants. The plants' leaves were then toxic to moth caterpillars. *Bt* was also found in cotton plants that grew in an area that had never been treated with *Bt*.[36]

Water

Bt is not expected to reproduce or multiply in water.[2] However, *Bt* has been found in many aquatic environments. *Bt* may reach natural waters from rain, wind, animal feces, and runoff.[30]

Air

Sunlight breaks down *Bt* spores quickly. In one study, over 80% could no longer grow within one hour of sun exposure.[3,37]

Can *Bt* affect birds, fish, or other wildlife?

Terrestrial animals

Bt is practically nontoxic and doesn't cause disease in birds, fish, and shrimp. No negative effects or infection was found in rats fed large doses of *Bt* strains or forced to inhale the spores.[6] It does not cause disease in mammals.[3] There is no evidence that *Bt* can cause a disease outbreak among wild animals.[2,8]

Bt is considered practically nontoxic to bobwhite quail and mallard ducks that were fed doses of 3,100 mg/kg (3.1 g/kg) each day for five days. No mallards died after ingesting a single dose of 10 g/kg *Bt*. *Bt* was also practically nontoxic to birds when inhaled. The LC_{50} was greater than 8,570 mg/kg. Scientists tested *Bt tenebrionis*, *israelensis*, *aizawai*, and *kurstaki* strains.[2]

Scientists have observed little to no direct toxicity to non-target insects and other arthropods. Spraying *Bt kurstaki* for spruce budworm did not affect soil-dwelling organisms.[38]

Bt strains had little to no toxicity to lacewings, predatory beetles, parasitic wasps, or earthworms when these were not targets of the strain.[2] *Bt* does not seem to hurt earthworms, although it has been found in their gut contents.[32] Other scientists found that *Bt* did not harm earthworms when used according to typical label instructions. However, high doses of the bacteria caused fatal infection in the earthworms.[39] In addition, a few studies also found that non-target moths were harmed.[40]

How naturally occurring *Bt* behaves in its environment is not yet well understood.[30,39,41] *Bt* has been found in nematodes, soil crustaceans, and earthworms.[39] Naturally occurring *Bt* can kill nematodes. It is also spread by nematodes.[5] Naturally occurring *Bt* may be taken up by plant roots and translocated throughout the plant, making the leaves toxic to moth caterpillars.[36]

Bees and other pollinators

Several studies have tested formulated products with *Bt* for bee toxicity. These studies did not account for the presence of other ingredients besides *Bt* in the products.[42,43,44] Some of these studies found effects on bumblebee and honeybee survival and behavior when the bees were exposed to products containing *Bt aizawai*, but these effects could have been from the other ingredients in the products. More research is needed. **See the text box about Technical Grade *Bt*.**

Scientists tested the toxicity of pure *Bt aizawai* to honeybees. They found that the 72-hour LD_{50} of the pure *Bt aizawai* strain was greater than 100 µg/bee. This is considered practically nontoxic.[45] The EPA also concluded that the *Bt* strains *tenebrionis*, *israelensis*, and *kurstaki* are low in toxicity to bees.[2]

Aquatic life

Nearly all the studies that looked at whether *Bt* may harm non-target aquatic animals found no effects. Some species of stoneflies, mayflies, and caddisflies were negatively affected in some studies but not others.[40]

Scientists found that *Bt kurstaki*, *israelensis*, *tenebrionis*, and *aizawai* strains were practically nontoxic to trout, bluegill sunfish, and sheepshead minnows. The LC_{50} values ranged from 8.7 to 49 billion cfu/L (colony-forming units per liter).[2] Scientists exposed young brook trout to concentrations of a formulated product containing *Bt israelensis* for 45 minutes. The brook trout did not seem to be affected until they were exposed to concentrations of 3 g/L or more of the product.[46]

Researchers exposed the tadpoles of European common frogs to a formulated product containing *Bt israelensis* at measured field concentrations of 1 mg/L. They also exposed tadpoles to 10 mg/L and 100 mg/L. The *Bt* product was sprinkled on the water six days apart. The researchers saw no effects on the tadpoles' development or overall health.[47]

Other scientists performed a similar experiment with the same species of frog. They exposed the tadpoles to three formulated products at three concentrations per product three times during the experiment. The smaller exposures mimicked those that would occur in the environment. The researchers considered the largest dose as a worst case. The exposed tadpoles showed evidence that detoxification processes in their bodies had occurred. The tadpoles had higher neuronal enzyme (AchE) activity during the first two exposures to all products at all concentrations. However, tadpole weight and survival were not affected.[48]

Water fleas (*Daphnia*) exposed to the *kurstaki* and *israelensis* strains showed moderate toxicity. The *aizawai* strains seemed to be highly toxic to water fleas. However, evidence suggested that toxicity in those tests may have been related to impurities from the production of *Bt*.[2]

Adult zebrafish were exposed to four of the toxins produced by *Bt kurstaki* at a concentration of 100 mg/L for 96 hours. The fish showed no sign of toxicity. However, zebrafish embryos and larvae exposed to the toxins showed developmental delays and toxic responses after being exposed for 96 hours to concentrations of 25, 50, 100, and 150 mg/L. The different toxins affected the fish differently.[49]

Bt strains including *aizawai* were practically nontoxic to grass shrimp, sheepshead minnows, or copepods, which live in estuaries or salt water.[2] Copepods are small relatives of crabs, shrimp, and barnacles.

Scientists looked at whether a single use of *Bt* may harm non-target aquatic insects or fish when *Bt* was applied to a river. They found no effects.[50] Some studies did find some impacts when products were used as the labels directed in aquatic systems. Many of the affected species were close relatives of black flies and mosquitoes. Some scientists have found that repeated applications of *Bt* may affect non-target organisms indirectly by affecting the food web.[40,51]

Where can I get more information?

For more detailed information about *Bacillus thuringiensis (Bt)*, call the National Pesticide Information Center, Monday - Friday, between 8:00am - 12:00pm Pacific Time (11:00am - 3:00pm Eastern Time) at 800-858-7378, or visit us on the web at npic.orst.edu. NPIC provides objective, science-based answers to questions about pesticides.

Date Published: May 13, 2022

Please cite as: Gervais, J.; Cocks, M.; Cross, A.; Jenkins, J. 2022. *Bacillus thuringiensis (Bt) Fact Sheet*; National Pesticide Information Center, Oregon State University Extension Services. npic.orst.edu/factsheets/btgen.html.

References:

1. Sanahuja, G.; Banakar, R.; Twyman, R. M.; Capell, T.; Christou, P. *Bacillus thuringiensis*: A Century of Research, Development and Commercial Applications. *Plant Biotechnol. J.* 2011, 9, 283–300. https://doi.org/10.1111/j.1467-7652.2011.00595.x.
2. *Reregistration Eligibility Decision (RED) Bacillus thuringiensis*; U.S. Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, U.S. Government Printing Office: Washington, DC, 1998.
3. Tomlin, C. D. S. *The Pesticide Manual, A World Compendium: Bacillus thuringiensis*, 14th ed.; British Crop Protection Council: Hampshire, UK, 2006, pp 58-62.
4. Dakhel, W. H.; Jarnoski, S. T.; Schell, S. Control of Pest Grasshoppers in North America. *Insects* 2020, 11 (566). https://doi.org/10.3390/insects11090566.
5. Ruan, L.; Crickmore, N.; Peng, D.; Sun, M. Are Nematodes a Missing Link in the Confounded Ecology of the Entomopathogen *Bacillus thuringiensis*? *Trends in Microbiol.* 2015, 23 (6), 341–346.
6. McClintock, J. T.; Schaffer, C. R.; Sjobl, R. D. A Comparative Review of the Mammalian Toxicity of *Bacillus thuringiensis*-Based Pesticides. *Pestic. Sci.* 1995, 45, 95–105.
7. National Pesticide Information Center. *NPIC Product Research Online (NPRO): Bacillus thuringiensis*. http://npic.orst.edu/NPRO/ (accessed November 2021).
8. *R.E.D. Facts Bacillus Thuringiensis*; U.S. Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, U.S. Government Printing Office: Washington, DC, 1998.
9. Organic Materials Review Institute. *OMRI Products List: Bacillus thuringiensis*. https://www.omri.org/us-list (accessed November 2021).
10. Broderick, N. A.; Raffa, K. F.; Handelsman, J. Midgut Bacteria Required for *Bacillus thuringiensis* Insecticidal Activity. *PNAS* 2006, 103 (41), 15196–15199.
11. Vachon, V.; Laprade, R.; Schwartz, J-L. Current Models of the Mode of Action of *Bacillus thuringiensis* Insecticidal Crystal Proteins: A Critical Review. *J. Invertebr. Pathol.* 2012, 111, 1–12.
12. Sanchis, V. From Microbial Sprays to Insect-Resistant Transgenic Plants: History of the Biospesticide *Bacillus thuringiensis*. A Review. *Agron. Sustain. Dev.* 2011, 31, 217–231.
13. Nishiitsutsuji-Uwo, J.; Ohsawa, A.; Nishi, M. S. Factors Affecting the Insecticidal Activity of Delta-Endotoxins of *Bacillus thuringiensis*. *J. Invertebr. Pathol.* 1977, 29 (2), 162–169.
14. Siegel, J. P. The Mammalian Safety of *Bacillus thuringiensis*-Based Insecticides. *J. Invertebr. Pathol.* 2001, 77, 13–21.
15. Valadares de Amorim, G.; Whittome, B.; Shore, B.; Levin, D. B. Identification of *Bacillus thuringiensis* Subsp. *Kurstaki* Strain HD1-Like Bacteria from Environmental and Human Samples after Aerial Spraying of Victoria, British Columbia, Canada, with Foray 48B. *Appl. Environ. Microb.* 2001, 67 (3), 1035–1043.

16. Lemos, A J. J.M.; Siqueira, H. A.A.; Wanderley-Teixeira, V.; Maia, F. C.L.; Teixeira, A. A.C.; Silva, E. J.; Oliveira, J. V. Effect of Sub-Lethal Doses of *Bacillus thuringiensis* Subsp. *Aizawai* and Deltamethrin with Regard to Fertility and Organ Toxicity in Pregnant Albino Rats. *Ex. Toxicol. Pathol.* 2013, 65, 489–495.
17. Ignoffo, C. M. Effects of Entomopathogens on Vertebrates. *Ann. NY Acad. Sci.* 1973, 217, 141–164.
18. Fisher, R.; Rosner, L. Toxicology of the Microbial Insecticide, Thuricide. *Agr. Food Chem.* 1959, 7 (10), 686–688.
19. *Bacillus thuringiensis Revised Preliminary Work Plan and Summary Document*; U.S. Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Office of Pesticide Programs, Biopesticides and Pollution Prevention Division, U.S. Government Printing Office: Washington, DC, 2014.
20. Petrie, K.; Thomas, M.; Broadbent, E. Symptom Complaints Following Aerial Spraying with Biological Insecticide Foray 48B. *N. Z. Med. J.* 2003, 116 (1170), U354.
21. Pearce, M.; Habbick, B.; Williams, J.; Eastman, M.; Newman, M. The Effects of Aerial Spraying with *Bacillus thuringiensis Kurstaki* on Children with Asthma. *C. J. Public Health* 2002, 93 (1), 21–25.
22. Bernstein, I. L.; Bernstein, J. A.; Miller, M.; Tierzieva, S.; Bernstein, D. I.; Lummus, Z.; Selgrade, M. K.; Doerfler, D. L.; Seligy, V. L. Immune Responses in Farm Workers after Exposure to *Bacillus thuringiensis* Pesticides. *Envir. Health Persp.* 1999, 107 (7), 575–582.
23. Doekes, G.; Larsen, P.; Sigsgaard, T.; Baelum, J. IgE Sensitization to Bacterial and Fungal Biopesticides in a Cohort of Danish Greenhouse Workers: The BIOGART Study. *Am. J. Ind. Med.* 2004, 46, 404–407.
24. Rubio-Infante, N.; Moreno-Fierros, L. An Overview of the Safety and Biological Effects of *Bacillus thuringiensis* Cry Toxins in Mammals. *J. Appl. Toxicol.* 2015, 36, 630–648.
25. Siegel, J. P.; Shadduck, J. A.; Szabo, J. Safety of the Entomopathogen *Bacillus thuringiensis* Ssp. *Israelensis* for Mammals. *J. Econ. Entomol.* 1987, 80, 717–723.
26. Koch, M. S.; Ward, J. M.; Levine, S. L.; Baum, J. A.; Vicini, J. L.; Hammond, B. G. The Food and Environmental Safety of *Bt* Crops. *Front. Plant Sci.* 2015, 6 (283). https://doi.org/10.3389/fpls.2015.00283.
27. Schnepf, E.; Crickmore, N.; Van Rie, J.; Lereclus, D.; Baum, J.; Feitelson, J.; Zeigler, D. R.; Dean, D. H. *Bacillus thuringiensis* and Its Pesticidal Crystal Proteins. *Microbiol. Mol. Biol. R.* 1998, 62 (3), 775–806.
28. Bizzarri, M. F.; Bishop, A. H. The Ecology of *Bacillus thuringiensis* on the Phylloplane: Colonization from Soil, Plasmid Transfer, and Interaction with Larvae of *Pieris Brassicae*. *Microb. Ecol.* 2008, 56, 133–139. https://doi.org/10.1007/s00248-007-9331-1.
29. Clark, B. W.; Phillips, T. A.; Coats, J. R. Environmental Fate and Effects of *Bacillus thuringiensis* (*Bt*) Proteins from Transgenic Crops: A Review. *J. Agr. Food Chem.* 2005, 53 (12), 4643–4653.
30. Argolo-Filho, R. C.; Loguercio, L. L. *Bacillus thuringiensis* Is an Environmental Pathogen and Host-Specificity Has Developed as an Adaptation to Human-Generated Ecological Niches. *Insects* 2014, 5, 62–91.
31. Petras, S. F.; Casida, Jr., L. E. Survival of *Bacillus thuringiensis* Spores in Soil. *Appl. Environ. Microbiol.* 1985, 50 (6), 1496–1501.

32. Hendriksen, N. B.; Hansen, B. M. Long-Term Survival and Germination of *Bacillus thuringiensis* Var. *Kurstaki* in a Field Trial. *Can. J. Microbiol.* 2002; 48, 3 2002, 48 (3), 256–261.
33. West, A. W.; Burges, H. D. Persistence of *Bacillus thuringiensis* and *Bacillus Cereus* in Soil Supplemented with Grass or Manure. *Plant Soil* 1985, 83 (3), 389–398.
34. Akiba, Y. Mocrobial Ecology of *Bacillus thuringiensis* VI. Germination of *Bacillus thuringiensis* Spores in Soil. *App. Ent. Zool.* 1986, 21 (1), 76–80.
35. Saleh, S. M.; Harris, R. F.; Allen, O. N. Fate of *Bacillus thuringiensis* in Soil: Effect of Soil PH and Organic Amendment. *Can. J. Microbiol.* 1970, 16, 677–680.
36. Monnerat, R. G.; Soares, C. M.; Capdeville, G.; Jones, G.; Martins, E. S.; Praca, L.; Cordeiro, B. A.; Braz, S. V.; Cavalcante dos Santos, R.; Berry, C. Translocation and Insecticidal Activity of *Bacillus thuringiensis* Living inside of Plants. *Microb. Biotechnol.* 2009, 2 (4), 512–520.
37. Cantwell, G. E.; Franklin, B. A. Inactivation by Irradiation of Spores of *Bacillus thuringiensis* Var. *Thuringiensis*. *J. Invertebr. Pathol.* 1966, 8, 256–258.
38. Addison, J. A.; Otvos, I. S.; Battigelli, J. P.; Conder, N. Does Aerial Spraying of *Bacillus thuringiensis* Subsp. *Kurstaki* (Btk) Pose a Risk to Nontarget Soil Microarthropods? *Can. J. For. Res.* 2006, 36, 1610–1620.
39. Belousova, M. E.; Malovichko, Y. V.; Shikov, A. E.; Nizhnikov, A. A.; Antonets, K. S. Dissecting the Environmental Consequences of *Bacillus thuringiensis* Application for Natural Ecosystems. *Toxins* 2021, 13 (355). https://doi.org/10.3390/toxins13050355.
40. Boisvert, M.; Boisvert, J. Effects of *Bacillus thuringiensis* Var. *Israelensis* on Target and Nontarget Organisms: A Review of Laboratory and Field Experiments. *Biocontrol Sci. Technol.* 2000, 10 (5), 517–561.
41. Raymond, B.; Johnston, P. R.; Nielsen-LeRoux, C.; Lereclus, D.; Crickmore, N. *Bacillus thuringiensis*: An Impotent Pathogen? *Trends in Microb.* 2010, 18 (5), 189–194.
42. Mommaerts, V.; Jans, K.; Smagghe, G. Impact of *Bacillus thuringiensis* Strains on Survival, Reproduction and Foraging Behaviour in Bumblebees (*Bombus Terrestris*). *Pest. Manag. Sci.* 2010, 66, 520–525.
43. Libardoni, G.; Neves, P. M. O. J.; Abati, R.; Sampaio, A. R.; Costa-Maia, F. M.; Vismara, E. dS.; Lozano, E. R.; Potrich, M. Possible Interference of *Bacillus thuringiensis* in the Survival and Behavior of Africanized Honey Bees (*Apis Mellifera*). *Sci. Rep.* 2021, 11 (3482). https://doi.org/10.1038/s41598-021-82874-1.
44. Steinigeweg, C.; Alkassab, A. T.; Beims, H.; Eckert, J. H.; Richter, D.; Pistorius, J. Assessment of the Impacts of Microbial Plant Protection Products Containing *Bacillus thuringiensis* on the Survival of Adults and Larvae of the Honeybee (*Apis Mellifera*). *Environ. Sci. Pollut. R.* 2021, 28, 29773–29780.
45. *Technical Overview of Ecological Risk Assessment - Analysis Phase: Ecological Effects Characterization*; U.S. Environmental Protection Agency, Office of Pesticide Programs, U.S. Government Printing Office, Washington DC, 2021.
46. Fortin, C.; Lapointe, D.; Charpentier, G. Susceptibility of Brook Trout (*Salvelinus Fonfinalis*) Fry to a Liquid Formulation of *Bacillus thuringiensis* Serovar. *Israelensis* (Teknar®) Used for Black Fly Control. *Can. J. Fish. Aquat. Sci.* 1986, 43, 1667–1670.

47. Schweizer, M.; Miksch, L.; Köhlera, H-R.; Triebskorn, R. Does *Bt*i (*Bacillus thuringiensis* Var. *Israelensis*) Affect *Rana Temporaria* Tadpoles? *Ecotox. Environ. Safe.* 2019, 181, 121–129.
48. Allgeier, S.; Frombold, B.; Mingo, V.; Bruhl, C. A. European Common Frog *Rana Temporaria* (Anura: Ranidae) Larvae Show Subcellular Responses under Field-Relevant *Bacillus thuringiensis* Var. *Israelensis* (Bti) Exposure Levels. *Environ. Res.* 2018, 162, 271–279.
49. Grisolia, C. K.; Oliveira, R.; Dominguesb, I.; Oliveira-Filho, E. C.; Monerat, R. G.; Soares, A. M.V.M. Genotoxic Evaluation of Different Delta-Endotoxins from *Bacillus thuringiensis* on Zebrafish Adults and Development in Early Life Stages. *Mutat. Res.* 2009, 672, 119–123.
50. Jackson, J. K.; Horwitz, R. J.; Sweeney, B. W. Effects of *Bacillus thuringiensis Israelensis* on Black Flies and Nontarget Macroinvertebrates and Fish in a Large River. *T. Am. Fish. Soc.* 2002, 131, 910–930.
51. Hershey, A. E.; Lima, A. R.; Niemi, G. J.; Regal, R. R. Effects of *Bacillus thuringiensis Israelensis* (Bti) and Methoprene on Nontarget Macroinvertebrates in Minnesota Wetlands. *Ecol. Appl.* 1998, 8 (1), 41–60.

NPIC is a cooperative agreement between Oregon State University and the U.S. Environmental Protection Agency. The information in this publication does not in any way replace or supercede the restrictions, precautions, directions, or other information on the pesticide label or any other regulatory requirements, nor does it necessarily reflect the position of the U.S. EPA.