NOSB NATIONAL LIST
FILE CHECKLIST

CROPS

MATERIAL NAME: **#8 Killed Microbial Pesticide**

✔ **NOSB Database Form**

✔ **References**

✔ **MSDS (or equivalent)**

✔ **TAP Reviews from:** **Margaret Mellon, Brian Baker, Jerry Feitelson, Daniel Pimentel, and Philip VanBuskirk**

NOSB/NATIONAL LIST
COMMENT FORM
CROPS

Material Name: #8 Killed Microbial Pesticide

Please use this page to write down comments, questions, and your anticipated vote(s).

COMMENTS/QUESTIONS:

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1. In my opinion, this material is:
_____ Synthetic _____ Non-synthetic.

2. This material should be placed on the proposed National List as:
_____Prohibited Natural _____Allowed Synthetic.

TAP REVIEWER COMMENT FORM for USDA/NOSB

Use this page or an equivalent to write down comments and summarize your evaluation regarding the data presented in the file of this potential National List material. Complete both sides of page. Attach additional sheets if you wish.

This file is due back to us by: Sept. 15, 1995

Name of Material: killed Microbial Pesticide

Reviewer Name: MARGARET THELLEN Ph.D., J.D.

Is this substance Synthetic or non-synthetic? Explain (if appropriate)

Synthetic

If synthetic, how is the material made? (please answer here if our database form is blank)

~~[illegible]~~ the organisms ~~were made~~ by modern gene therapy technologies that require multistep modifications of DNA molecules. These chemical processes are essential to the production organisms, and result in them being considered Synthetics under the FOFPA.

This material should be added to the National List as:

____ **Synthetic Allowed** ____ **Prohibited Natural**

or, ____ **Non-synthetic (This material does not belong on National List)**

or, X Synthetic (This material does not belong on the National List?)

Are there any use restrictions or limitations that should be placed on this material on the National List?

Please comment on the accuracy of the information in the file:

Any additional comments? (attachments welcomed)

to be provided

Do you have a commercial interest in this material? ____ **Yes;** X **No**

Signature Margaret Thellen **Date** September 1

TAP REVIEWER COMMENT FORM for USDA/NOSB

Use this page or an equivalent to write down comments and summarize your evaluation regarding the data presented in the file of this potential National List material. Complete both sides of page. Attach additional sheets if you wish.

This file is due back to us by: Sept. 19, 1995

Name of Material: Killed Microbial Pesticide

Reviewer Name: Brian Baker

Is this substance Synthetic or non-synthetic? Explain (if appropriate) Synthetic

If synthetic, how is the material made? (please answer here if our database form is blank) Recombination of DNA.

This material should be added to the National List as:

____ **Synthetic Allowed** ____ **Prohibited Natural**

or, ____ **Non-synthetic (This material does not belong on National List)**

✓ Synthetic, This material does not belong on National List

Are there any use restrictions or limitations that should be placed on this material on the National List?

Please comment on the accuracy of the information in the file:

The attachments are for non-transgenic B.t, ~~and does not~~ as well as transgenic, and does not consider the killed microbial pesticide

Any additional comments? (attachments welcomed)

See attached
Comments will follow

Do you have a commercial interest in this material? ____ **Yes;** ✓ **No**

Signature _____ **Date** 9/19/95

Killed Microbial Pesticides
This is potentially a broad category of exempted pesticides that are being evaluated based on a single commercial product: Mycogen Corporation's *Pseudomonas florescens* that produces the delta endotoxin for *Bacillus thuringiensis* by recombination of DNA (for simplicity, abbreviated PfBt). There are literally hundreds of natural toxicants that could potentially be manufactured using as great or greater a number of host micro-organisms. Among those toxins that are known subjects of commercially applied research are pyrethrum and scorpion venom.

In consideration of the 6518(m) criteria:

1) Insufficient data. The potential that a more toxic and persistent form of B.t. coincides with the release of transgenic plants that also express the B.t. toxin. The combination of the two theoretically could increase selection pressure for B.t. resistant strains of insects. Mycogen is also a manufacturer of corn seeds engineered to produce the Bt toxin. Employees of the corporation have expressed the opinion that Bt corn should also be allowed in organic production. More generally, the use of micro-organisms that have been engineered to express broad-spectrum insecticidal properties could prove devastating to beneficial populations and create a pesticide treadmill.

2) Insufficient data. For PfBt, the mode of action is similar to that of Bt. However, PfBt is more persistent and more toxic than cultured Bt. For other products, the toxicity studies have not been performed.

3) Insufficient data. Many of these processes are considered confidential business information, and are therefore not possible to evaluate without access to the process steps.

4) Insufficient data. The toxicology profile submitted to the TAP for consideration are not relevant to the potential universe that would be created by this category. Indeed, most of the toxicology studies use the entire B.t. organism, not the delta endotoxin. While it is technically correct to say that the delta endotoxin crystal in PfBt is found in naturally occurring Bt, the protein in PfBt is a simpler molecule that is a truncated version of the protein molecule found in Bt. It is possible that the delta endotoxin is metabolized differently than the entire toxin in humans and other organisms. Therefore the toxicology data presented is invalid even for the *Pseudomonas florescens* that expresses the B.t. delta endotoxin.

5) Insufficient data. Most formulations under consideration are for foliar, not soil use.

6) B.t. is a familiar substitute for the products that express the B.t. toxin. Pyrethrum extracted from pyrethrum flowers are a substitute for organisms that express pyrethrin. Maintenance of habitats for spiders and other beneficials offer a potential alternative to scorpion venom. Botanicals can substitute for the other potential broad-spectrum insecticides that might qualify. These are in additional to numerous cultural and biological practices, such as rotations, intercropping, beneficial releases, resistant variety selection (through classical breeding programs).

7) No private organic certifier at present allows the use of these products. The International Federation of Organic Agriculture Movements passed a resolution to categorically prohibit rDNA techniques in organic production. This resolution is likely to be supported in the Codex Alimentarius process. Data is insufficient and organic use is unprecedented even for PfBt.

Recommendation:
Synthetic: Yes.
Allowed: No.

Signed: _____ 9/21/95
Brian Baker — Date

TAP REVIEWER COMMENT FORM for USDA/NOSB

Use this page or an equivalent to write down comments and summarize your evaluation regarding the data presented in the file of this potential National List material. Complete both sides of page. Attach additional sheets if you wish.

This file is due back to us by: Sept 15, 1995

Name of Material: Killed Microbial Pesticide

Reviewer Name: Jerry Feitelson

Is this substance Synthetic or non-synthetic? Explain (if appropriate)

Synthetic

If synthetic, how is the material made? (please answer here if our database form is blank)

This material should be added to the National List as:

X Synthetic Allowed ____ Prohibited Natural

or, ____ Non-synthetic (This material does not belong on National List)

Are there any use restrictions or limitations that should be placed on this material on the National List?

No.

Please comment on the accuracy of the information in the file:

See attached summary

Any additional comments? (attachments welcomed)

See attached summary

Do you have a commercial interest in this material? X Yes; ____ No

Signature Jerald Feitelson **Date** 18 Sept 1995

NOSB Materials Database

Identification

Common Name **Killed Microbial Pesticide** **Chemical Name**

Other Names *Bacillus thuringiensis*

Code #: CAS **Code #: Other**

N. L. Category Synthetic Allowed **MSDS**

Chemistry

Family

Composition *Bacillus thuringiensis* (*B.t.*) and *Pseudomonas fluorescens* (*P.f.*)

Properties Specific to certain insects. The *P.f.* contributes the ability to resist wash off and degradation. Can be grown in an aerobic, submerged culture fermentation, just like *B.t.*

How Made

B.t. strains are selected for superior potency against a target pest. A non-pathogenic strain of *P.f.* was selected to serve as the parent host. The gene that encodes the δ-endotoxin crystal protein in *B.t.* is transferred to a non-conjugative and non-transmissable plasmid in the *P.f.* parent. The resulting hybrid is capable of producing the identical toxin produced by the *B.t.* parent. The cells of the *P.f.* parent are then killed in a special way which fixes the cell wall rather than lyses it, so that the toxin is encapsulated within the dead cell.

Use/Action

Type of Use Crops and Non-Crops

Use(s) pest control for insects. Products have been developed with action on Colorado potato beetle, elm leaf beetle, diamond-back moth, cabbage looper, imported cabbageworm, european corn borer, tobacco budworm, and beet armyworm. (See attached Sheet)

Action Toxin ~~works~~ mode of action is the same as regular *B.t.* but the encapsulation increases the effective life as a pest control. Must be ingested to be effective. Toxin attacks the gut lining of insects bearing appropriate receptor molecules, thus disrupting the digestive system. (solubility conditions, proteolytic activation, and)

Combinations

Status

OFPA

N. L. Restriction Toxins from microorganisms, such as Bacillus thuringiensis, which have been genetically manipulated may be allowed provided that such substances do not contain living genetically manipulated organisms.

EPA, FDA, etc

Safety Guidelines See attachment **Directions**

Registration **State Differences**

Historical status

International status La

USDA/TAP Comments on Material Database

Killed Microbial Pesticide

Chemistry
Essentially correct as written; some typographical errors corrected on the original sheet.

Use/Action
Type of Use: Crops [and Non-Crops]

Use(s): Products have been developed with insecticidal activity on Lepidopteran pests such as diamondback moth, cabbage looper, imported cabbageworm, European corn borer, tobacco budworm, cotton bollworm, and beet armyworm. In addition, different products can control certain Coleopteran pests, such as Colorado potato beetle, elm leaf beetle and cottonwood leaf beetle.

Action: Essentially correct as written; some additions made on the original sheet.

Status

EPA, FDA, etc.: There are currently 5 killed microbial products based on the delta endotoxin proteins of *Bacillus thuringiensis* registered with the U.S. EPA. They are as follows: M-Trak® Bioinsecticide (EPA Reg. No. 53219-2); MVP® Bioinsecticide (EPA Reg. No. 53219-3); M-Peril® Bioinsecticide (EPA Reg. No. 53219-9); Mattch™ Bioinsecticide (EPA Reg. No. 53219-10); and MVP®II Bioinsecticide (EPA Reg. No. 53219-12). These killed microbial pesticides are exempt under 40 CFR §180 from the requirement of a tolerance for residues on all raw agricultural commodities.

State Differences: MVP, M-Trak and M-Peril are currently registered in all states. Mattch and MVPII state applications are being filed in all states for registration.

Historical Status: The first Killed Microbial Products based on the delta endotoxin proteins of *Bacillus thuringiensis*, MVP and M-Trak, were initially registered by the EPA in June 1991.

International Status: MVP Bioinsecticide is currently registered in the following countries: Argentina, France, Guatemala, Honduras, Jamaica, Japan, Korea, Malaysia, Mexico, Pakistan, Puerto Rico, Spain, St. Lucia, Switzerland, Taiwan, Trinidad, and United Arab Emirates

The Commission du Génie Biomoléculaire of France has officially determined that MVP Bioinsecticide is not a Genetically Modified Organism (GMO) because the organism is killed, and the plasmid DNA was rendered biologically inert. Thus, there is no possibility of transmission of genetic information from MVP to other organisms in the biosphere.

OFPA Criteria

1. The potential of each substance for detrimental chemical interactions with other materials used in organic farming systems:

Killed Microbial Pesticides cannot interact in a detrimental way with other materials used in organic farming systems. Biotoxin genes from *Bacillus thuringiensis*, for example, have been cloned and expressed in the Gram negative bacterium, *Pseudomonas fluorescens*. Unlike *B.t.*, the cells of *P.f.* do not lyse during stationary growth, nor do they form a spore that would make them very difficult to kill. The existence of the dead cell results in a product incapable of reproducing or proliferating in the environment.

2. The toxicity and mode of action of the substance and of its breakdown products or any contaminants, and their persistence and areas of concentration in the environment:

Toxicity
A wide variety of toxicology tests have been conducted with Killed Microbial *B.t.* based products, which were required by the EPA for product registration. These tests were designed to evaluate the pathogenicity, unusual persistence, and toxicity of the killed microbial pest control agent and any other microbial contaminants. No toxic effects were observed for any of the organisms tested, including mammals, birds, fish, aquatic invertebrates, plants and beneficial insects such as honeybees, ladybug, beetles, lacewing, and parasitic wasps.

Mode of Action
Killed Microbial *B.t.* based products have a highly targeted mode of action on specific insect pests. The toxin crystal must be ingested to be effective; there is no contact activity. In the insect gut, in the presence of certain pH and enzyme conditions, the crystal is solubilized and processed to an active state. In the activated state, the toxin protein passes through the "fixed" cell wall and attacks the gut lining of insects bearing appropriate receptor molecules. If it is eaten by an insect or any other organism lacking the appropriate gut enzymes, pH, or receptors, there is no toxic effect. Different toxins require different receptors - partially explaining insect specificity and low mammalian or non-target toxicity. Feeding behavior of a susceptible insect ceases within minutes of toxin ingestion. The encapsulated insecticidal protein fatally disrupts the digestive system of susceptible pests, causing starvation and death approximately one to five days after toxin consumption.

Breakdown Properties
The "hybrid" between two naturally occurring bacteria, *B.t.* and *P.f.*, biodegrades by natural processes into their respective natural biochemical components. Because of the natural origin of the components in the Killed Microbial products, the pesticidal material will ultimately decompose to carbon dioxide, ammonia, and water. These breakdown products will be naturally assimilated by other organisms in the environment.

3. The probability of environmental contamination during manufacture, use, misuse or disposal of such substance:

The encapsulated cells are dead, can neither survive nor increase in the environment, nor move independently to unanticipated locations. Furthermore, the horizontal transfer of genetic material is effectively prevented because the DNA is completely biologically inactivated. The result is a stable, non-toxic dead cell capsule with less potential for adverse environmental impact than conventional *B.t.* products.

Killed Microbial technology has already received full product registration approval by the EPA Office of Pesticide Programs. The Office of Pollution Prevention and Toxics, which regulates the manufacture and disposal of microbes used to produce pesticides, has additionally reviewed and validated cell kill technology. These results confirm that Killed Microbial Pesticide products use an environmentally safe delivery system for highly specific *B.t.* δ-endotoxins.

4. **The effect of the substance on human health:**

In the Draft Registration Standard for *Bacillus thuringiensis*, EPA Case No. 0247 dated December 1986, the EPA stated that the δ-endotoxin in *B.t.* "has no known toxic pathogenic effect in humans or other mammals." All toxicity data used to support Mycogen's killed microbial products were consistent with the non-toxic nature of *B.t.* determined years before.

5. **The effects of the substance on biological and chemical interactions in the agroecosystem, including the physiological effects of the substance on soil organisms (including the salt index and solubility of the soil), crops and livestock:**

Killing of the microbial cell after biotoxin synthesis and "fixing" the cell wall eliminate any chance of detrimental biological or chemical interactions of Killed Microbial Pesticide products in the agroecosystem. Killed Microbial Pesticide technology ensures that the toxin maintains a highly targeted mode of action on specific insect pests. They have also been found to be active against targeted insects that have developed a resistance to synthetic chemical pesticides. Furthermore, there are no antibacterial, antifungal, plant pathogenic, or mammalian effects from the use of Killed Microbial Pesticide products in any agricultural system.

The "hybrid" used in Killed Microbial Pesticides consists of components from two naturally derived bacterial species. *Bacillus thuringiensis* occurs naturally in soil; *Pseudomonas fluorescens* exists on the leaves of plants. The breakdown of this product is through natural biodegradation processes. The tolerance exemption established by the EPA confirms that bioinsecticides employing *B.t.* pose little or no threat to environmental ecosystems and that any adverse physiological effects on soil organisms will be minimal.

6. **The alternatives to using the substance in terms of practices or other available materials**

Killed Microbial Pesticides provide increased efficacy over conventional *B.t.* products due to greater foliar persistence and the use of a selected toxin. The specificity of the toxin ensures a high degree of safety to applicators and greater control for the targeted pest. Unlike *B.t.* products, Killed Microbial Pesticides have increased residual activity of the active ingredient. *B.t.* crystals are typically degraded within 24-48 hours of application due to effects of UV light, heat, proteases, and other environmental degradation processes. Killed Microbial Pesticides reduce this problem by encapsulation of the crystalline toxin within the killed, "fixed" *P.f.* cell. This provides more protection from environmental conditions and rapid breakdown. Higher persistence levels may allow for reductions in the recommended field application rate, while still maintaining high levels of control.

It is important to reiterate that since *P.f.* produces no spores, Killed Microbial Pesticide products have less potential for adverse environmental and human impact than conventional *B.t.* products. Furthermore, as a result of the protected state of the biotoxin, the range of

materials used in formulations for additional stabilization, dispersing, wetting, suspending, and sticking properties is significantly expanded beyond those that can be used with the spores and naked crystal protein crystal preparations in current commercial *B.t.* products.

Another advantage of Killed Microbial Pesticides is a complete absence of the ability to produce β-exotoxin, a potent mutagen and teratogen often found in *B.t.* cultures. *P.f.* cells cannot produce this very toxic metabolic byproduct, and thus the risk to human and animal health is reduced.

In summary, the advantages of cellular bioencapsulation of protein biotoxins in nonliving cells include:

1) Killed Microbial Pesticide products cannot spread from the site of application, thereby eliminating concerns about containing genetically engineered microorganisms following their intentional release into the environment;
2) lack of cell lysis in stationary phase; stabilization occurs in an intact cellular state;
3) *P.f.* cells are spore-free and easily killed;
4) no possible β-exotoxin production;
5) easy and reproducible production of the δ-endotoxin to yield the maximum dose of a variety of active biotoxins;
6) stability to a wide range of formulation reagents;
7) excellent shelf life properties;
8) efficacy with good residual activity (stability) in agricultural applications.

7. **Its compatibility with a system of sustainable agriculture:**

Sustainable land use systems are designed to incorporate nature's regenerative tendencies into farm systems. The primary focus is to rely on the recycling of natural resources for soil fertility and pest management with only minimal use of pesticides. The use of Killed Microbial Pesticides, which neither contaminates the environment nor leaves undesirable residues, with other sustainable agricultural practices could prove to be very profitable for food producers and safe for the consumer of organic products.

Killed Microbial Pesticides perfectly fit into sustainable agriculture systems because of their highly specific mode of action. Furthermore, these products are totally compatible when used in combination or rotation with other pest control measures. They are responsive to natural cycles and biological interactions.

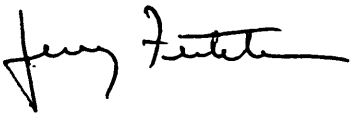
Of particular note, Killed Microbial Pesticide products are very appropriate in combination with parasite-predator release measures. Because of the toxins' specificity, targeted larvae are paralyzed on the leaf surface providing an excellent opportunity for natural parasitism and predation without any negative effects on the beneficial insect population. This aspect of biopesticides is particularly distinguishing from synthetic chemical pesticides, which often damage the delicate environmental balance between pests and beneficials. Killed Microbial Pesticide products can halt crop damage, while paralyzing the pest larvae as suitable hosts for parasites and predators, and concurrently lacking any deleterious effects on the parasites and predators preying upon the pests.

A final comment concerns the fact that Killed Microbial Pesticides are produced through recombinant DNA technology. This point was clearly communicated to the NOSB through the

July 1993 Petition (Feitelson and Debus) and by two personal presentations: at Kutztown, PA and Cottage Grove, OR. These interactions were extremely useful in helping me to understand the philosophies and concerns of organic growers. As a result, I strongly believe that Killed Microbial Pesticides are consistent with the organic philosophy and should be added to the National List as an Approved Synthetic Compound. This will help many farmers make the transition to organic methods of production.

The above comments and enclosed information are provided in an attempt to accurately complete the NOSB Materials Database for the review of Killed Microbial Pesticides as an acceptable material under the Organic Foods Production Act.

Yours sincerely,



Jerald S. Feitelson, Ph.D.
Science Fellow
Reviewer for the USDA/TAP

TAP REVIEWER COMMENT FORM for USDA/NOSB

Use this page or an equivalent to write down comments and summarize your evaluation regarding the data presented in the file of this potential National List material. Complete both sides of page. Attach additional sheets if you wish.

This file is due back to us by: _____

Name of Material: Killed Microbial Pesticide

Reviewer Name: David Pimentel

Is this substance Synthetic or non-synthetic? Explain (if appropriate)

non-synthetic

If synthetic, how is the material made? (please answer here if our database form is blank)

This material should be added to the National List as:

____ Synthetic Allowed ____ Prohibited Natural

or, ✓ Non-synthetic (This material does not belong on National List)

Are there any use restrictions or limitations that should be placed on this material on the National List?

none

Please comment on the accuracy of the information in the file:

Any additional comments? (attachments welcomed)

Do you have a commercial interest in this material? ____ Yes; ✓ No

Signature David Pimentel **Date** 9/12/95

Please address the 7 criteria in the Organic Foods Production Act:
(comment in those areas you feel are applicable)

(1) the potential of such substances for detrimental chemical interactions with other materials used in organic farming systems;

Should be minimal

(2) the toxicity and mode of action of the substance and of its breakdown products or any contaminants, and their persistence and areas of concentration in the environment;

minimal

(3) the probability of environmental contamination during manufacture, use, misuse or disposal of such substance;

low

(4) the effect of the substance on human health;

none known

(5) the effects of the substance on biological and chemical interactions in the agroecosystem, including the physiological effects of the substance on soil organisms (including the salt index and solubility of the soil), crops and livestock;

Low

(6) the alternatives to using the substance in terms of practices or other available materials; and

—

(7) its compatibility with a system of sustainable agriculture.

Compatible.

CORNELL
UNIVERSITY

College of Agriculture and Life Sciences

Department of Entomology
Comstock Hall
Ithaca, New York 14853-0901

Facsimile: 607 255-0939

21 September 1995

Dr. Z. Sonneborne

FAX 408-761-8999

Dear Dr. Sonneborne:

After more study, I consider the killed microbial pesticide not natural but synthetic.

Thank you for your interest in this issue.

Sincerely yours,

David Pimentel
Professor

TAP REVIEWER COMMENT FORM for USDA/NOSB

Use this page or an equivalent to write down comments and summarize your evaluation regarding the data presented in the file of this potential National List material. Complete both sides of page. Attach additional sheets if you wish.

This file is due back to us by: Sept. 19, 1995

Name of Material: Killed Microbial Pesticide

Reviewer Name: Philip Van Buskirk

Is this substance Synthetic or non-synthetic? Explain (if appropriate)

Synthetic

If synthetic, how is the material made? (please answer here if our database form is blank)

This material should be added to the National List as:

✓ Synthetic Allowed ___ Prohibited Natural

or, ___ Non-synthetic (This material does not belong on National List)

Are there any use restrictions or limitations that should be placed on this material on the National List?

NO.

Please comment on the accuracy of the information in the file:

good

Any additional comments? (attachments welcomed)

NO.

Do you have a commercial interest in this material? ___ Yes; ✓ No

Signature Philip Van Buskirk **Date** 9/9/95

Identification

Common Name **Killed Microbial Pesticide** **Chemical Name**

Other Names *Bacillus thuringiensis*

Code #: CAS **Code #: Other**

N. L. Category Synthetic Allowed **MSDS**

Chemistry

Family

Composition *Bacillus thuringiensis* (*B.t.*) and *Pseudomonas fluorescens* (*P.f.*)

Properties Specific to certain insects. The *P.f.* contributes the ability to resist wash off and degradation. Can be grown in an aerobic, submerged culture fermentation, just like *B.t.*

How Made

B.t. strains are selected for superior potencey against a target pest. A non-pathogenic strain of *P.f.* was selected to serve as the parent host. The gene that encodes the -endotoxin crystal protein in *B.t.* is transferred to a non-conjugative and non-transmissable plasmid in the *P.f.* parent. The resulting hybrid is capable of producing the identical toxin produced by the *B.t.* parent. The cells of the *P.f.* parent are then killed in a special way which fixes the cell wall rather than lyses it, so that the toxin is encapsulated within the dead cell.

Use/Action

Type of Use Crops

Use(s) pest control for insects. Products have been developed with action on Colorado potato beetle, elm leaf beetle, diamond-back moth, cabbage looper, imported cabbageworm, european corn borer, tobacco budworm, and beet armyworm.

Action Toxin works the same as regular *B.t.* but the encapsulation increases the effective life as a pest control. Must be ingested to be effective. Toxin attacks the gut lining of insects bearing appropriate receptor molecules, thus disrupting the digestive system.

Combinations

Status

OFPA

N. L. Restriction Toxins from microorganisms, such as Bacillus thuringiensis, which have been genetically manipulated may be allowed provided that such substances do not contain living genetically manipulated organisms.

EPA, FDA, etc

Safety Guidelines **Directions**

Registration **State Differences**

Historical status

Internationl status

OFPA Criteria

2119(m)1: chemical interactions

The existence of the dead cell results in a product which the manufacturer claims cannot interact in a detrimnental way with the environment. The cells cannot replicate or spread.

2119(m)2: toxicity & persistence

No toxic effects observed on mammals, birds, fish, aquatic invertebrates, plants and beneficial insects. Organism biodegrades by natural processes into biological components.

2119(m)3: manufacture & disposal consequences

2119(m)4: effect on human health

Non-toxic nature of *B.t.* on humans is well documented. It is exempt from tolerance by the EPA with all the toxicological data they require not showing any deleterious effects.

2119(m)5: agroecosystem biology

The toxin is specific to the target insect species, and there is no evidence that it has any effect on any other organisms. The issue of speeding insects developing resistance to *B.t.* has not been addressed.

2119(m)6: alternatives to substance

Regular *B.t.*; biological controls, habitat enhancement, resistant varieties, cultural controls.

2119(m)7: Is it compatible?

This is a product of genetic engineering. It raises the broader question of whether this technoloy is compatible with organic farming, regardless of the specific end product.

References

40 CFR Part 180.. MVP Bioinsecticide; Tolerance Exemption, Federal Register, Vol. 56, No. 119, 6-20-91

Feitelson, J. and Debus, T. 1993. A Petition to the NOSB Concerning the Cellcap® Bioencapsulation and Delivery System, written communication, 7/93

McEwen, F. L. and G. R. Stephenson. 1979. The use and significance of pesticides in the environment. NY: John Wiley and Sons, Inc.

National Coalition Against the Misuse of Pesticides. 1986 (Dec.) Pesticides & you. Washington, D.C.

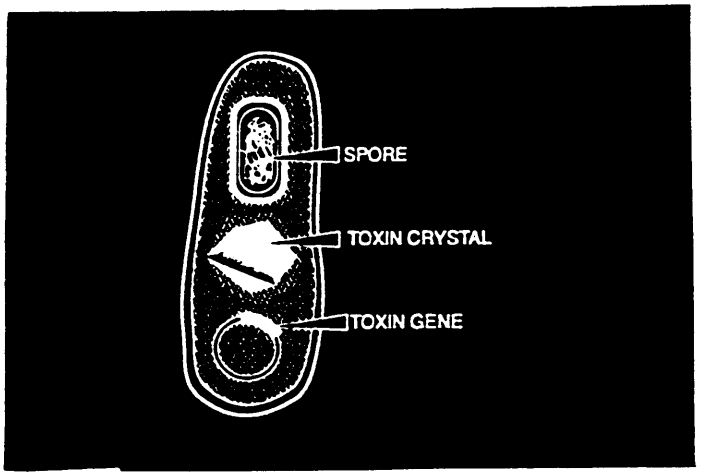
Nor-Am Chemical Company. 1985. Material safety data sheet: Bacillus thuringiensis. Wilmington, DE.

Sittig, M. 1980. Pesticide manufacturing and toxic materials control encyclopedia. Parkridge, NJ: Noyes Data Corporation.

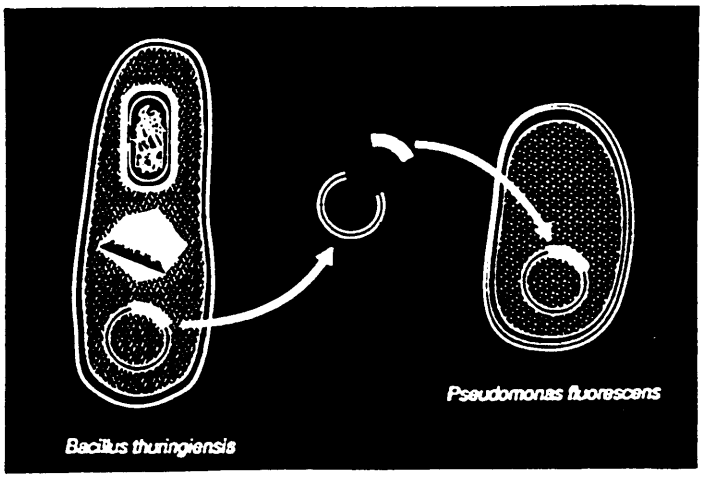
U. S. Environmental Protection Agency. 1986. Pesticide fact sheet for Bacillus thuringiensis. Fact sheet # 93. Office of Pesticide Programs. Washington, DC.

Ware, G. W. 1982. Fundamentals of pesticides. A self-instruction guide. Fresno, CA: Thomas Publications.

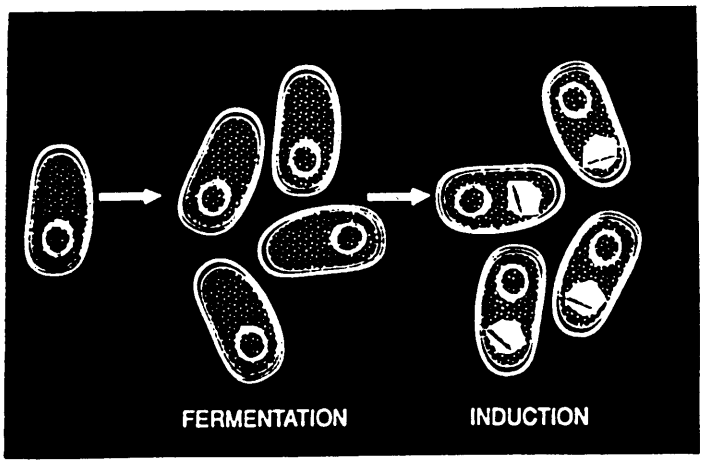
Figure 1: Schematic diagrams showing sequence of steps involved in the CellCap® process, including the transfer of a selected *Bacillus thuringiensis* (B.t.) delta endotoxin gene into *Pseudomonas fluorescens* (P.f.) and the formation and fixation steps in manufacturing.



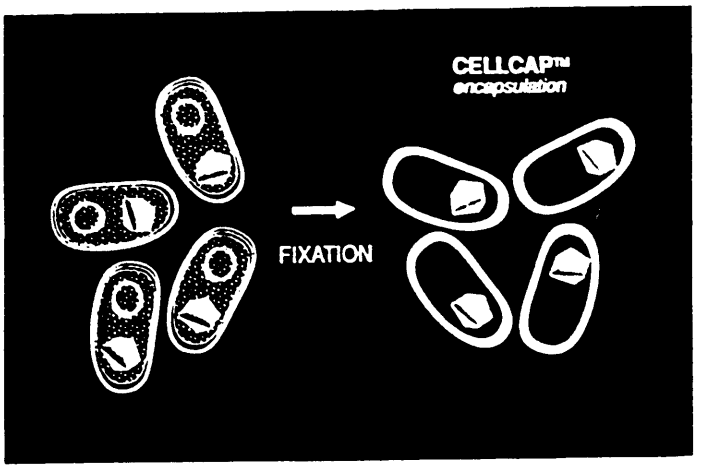
A) Natural B.t. cell that has sporulated, but before cell wall lysis, showing spore, toxin crystal, and plasmid carrying delta endotoxin gene.



B) Transfer of gene into genome of P.f. by isolating and inserting the gene into a plasmid vector that is then transformed into the P.f. cell.



C) CellCap production involves liquid fermentation. Early stages of the process increases cell numbers. Induction, later in the process with a specific nutrient initiates production of toxin within the cells and formation of the protein crystal.



D) Chemical fixation process kills and fixes P.f. cells encapsulating the delta endotoxin crystal within the cell wall of the dead cells.

E X T O X N E T: Extension Toxicology Network

Source: Cornell University, March 22, 1988

BACILLUS THURINGIENSIS (B.t.)

TRADE OR OTHER NAMES

Berliner (B.t. variety kurstaki): Dipel, Thuricide, Bactospeine, Leptox, Novabac. Due to changing regulations, these names may not be up-to-date; check with most recent Farm Chemicals Handbook for current trade names.

REGULATORY STATUS

This microbial insecticide was originally registered in 1961 as a general use insecticide. A registration standard, issued in 1986 by the U.S. Environmental Protection Agency (EPA), required manufacturers, or 'registrants', to make minor changes in label precautions and to provide additional data on the effects of B.t. on nontarget organisms. While EPA considers the toxicological data base for B.T. complete, the Agency is still requiring more ecological effects data. Check with specific state regulations for local restrictions which may apply.

Bacillus thuringiensis (B.t.) is a naturally-occurring soil bacterium that produces poisons, or 'toxins', which cause disease in insects. A number of insecticides, chemicals used to kill pest insects, are based on these toxins(8). B.t. is considered ideal for pest management because of its specificity to pests, in combination with its safety to humans and natural enemies of many crop pests(14). There are different strains of B.t , each with specific toxicity to particular types of insects: B.t. aizawai (B.t.a.) is used against wax moth larvae in honeycombs; B.t. israelensis (B.t.i.) is effective against mosquitoes, blackflies and some midges; B.t. kurstaki (B.t.k.) controls various types of lepidopterous insects, including the gypsy moth and cabbage looper. A new strain, B.t. san diego, has been found to be effective against certain beetle species and the boll weevil. In order to be effective, B.t. must be eaten by insects in the immature, feeding stage of development refered to as larvae. It is ineffective against adult insects. Monitoring the target insect population insures that insects are in the vulnerable larval stage(9). More than 150 insects, mostly lepidopterous larvae, are known to be susceptible in some way to B.t.(5).

A bacterium is a primitive form of cells, called procaryotic cells, which are neither plant nor animal. Like certain other members of the plant kingdom, such as ferns and mushrooms, B. t. forms asexual reproductive cells, called spores, which enable it to survive in adverse conditions. During the process of spore formation, B.t. also produces unique crystalline bodies as a companion product. The spores and crystals of B.t. must be eaten before they can act as poisons; B.t. is therefore referred to as a stomach poison. It is not a contact poison: It does not act by being applied and absorbed through the external covering of susceptible insects(7). B.t. crystals dissolve in response to intestinal conditions of susceptible insect larvae. This paralyzes the cells in the gut, interfering with normal digestion and triggering the insect to stop feeding on, and damaging, host plants. B.t. spores can then invade other insect tissue, multiplying in the insect's blood, until it dies. Death can occur within a few hours to a few weeks of B.t. application, depending on the insect species and the amount of B.t. ingested(13,7).

TOXICOLOGICAL EFFECTS

Acute Toxicity (Effects of one, or short-term, exposure)

No complaints were made after eighteen humans ate one gram (g) of commercial B.t. preparation daily for five days, on alternate days; some inhaled 100 milligrams (mg) of the powder daily, in addition to the dietary dosage(6). Humans that ate one g/day of B.t.k. for three consecutive days were not poisoned, nor infected(12).

Since it was one of the first disease-causing chemicals, or pathogens, registered for use against insects in the U.S., it had to undergo a testing program which was more thorough than that which the EPA currently requires. As a result, there are no data gaps in the toxicity information required by the EPA for registration purposes. A wide range of studies have been conducted on test animals, using several routes of exposure. (The highest dose tested was 6.7 x 10 to the 11th power spores per animal.) The results of these tests suggest that the use of B.t. products can cause few, if any, negative effects. B.t. did not have acute toxicity in other tests conducted on experimental birds, dogs, guinea pigs, mice, rats, humans, or other animals. When rats were injected with B.t.k., no toxic or virus-like effects were seen.

Very slight irritation was observed in test animals from inhalation and skin, or dermal, exposure; this may have been caused by the physical rather than the biological properties of the B.t. formulation tested(14). Mice survived one or more 1-hour periods of breathing mist that contained as many as 6.0 x 10 to the 10th power spores of B.t. per cubic meter(6). No toxic effects were observed in rats that had a B.t. formulation put directly into their lungs, at rates of five mg/kg of body weight(1).

The amount of formulated insecticide that killed 50% of the rats experimentally fed the material, ranges from 2.65 to greater than five grams per kilogram (g/kg)(1,12). This amount is referred to as the lethal dose fifty (LD50) for Bt in rats. Single oral dosages of up to 10,000 milligram per kilogram (mg/kg) of body weight did not produce toxicity in mice, rats or dogs(1).

The skin, or dermal, LD50 for formulated product in rabbits is 6.28 g/kg. Some reversible abnormal redness of the skin was observed when one mg/kg/day of formulated B.t. product was put on scratched skin for 21 days; no general, systemic, poisoning was observed. A single dermal application of 7.2 g/kg of B.t. was not toxic to rabbits(1).

B.t. is considered an eye irritant. One hundred grams of formulated product applied in each eye of test rabbits caused continuous congestion of the iris as well as redness and swelling of the conjunctiva, the membrane that lines the inner surface of the eyelids(2).

Chronic Toxicity (Effects of long-term, repeated exposure)

No complaints were made by eight men after they were exposed for seven months to fermentation broth, moist bacterial cakes, waste materials, and final powder created during the commercial production of B.t.(6).

There is no evidence of chronic B.t. toxicity in dogs, guinea pigs, rats, humans or other test animals Thirteen-week dietary administration of B.t. to rats at dosages of 8,400 mg/kg did not produce toxic effects(14).

Reproductive Effects (Effects of exposure on reproduction)

This literature review did not produce any information on the effects of B.t. exposure to reproductive systems.

Teratogenic Effects (Deformities in unborn offspring related to exposure)

There is no evidence indicating that formulated B.t. can cause birth defects in mammals(1).

Mutagenic Effects (Permanent changes in hereditary material related to exposure)

Thuringiensis appears to have mutagenic potential in plant tissue. Extensive use of B.t. on food plants might be hazardous, given its mutagenic potential(6).

Carcinogenic Effects (Cancer production related to exposure)

Tumor-producing effects were not seen in two-year chronic studies during which rats were given dietary doses of 8,400 mg/kg of B.t. formulation(1).

Organ Toxicity (Harmful effects on organs)

No additional information was found on the harmful effects of B.t. to organs.

Fate in Humans and Animals

While B.t. interferes with insect digestion, it does not persist in the digestive systems of mammals that ingest it.

ECOLOGICAL EFFECTS

In general, the plentiful data on the ecological effects of B.t. indicate a lack of adverse effects on nontarget insects, fish, plants, birds, mammals and humans. However, the EPA determined that most of the ecological studies on B.t. "lack key elements of information which preclude definitive conclusions." More studies have been required of registrants on the effects of B.t. on birds, fish and nontarget plants(12).

Harmful Effects on Birds

No negative effects from B.t. have been reported in birds(2). It biodegrades and does not persist in the digestive systems of birds(9). The LD50 for bobwhite quail was greater than 10 g B.t./kg body weight. When autopsies were performed on these birds, no pathology was attributed to B.t. Field observations of 74 bird species did not reveal any population changes after aerial spraying of B.t. formulation(1).

Harmful Effects on Fish

B.t. has not been reported as having harmful effects in fish(2). Rainbow trout and bluegills exposed for 96 hours to B.t. technical material, at concentrations of 560 and 1,000 parts per million (ppm), did not show adverse effects. A small marine fish (Anguilla anguilla) was not negatively affected by exposure to 1,000-2,000 times the level of B.t.expected during spray programs. Field observations of populations of brook trout, common white suckers, and smallmouth bass, did not reveal adverse effects one month after aerial application of B.t. formulation(1).

Harmful Effects on Other Animals/Insects (Nontarget species)

As of 1986, EPA had not completed its assessment of the potential impact of certain uses of B.t. on endangered and/or threatened species of moths and butterflies. Concern was expressed regarding its potential to kill endangered species of butterflies, along with target pests; inconclusive evidence indicated that high concentrations of B.t.k. can be toxic to bees and earthworms, and possibly to brine shrimp and mussells(9). Users of B.t. are encouraged to consult local officials or the nearest EPA regional office responsible for protecting endangered species before using B.t. products in counties where susceptible endangered species of Lepidoptera are known to be present. (In California: Los Angeles, Contra Costa, Mendocino, San Francisco, San Mateo, Monterey, and Kern Counties; Date and Monroe Counties in Florida; Pacific and Tillamook Counties in Washington; and Lane County in Oregon)(12). Death occurs in some nontarget insect species when B.t. is applied at rates used for mosquito control. Results of other experimental testing do not suggest that B.t. adversely affects nontarget insects or aquatic invertebrates. It has not been observed having negative effects on frogs and salamanders(2). Applications of labeled rates of formulated B.t. have not been toxic to beneficial or predator insects(1). Treatment of honeycombs with B.t. var. aizawai will not have a detrimental effect upon bees, nor on the honey produced(4).

ENVIRONMENTAL FATE

B.t. is a naturally-occurring pathogen that readily breaks down in the environment. As a biological entity, it is subject to death and inactivation in the same fashion as all living things(5,1). Due to its short biological half-life and its specificity, it is less likely than other chemical pesticides to develop field resistance. Research indicates that under normal environmental stresses, such as sun and rain, it does not develop resistance. In enclosed situations, however, B.t. resistance has been reported in a stored grain pest, the Indian meal moth(9). Because this material readily biodegrades in the environment, it poses little or no disposal problem(11).

Breakdown of Chemical in Soil and Groundwater

Under suitable conditions, B.t. can persist for several months in soil. Its spores are released into the soil from decomposing dead insects after they have been killed by the bacterium. B.t. is rapidly inactivated in soils that have a pH below 5.1(1,5).

Microbial pesticides such as B.t. are classified as immobile because they do not move, or leach, with groundwater; their rapid biological breakdown and low toxicity make them less dangerous to groundwater.

Breakdown of Chemical in Water

The EPA has not issued restrictions for the use of B.t. around bodies of water. It can be effective for up to 48 hours in water, gradually settling out, or adhering to suspended organic matter(2).

Breakdown of Chemical in Vegetation

Since it does not spread, B.t. must be applied to the parts of the plants that are normally attacked by lepidopterous larvae, or to the particular zones of water in which dipterous larvae feed. It is relatively short-lived on foliage because the ultraviolet (UV) light of the sun destroys it very rapidly, and rain washes it onto the soil. The bacterium is nonphytotoxic, or not poisonous to plants, and has not shown any adverse effect upon seed generation or plant vigor(2).

PHYSICAL PROPERTIES/GUIDELINES

The insecticidal action of B.t. is attributed to protein crystals produced by the bacillus. The vegetative cells of B.t. are approximately one micrometer (mcm) in width and 5 mcm in length, and are motile (12). The commercial product contains about 2.5 x 10 to the 11th power viable spores per gram. Typical agricultural formulations that contain spores and protein crystals include wettable powders, spray concentrates, liquid concentrates, dusts, baits, and time release rings(6,14,4).

B.t. products should be stored in a cool, dry place. Some loss of effectiveness can be expected in products stored for more than six months(2). Formulated products are compatible with most insecticides, acaricides, fungicides and plant growth regulators; they are not compatible with captafol, dinocap, alkaline sprays or, under some conditions, leaf, or foliar, nutrients(14,4).

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