1-29-88

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

JAN 29 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

Subject: Review of Toxicology Data for registration of the microbial pesticide control agent Bacillus thuringiensis var. san diego.

To: Willie Nelson (PM-17)
Registration Division (TS-767C)

From: Roy D. Sjoblad, Ph.D.
Microbiologist, Mission Support Staff
Toxicology Branch (TS-769C)

R. D. Sjoblad 1/27/88

Through: Reto Engler, Ph.D.
Chief, MSS, Toxicology Branch

Reto Engler 1/28/88

Theodore M. Farber, Ph.D.
Chief, Toxicology Branch

1/29/88

Microbial pest control agent: Bacillus thuringiensis var. san diego;

Product/trade name: M-ONE insecticide

Company code: MYX-805, LX136-06,
B. thuringiensis var. M-7

EPA record No.: 205060 Caswell No.: 66F

EPA ID No.: 53219-R Tox. Branch Project No.: 8-0144

Action requested: Review Toxicology data provided to support registration of B. thuringiensis var. san diego

Recommendation: The data show that the test material containing B. thuringiensis var. san diego is not pathogenic nor infective for the test animals. However, the data show that the test material can be toxic to test animals when administered as an aerosol or by intravenous injection, and lethally toxic when injected intravenously

at high concentrations. The cause of the observed toxicity has not been established; and a microbially-produced toxin cannot be ruled out. It is recommended that the Registrant further investigate the cause of toxicity and lethality of their test material to mice and hamsters upon intravenous injection. It is not recommended that registration of B. thuringiensis var. san diego be delayed until these data are developed. However, it is recommended that the proposed label for M-ONE insecticide be modified to include the requirement for appropriate applicator protective clothing; namely gloves, dust mask or equivalent pulmonary tract covering, and goggles. In addition, it should be stated that contact with skin, and especially open cuts or wounds is to be avoided.

Summary: The following studies were submitted for review. Toxicology Branch classifications of the studies are indicated in parentheses.

Acute oral toxicity/infectivity-rat	(Acceptable)
Acute dermal toxicity/infectivity-rat	(Acceptable)
Primary dermal irritation-rabbit	(Acceptable)
Primary eye irritation-rabbit	(Acceptable)
Intravenous toxicity/infectivity-mouse	(Supplementary)
Intravenous toxicity/infectivity-hamster	(Supplementary)
Delayed-type hypersensitivity-guinea pig	(Acceptable)
Acute inhalation toxicity/infectivity	(Acceptable)

In addition, certain information in the Product Chemistry volume (i.e., taxonomy, presence of beta-exotoxin, subcutaneous toxicity to mice, and microbial contaminants) is considered pertinent to mammalian toxicology issues, and also is reviewed.

The study data indicated that the test substance was not pathogenic or infective for test animals when administered by oral, dermal, pulmonary or subcutaneous routes of exposure. No significant signs of toxicity were observed in test animals dosed by oral dermal, or subcutaneous routes of exposure. Aerosol exposure caused signs of toxicity that included red nasal discharge, salivation, and soft stool.

The test material was mildly irritating (TOX category III) to the skin and eyes of rabbits. The test material did not cause a delayed-type hypersensitivity reaction in guinea pigs.

Mortality was observed in mice and hamsters after intravenous dosing with the test material containing B. thuringiensis at $\geq 5.2 \times 10^4$ CFU per test animal. The presence of a toxin in the dosing material that can cause mortality upon intravenous injection cannot be ruled out. A protein toxin (28,000 MW) lethal to mice has been found in isolates of B. thuringiensis var. israelensis and also in an isolate of B. thuringiensis var. morrisoni (personal communication, C. Kawanishi, EPA, RTP). Lethal effects appear due, at least in part, to hemorrhagic activity, and signs of toxicity include hypothermia and tremors.

[redacted] not been exempted from the requirement for a tolerance according to CFR 40 §180.1001.

Reviewed by: R. D. Sjoblad, Ph.D.
MSS, Tox. Branch (TS-769C) R.D. Sjoblad 1/27/88

Secondary Reviewer: R. Engler, Ph.D.
Chief, MSS, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Acute oral toxicity/infectivity TOX. CHEM NO.: 66F

ACCESSION NUMBER: 40063604

TEST MATERIAL: BT M-7 (Bacillus thuringiensis var. san diego)

STUDY NUMBER: 6392-85

SPONSOR: Mycogen Corporation

TESTING FACILITY: Bio/dynamics Inc.

TITLE OF REPORT: "Acute oral toxicity/infectivity study in rats."

AUTHORS: C.S. Auletta and I. W. Daly

REPORT ISSUED: November 6, 1986

CONCLUSION: The test MPCA, B. thuringiensis var. san diego, is not pathogenic for, nor infective in, rats after administration by the oral route. The preparation used for dosing elicited no toxicity signs in the test animal.

Classification: Acceptable

A. Materials:

1. Test compound: BT M-7, containing 1.4 to 7.0 x 10^8 colony-forming units (CFU)/gram, Density: 1.08 g/ml.

2. Test animals: Albino rat; Strain: CD^R (Sprague-Dawley derived) Age: 9-12 weeks; Weight: 265-274 g (males) and 221 to 246 g (females); Supplier: Charles River Breeding Laboratories.

B. Study design:

Five female and 5 male rats were dosed orally with BT M-7 at 5 g/kg. Animals were observed for 14 days after dosing for viability and for clinical signs of toxicity. Body weights were determined at pre-test and at day 7 and day 14 after dosing. Urine and feces were collected from each animal at 24, 48, and 72 h after dosing, and from several animals at pre-test, and were analyzed for the microbial pest control agent (MPCA).

At day 14 after dosing, animals were sacrificed, and the following tissues/organs/body fluids were analyzed for the presence of the MPCA:

spleen	stomach
liver	small intestine
lungs	intestinal contents
kidneys	peritoneal fluid
heart	blood
	urine

Urine and feces of four non-treated test animals also were examined for the presence of the MPCA. Standard microbiological techniques were used to enumerate the MPCA, and MPCA identification was based on colony morphology, Gram stain reaction, microscopy, and/or appropriate biochemical reactions.

Results:

No test animals died from administration of the test substance. All test animals gained weight during the study. None of the test animals showed any unusual pharmacologic or toxicologic signs. No unusual gross post-mortem signs attributable to the test substance were observed in any test animal. The test MPCA was not detected in spleen, liver, kidneys, heart, stomach, small intestine, peritoneal fluid, blood and urine of any test animals. The MPCA, at <10 CFU/g sample, was detected in the lungs and intestinal contents of one male rat.

In general, the numbers of MPCA enumerated from feces increased slightly from 24 hours after dosing to 48 hours after dosing (e.g., from 10^6 CFU/g to 10^7 CFU/g) and then declined at 72 hours after dosing to 10^4 - 10^5 CFU/g. The MPCA was not detected in feces of non-treated animals.

Discussion:

The test MPCA can survive passage through the intestinal tract, however, the data showed that the MPCA is not infective for the test animals. Sporadic detection of small numbers of the MPCA in isolated tissues/organs of a limited number of test animals is not sufficient to allow for the conclusion that the MPCA is infective for the test animal.

The dose level of the MPCA (5 g/kg of a preparation containing 1.4 to 7 x 10^8 CFU/g) was sufficient for the purposes of this study.

Reviewed by: R. D. Sjoblad, Ph.D. R. D. Sjoblad 1/27/88
MSS, Tox. Branch (TS-769C)

Secondary Reviewer: R. Engler, Ph.D.
Chief, MSS, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Acute dermal toxicity/infectivity TOX. CHEM NO.: 66F

ACCESSION NUMBER: 40063605

TEST MATERIAL: BT M-7 (Bacillus thuringiensis var. san diego)

STUDY NUMBER: 6393-85

SPONSOR: Mycogen Corporation

TESTING FACILITY: Bio/dynamics Inc.

TITLE OF REPORT: "Acute dermal toxicity/infectivity study in rats."

AUTHORS: C.S. Auletta and I. W. Daly

REPORT ISSUED: November 7, 1986

CONCLUSION: The test MPCA, B. thuringiensis var. san diego, is not pathogenic for, nor infective in, rats after administration by the dermal route. The preparation used for dosing elicited no significant signs of toxicity in the test animal.

Classification: Acceptable

A. Materials:

1. Test compound: BT M-7, containing 1.4 x 10^9 colony-forming units (CFU)/gram; Density: 1.08 g/ml.

2. Test animals: Albino rat; Strain: CD^R (Sprague-Dawley derived) Age: 9-12 weeks; Weight: 295-316 g (males) and 237 to 260 g (females); Supplier: Charles River Breeding Laboratories.

B. Study design:

Five female and 5 male rats were dosed dermally (at a clipped, abraded skin site of the dorsal area of the trunk) with BT M-7 at 2 g/kg. The animals then were wrapped in gauze, and then in an impervious plastic sleeve. Wrappings were removed at 24 h and excess test material was removed. Animals were observed for 14 days after dosing for viability and for clinical signs of toxicity. Body weights were determined at pre-test and at day 7 and day 14 after dosing.

At day 14 after dosing, animals were sacrificed, and the following tissues/organs/body fluids were analyzed for the presence of the MPCA:

spleen	skin (treated area)
liver	urine
lungs	blood
kidneys	
heart	

Standard microbiological techniques were used to enumerate the MPCA, and MPCA identification was based on colony morphology, Gram stain reaction, microscopy, and/or appropriate biochemical reactions.

C. Results:

No test animals died from administration of the test substance. All test animals gained weight during the study. Other than urinary staining (at 4 and/or 24 h after dosing) and fecal staining or unthrifty coat (at 24 h only) no other unusual pharmacologic or toxicologic signs were observed. No unusual gross post-mortem signs attributable to the test substance were observed in any test animal. The test MPCA was not detected in any of the tissues/organs/body fluids analyzed, except for the treated skin. The MPCA was detected on the treated skin of 6 animals and at levels ranging from 10^1 to 4.6×10^2 CFU/animal skin sample.

D. Discussion:

The dose level was sufficiently high with respect to CFU applied to each test site (i.e., $>10^8$ CFU/animal).

Reviewed by: R. D. Sjoblad, Ph.D.
MSS, Tox. Branch (TS-769C) R.D. Sjoblad 1/31/88

Secondary Reviewer: R. Engler, Ph.D.
Chief, MSS, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Primary dermal irritation TOX. CHEM NO.: 66F

ACCESSION NUMBER: 40063609

TEST MATERIAL: BT M-7 (*Bacillus thuringiensis* var. *san diego*)

STUDY NUMBER: 6394-85

SPONSOR: Mycogen Corporation

TESTING FACILITY: Bio/dynamics Inc.

TITLE OF REPORT: "Primary dermal irritation study in rabbits".

AUTHORS: C.S. Auletta and I. W. Daly

REPORT ISSUED: September 10, 1986

CONCLUSION: The preparation used for dosing, BT M-7, can be considered as a mild irritant, and classified in TOX category III.

Classification: Core Minimum

A. Materials:

1. Test compound: BT M-7, containing 3 x 10^8 colony-forming units (CFU)/gram; Density: 1.08 g/ml.

2. Test animals: New Zealand White albino rabbits; Age: young adults; Supplier: Hazleton-Dutchland, Inc.

B. Study design:

Four female and 6 male rabbits were dosed dermally (at clipped, intact and at abraded skin sites on the back of each animal) with BT M-7 at 0.5 ml. The test site was covered by gauze, and then animals were wrapped in plastic sheets. Wrappings were removed at 24 h and excess test material was removed. Dermal observations were scored (Draize system) at 30 min after removal of the wraps, at 72 h, and then until no signs of irritation were noticeable.

C. Results:

No test animals died from administration of the test substance. At 24 h after dosing all test animals exhibited well-defined to moderate to severe erythema, and very slight to slight edema. At 72 h, most animals exhibited very slight to well-defined erythema and no edema or only very slight edema. Responses were comparable at intact and at abraded skin sites. All sites were free of signs of dermal irritation at 9 days after dosing.

The primary irritation index was calculated as 2.7 of a maximum possible score of 8.0.

Reviewed by: R. D. Sjoblad, Ph.D.
MSS, Tox. Branch (TS-769C) R. D. Sjoblad 1/12/88

Secondary Reviewer: R. Engler, Ph.D.
Chief, MSS, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Primary eye irritation TOX. CHEM NO.: 66F

ACCESSION NUMBER: 40063610

TEST MATERIAL: BT M-7 (*Bacillus thuringiensis* var. *san diego*)

STUDY NUMBER: 6395-85

SPONSOR: Mycogen Corporation

TESTING FACILITY: Bio/dynamics Inc.

TITLE OF REPORT: "Eye irritation study in rabbits".

AUTHORS: C.S. Auletta and I. W. Daly

REPORT ISSUED: January 14, 1987

CONCLUSION: The preparation used for dosing, BT M-7, can be considered as a mild eye irritant, and classified in TOX category III.

Classification: Acceptable

A. Materials:

1. Test compound: BT M-7, containing 1.4 to 7 x 10^8 colony-forming units (CFU)/gram, Density: 1.08 g/ml.

2. Test animals: New Zealand White albino rabbits; Age: young adults; Supplier: Hazleton-Dutchland, Inc.

B. Study design:

The right eye of 5 female and 4 male rabbits was dosed with BT M-7 at 0.1 ml. The treated and control eyes of six animals were not washed after dosing, while the eyes of the other three animals were washed for 1 min with lukewarm water at 20 seconds after dosing. Ocular irritation was scored (Draize scale) at 24, 48, and 72 h and at 4 and 7 days after dosing.

C. Results:

Eight of nine test animals exhibited slight redness (value = 1) of the conjunctivae at 48 h after dosing, and seven of these animals exhibited slight redness also at 24 h after dosing. At 72 h after dosing, no signs of redness were noticed. Except for slight discharge in the eye of one test animal at 24 h after dosing, no other signs of eye irritation were noticed at any examination period.

Reviewed by: R. D. Sjoblad, Ph.D.
MSS, Tox. Branch (TS-769C) R. D. Sjoblad 1/27/88

Secondary Reviewer: R. Engler, Ph.D.
Chief, MSS, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Intravenous toxicity/infectivity TOX. CHEM NO.: 66F

ACCESSION NUMBER: 40063608

TEST MATERIAL: BT M-7 (Bacillus thuringiensis var san diego)

STUDY NUMBER: 6397-85

SPONSOR: Mycogen Corporation

TESTING FACILITY: Bio/dynamics

TITLE OF REPORT: "An intravenous toxicity/infectivity study in mice with Bacillus thuringiensis (BT M-7)."

AUTHORS: C.S. Auletta, I.W. Daly

REPORT ISSUED: November 10, 1986

CONCLUSION: The test material (BT M-7) was not toxic to mice when injected intravenously as a suspension containing 1.1×10^4 B. thuringiensis CFU/test animal. Also the bacterium was not infective for, or pathogenic to, mice when injected intravenously. The test material at higher dose levels (i.e., at $\geq 5.2 \times 10^4$ B. thuringiensis CFU/animal) caused mortality and toxicity (e.g., tremors and hypothermia) in the test animals.

Classification: Supplementary (because the causes of mortality and toxicity at high dose levels were not determined).

A. Materials:

1. Test compound: BT M-7, containing 1.1×10^5 colony forming units (CFU)/gm; Density: 1.08 g/ml.

2. Test animals: Mouse; Strain: CD-1 (1 CR) BR; Age: 41 days; Weight: 25-32 g (males) and 19-26 g (females); Supplier: Charles River Breeding Laboratories.

B. Study design:

Twenty female and 20 male mice each were dosed by intravenous injection (into the tail vein) with 0.1 ml (i.e., with approximately 10^4 bacteria) of the test substance. Animals were observed for viability and for clinical signs of toxicity. Body weights were determined at pre-test and weekly thereafter. Food consumption was determined weekly, beginning at 1 week prior to dosing. Ten female and

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ten male mice were treated with cortisone (subcutaneous injection with 125 mg/kg, at one day prior to dosing with the test substance) for the purpose of providing an "immunodepressed" group of test animals. Twenty animals (5/sex/group) were sacrificed at 2 weeks after dosing and complete gross post-mortem examination was performed on all animals. The spleen, liver, lungs, and brain (1 g of each organ/animal), and 1 ml of blood/animal were analyzed for the presence of B. thuringiensis var. san diego. The remaining twenty animals were sacrificed and analyzed, as above, at 4 weeks after dosing.

Several pilot studies were done to determine toxic and pathogenic effects of high levels of the test substance when administered to mice by the intravenous route, and to establish a sub-lethal dose for use in the primary study.

C. Results:

All animals survived throughout the study, and no physical abnormalities were observed in any test animal. Body weight gains and food consumption appeared normal throughout the study. No abnormalities, that could be attributed to intravenous administration of the test substance, were observed upon gross post-mortem examination. B. thuringiensis was not detected in the blood, brain, or lungs of any test animal. The bacterium was recovered from the spleens of 18 of the 20 test animals and from the livers of 17 of the 20 test animals sacrificed at 2 weeks after dosing. The numbers of bacteria recovered ranged in the spleens or livers of these animals ranged from 10 to 160/g of each organ. At the 4 week post-dosing sacrifice time , B. thuringiensis was recovered from the spleens of 18/20 test animals and from the livers of 19/20 test animals. The numbers of bacteria recovered ranged from <10 to 190/g of each tissue. In general, the test animals remained free from infection, as indicated by a general lack of detection of other bacteria in lungs and blood. B. thuringiensis was the only bacterium detected in the liver and spleens of the test animals.

In the pilot studies, all test mice were immunodepressed via subcutaneous injection of cortisone (125 mg/kg) on the day prior to intravenous injection of the B. thuringiensis test material (i.e., BT M-7). In one pilot study, 5 female and 5 male mice were dosed by tail vein injection of a preparation of BT M-7 containing 1.5×10^8 bacteria. Three of the five female mice died immediately after dosing. The remaining two female mice and one male mouse died within 24 h after dosing. The remaining 4 male mice were found moribund at 24 h after dosing.

In a separate pilot study, mice were injected with serially diluted preparations of BT M-7, at doses of 4.2×10^7, 1.3×10^6, 1.3×10^5, or 4.2×10^2 B. thuringiensis CFU/kg body weight. There were 5 female and 5 male mice/group. Mortalities were observed only in the high dose group, where 3/5 male mice and 2/5 female mice died immediately after dosing. Two additional female mice died within 6 days after

dosing. There was no apparent significant effect of the test substance on body weight gains. Clinical signs of toxicity observed in animals from the lower three dose groups, and in survivors of the high dose group, included hypopnea, fecal and urinary staining, hyperpnea, fine and coarse tremors, and hypothermia. In general, the severity of these abnormalities was dose related. Gross post-mortem examination of animals in the lower three dose groups revealed no test material related abnormalities. Also, no significant abnormalities were observed in the high dose group animals which died immediately after dosing. The single female in the high dose group that survived to study termination (i.e., to 14 days) showed an enlarged spleen, and one female that died on study showed slight pallor of the liver.

D. Discussion:

1. No determinations were made on the effectiveness with which the cortisone injection caused immune system suppression in mice. However, this is not considered necessary for the purposes of this study, since the conclusions based on the study results are not affected by this omission.

2. The cause of mortality from intravenous injection of high concentrations of the test material containing $\geq 4.2 \times 10^7$ B. thuringiensis CFU was not determined. The observed toxicity and mortality are not inconsistent with the presence of a bacterial toxin (28,000 MW), such as has been isolated from B. thuringiensis var. israelensis, and also from B. thuringiensis var. morrisoni (personal communication, C. Kawanishi, EPA, RTP). It also is not clear as to the extent which a general toxic shock reaction to components of the dosing material contributed to the observed results. The data do indicate that the adverse effects comprised a toxigenic response, and did not result from infectivity of the bacterium. It is recommended that the Registrant further resolve the cause(s) of the toxicity and mortality due to intravenous injection of the test material. As part of this, the Registrant should determine whether their strain of B. thuringiensis contains a protein toxic to mammals, and its relationship to the 28k protein from B. thuringiensis var. israelensis.

3. This reviewer concurs with the Registrant that recovery of B. thuringiensis at low levels from the spleens and livers of test animals is not an unusual event, and correlates instead with the normal phagocytic processes of these organs. This concurrence is supported by the lack of detection of the bacterium in blood, or in other organs.

Reviewed by: R. D. Sjoblad, Ph.D.
MSS, Tox. Branch (TS-769C) R. D. Sjoblad 1/27/88

Secondary Reviewer: R. Engler, Ph.D.
Chief, MSS, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Intravenous toxicity/infectivity TOX. CHEM NO.: 66F

ACCESSION NUMBER: 40063607

TEST MATERIAL: BT M-7 (*Bacillus thuringiensis* var *san diego*)

STUDY NUMBER: 6398-85

SPONSOR: Mycogen Corporation

TESTING FACILITY: Bio/dynamics, Inc.

TITLE OF REPORT: "An intravenous toxicity/infectivity study in hamsters with *Bacillus thuringiensis* (BT M-7)."

AUTHORS: C.S. Auletta, I.W. Daly

REPORT ISSUED: November 10, 1986

CONCLUSION: The test material (BT M-7) was not toxic to hamsters when injected intravenously as a suspension containing 1.1×10^4 *B. thuringiensis* CFU/test animal. Also the bacterium was not infective for, or pathogenic to, hamsters when injected intravenously. The test material at higher dose levels (i.e., at $\geq 5.2 \times 10^4$ *B. thuringiensis* CFU/animal) caused mortality. Signs of toxicity at high dose levels included coarse tremors, hypothermia, subcutaneous hemorrhage at the injection site, and mottled red lung lobes.

Classification: Supplementary (because the causes of mortality and toxicity at high dose levels were not determined).

A. Materials:

1. Test compound: BT M-7, containing 1.1×10^5 colony forming units (CFU)/gm; Density: 1.08 g/ml.

2. Test animals: Hamster, Syrian Golden; Age: 41 days; Weight: 72-90 g (males) and 85-102 g (females); Supplier: Charles River Breeding Laboratories.

B. Study design:

Twenty female and 20 male hamsters each were dosed by intravenous injection (via cephalic vein) with 0.1 ml (i.e., with approximately 10^4 bacteria) of the test substance. Animals were observed for viability and for clinical signs of toxicity. Body weights were determined at pre-test and weekly thereafter. Food consumption was determined weekly during the study period. Ten female and

ten hamsters were treated with cortisone (subcutaneous injection with 125 mg/kg, at one day prior to dosing with the test substance) for the purpose of providing an "immunodepressed" group of test animals. Twenty animals (5/sex/group) were sacrificed at 2 weeks after dosing and complete gross post-mortem examination was performed on all animals. The spleen, liver, lungs, and brain (1 g of each organ/animal), and 1 ml of blood/animal were analyzed for the presence of B. thuringiensis var. san diego. The remaining twenty animals were sacrificed and analyzed, as above, at 4 weeks after dosing.

Several pilot studies were done to determine toxic and pathogenic effects of high levels of the test substance when administered to hamsters by the intravenous route, and to establish a sub-lethal dose for use in the primary study.

C. Results:

All animals survived throughout the study, and no physical abnormalities were observed in any test animal. Body weight gains and food consumption appeared normal throughout the study. No abnormalities, that could be attributed to intravenous administration of the test substance, were observed upon gross post-mortem examination. B. thuringiensis was not detected in the blood or brain of any test animal. Small numbers (<10/g) were recovered from the lungs of three animals. The bacterium was recovered from the spleens of 20 of the 20 test animals and from the livers of 18 of the 20 test animals sacrificed at 2 weeks after dosing. The numbers of bacteria recovered in the spleens or livers of these animals ranged from <10 to 80/g of each organ. At the 4 week post-dosing sacrifice time, B. thuringiensis was recovered from the spleens of 17/20 test animals and from the livers of 19/20 test animals. The numbers of bacteria recovered ranged from <10 to 100/g of each tissue. In general, the test animals remained free from infection, as indicated by a general lack of detection of other bacteria in lungs and blood. B. thuringiensis was the only bacterium detected in the liver and spleens of the test animals.

In the pilot studies, all test hamsters were immunodepressed via subcutaneous injection of cortisone (125 mg/kg) on the day prior to intravenous injection of the B. thuringiensis test material (i.e., BT M-7). In one pilot study, female and male hamsters were dosed by cephalic vein injection of BT M-7 preparations containing either 4.7×10^6, 1×10^5, or 5.2×10^4 B. thuringiensis CFU/test animal. At 2 days after dosing, 1/12 hamsters in the high dose group was found moribund; and 5/6 and 4/6 hamsters were found dead, dying or moribund in the mid and low CFU dose groups, respectively.

In a separate pilot study, hamsters were injected with serially diluted preparations of BT M-7, at doses of 1.2×10^7, 3.8×10^5, 3.8×10^4, or 1.2×10^2 B. thuringiensis CFU/kg body weight. There were 5 female and 5 male hamsters/group. Mortalities were observed only in the high dose group, where

7/10 hamsters died within 4 h after dosing. The remaining three hamsters died within 1 day after dosing. There was no significant long-term effect of the test substance on body weight gains. Clinical signs of toxicity observed in animals from the highest two dose groups included tachypnea, dyspnea, ataxia, coarse tremors, hypothermia, labored breathing, and leaning to the right. No test substance related toxicological signs were observed in test animals from the two lowest dose groups. Gross post-mortem examination of animals in the lower three dose groups revealed no test material related abnormalities. All test animals in the highest dose group exhibited subcutaneous hemorrhage at the injection site, and also pulmonary lesions (i.e., mottled red lung lobes, and thoracic cavity filled with fluid).

D. Discussion:

1. No determinations were made on the effectiveness with which the cortisone injection caused immune system suppression in hamsters. However, this is not considered necessary for the purposes of this study, since the conclusions based on the study results are not affected by this omission.

2. The cause of mortality from intravenous injection of high concentrations of the test material containing $\geq 5.2 \times 10^4$ B. thuringiensis CFU was not determined. The observed toxicity and mortality are not inconsistent with the presence of a bacterial protein toxin (28,000 MW) such as has been isolated from B. thuringiensis var. israelensis, and also from B. thuringiensis var. morrisoni (personal communication, C. Kawanishi, EPA, RTP). It also is not clear as to the extent to which a general toxic shock reaction to components of the dosing material contributed to the observed results. The data do indicate that the adverse effects comprised a toxigenic response, and did not result from infectivity of the bacterium. It is recommended that the Registrant further resolve the cause(s) of the toxicity and mortality due to intravenous injection of the test material. As part of this, the Registrant should determine whether their strain of B. thuringiensis contains a protein toxic to mammals, and its relationship to the 28k protein from B. thuringiensis var. israelensis.

3. This reviewer concurs that recovery of B. thuringiensis at low levels from the spleens and livers of test animals is not an unusual event, and correlates instead with the normal phagocytic processes of these organs. This concurrence is supported by the lack of detection of the bacterium in blood, or in other organs.

Reviewed by: R. D. Sjoblad, Ph.D.
MSS, Tox. Branch (TS-769C) R. D. Sjoblad 1/22/88

Secondary Reviewer: R. Engler, Ph.D.
Chief, MSS, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Delayed-type hypersensitivity TOX. CHEM NO.: 66F

ACCESSION NUMBER: 40063611

TEST MATERIAL: BT M-7 (*Bacillus thuringiensis* var *san diego*)

STUDY NUMBER: 6396-85

SPONSOR: Mycogen Corporation

TESTING FACILITY: Bio/dynamics, Inc.

TITLE OF REPORT: "Guinea pig maximization test. Test material BT M-7

AUTHORS: C. S. Auletta, I. W. Daly

REPORT ISSUED: November 7, 1986

CONCLUSION: Under the conditions of the study, the test material, BT M-7, was not a dermal sensitizer in guinea pigs.

Category: Acceptable

A. Materials:

1. Test compound: BT M-7, containing 2.5×10^9 colony-forming units (CFU)/g; Density: 1.08 g/ml.

2. Test animals: Guinea pig, Hartley albino; Age: 5-6 weeks; Weight: 295-374 g; Supplier: Hazleton-Dutchland, Inc.

B. Study design:

1. Range-finding study:

Two test animals were given intradermal injections (at two sites/animal) of 0.1 ml of a 5.0% (v/v) concentration of the test material in distilled water. Local necrosis was observed at all test sites at both 24 and at 48 h after injection. Three male and 3 female animals were clipped (dorsal and lateral surfaces) and test material dilutions in distilled water were topically

applied to each test animal. Each test animal was treated with test material concentrations of 100%, and 50, 25, and 10% (v/v; in distilled water). For dosing, filter paper (2x2 cm square) was treated to saturation with each test material preparation. The treated filter paper patches were placed on each test site, and sites then were covered with plastic sheeting, which in turn was secured by wrapping the torso of each animal with elastic adhesive bandage. Wrappings and patches were removed at 24 h and observations for dermal irritation were made at 24 and 48 h after patch removal. Moderate to severe erythema (6/6 animals) and edema (3/6 animals) were observed with undiluted test material at 24 h. At 48 h, slight to moderate erythema (6/6 animals), and edema (2/6 animals) were observed. The 50% dilution preparation of test material elicited only slight erythema at 24 h and very slight to no erythema at 48 h. The 25% and 10% dilution preparations elicited no dermal reaction. On the basis of these range-finding studies, a 5.0% dilution of BT M-7 was used for the induction phase, and a 25% dilution of BT M-7 was used for the challenge phase of the guinea pig maximization study.

2. Maximization test:

The procedure used for the maximization test was based on that reported by B. Magnusson and A.M. Kligman (1970, Journal of Investigative Dermatology, vol 57, pp. 268-276).

The following were prepared for use in the intradermal injection induction phase of the test:

a.) adjuvant only (50% FCA/water emulsion).

b.) positive control agent (0.1% DNCB in propylene glycol).

c.) 5% BT M-7, in distilled water; containing 4.2×10^8 *B. thuringiensis* CFU/gram.

d.) DNCB, 0.1% in FCA/water emulsion.

e.) BT M-7, 5.0% in FCA/water emulsion; containing 2.7×10^8 *B. thuringiensis* CFU/gram

The following were prepared for use in the topical induction and topical challenge phases of the study:

f.) test material for topical induction: 50% BT M-7 in distilled water.

g.) test material for topical challenge: 25% BT M-7 in distilled water; containing 1×10^9 B. thuringiensis CFU/gram.

h.) positive control: 0.1% DNCB in 70% ethanol.

The experimental design for the study was as follows:

Animal group	No. of animals	Test/control Material	Test/control material (a. through f. above) applied: Intradermal induction Site: 1	2	3	Topical induction	Challenge
IA	5M, 5F	DNCB	a	b	d	h	h
IB*	3M, 3F	DNCB	--	--	--	--	h
IIA	5M, 5F	BT M-7	a	c	e	f	g
IIB*	3M, 3F	BT M-7	--	--	--	--	g

*Groups IB and IIB serve as "irritation control" groups (i.e., to differentiate dermal reactions produced by irritation from reactions produced by sensitization).

Intradermal injections were made in the clipped shoulder regon of each test animal. Topical applications were made on the clipped shoulder region. For the challenge phase, topical applications were made on the clipped skin of the flanks. Topical induction applications were made at 7 days after the intradermal injection portion of the induction phase. Hair was reclipped on the day prior to the topical induction. Procedures for topical applications were as described above for the range-finding study. The topical challenge phase of the study was done at 21 days after the initial intradermal injections. Patches containing challenge material were allowed to remain in contact with the animal skin for 24 h. Dermal observations were made at 24 h and at 48 h after patch removal.

During the study, test animals were observed for mortality and for clinical signs of toxicity.

C. Results:

No dermal reaction was observed in any test animal from Group IIA at 24 h or at 48 h after challenge with 25% BT M-7. Also, no dermal reactions were observed in any animal from the irritation control groups IIB and IB at 24 h and 48 h after challenge with 25% BT M-7 or with 0.1% DNCB, respectively.

Animals (9/10) in the positive control group IA showed slight, but well-defined erythema at 24 hours after challenge with 0.1% DNCB, which lessened to very slight (barely perceptible) erythema in 5/10 test animals at the 48 h observation.

Reviewed by: R. D. Sjoblad, Ph.D.
MSS, Tox. Branch (TS-769C) R. D. Sjoblad 11/20/88

Secondary Reviewer: R. Engler, Ph.D.
Chief, MSS, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Acute inhalation toxicity/infectivity TOX. CHEM NO.: 66F

ACCESSION NUMBER: 40063606

TEST MATERIAL: BT M-7 (*Bacillus thuringiensis* var. *san diego*)

STUDY NUMBER: 86-7908

SPONSOR: Mycogen Corporation

TESTING FACILITY: Bio/dynamics Inc.

TITLE OF REPORT: "An acute inhalation toxicity/infectivity study of BT M-7 in the rat."

AUTHORS: C.S. Auletta and I. W. Daly

REPORT ISSUED: November 7, 1986

CONCLUSION: The test MPCA, *B. thuringiensis* var. *san diego*, is not pathogenic for, nor infective in, rats after administration as an aerosol for 4 hours. The preparation used for dosing elicited signs of toxicity in the test animals included red nasal discharge, which persisted for as much as 12-15 days after dosing.

Classification: Acceptable

A. Materials:

1. Test compound: BT M-7, containing 2.5×10^8 colony-forming units (CFU)/gram, Density: 1.08 g/ml.

2. Test animals: Albino rat; Strain: CD^R (Sprague-Dawley derived) Age: 7 weeks (male), 8 weeks (female) ; Weight: 227-243 g (males) and 182-187 g (females); Supplier: Charles River Breeding Laboratories.

B. Study design:

Five female and 5 male rats were exposed for 4 hours to an atmosphere containing BT M-7 at an analytical concentration of 1 mg/l. Animals were observed during exposure and also for 14 days after dosing for viability and for clinical signs of toxicity.

Body weights were determined at pre-test and at days 2, 3, 5, 8, and 15 (just prior to sacrifice and necropsy) after dosing.

At day 15 after dosing, animals were sacrificed and subjected to a complete gross post-mortem examination. The following tissues/organs/body fluids were analyzed for the presence of the MPCA:

spleen	nasal passages
liver	trachea
lungs	bronchi
kidneys	blood
heart	
urine	

Standard microbiological techniques were used to enumerate the MPCA, and MPCA identification was based on colony morphology, Gram stain reaction, microscopy, and/or appropriate biochemical reactions.

C. Results:

Analyses of samples gave an average number of B. thuringiensis as 1.4×10^5 CFU/gram. A total of 307.7 grams of BT M-7 was used during the exposure phase of the study. The nominal exposure concentration of BT M-7 was determined to be 71 mg/l. The average mass median aerodynamic diameter of particles was 6.5 microns, with an average geometric deviation of 2.2. It was calculated that approximately 71% of the particles were <10 microns in diameter.

No test animals died from exposure to aerosols of the test substance. All test animals gained weight during the study from 3 days after exposure to study termination. For most animals, body weights at day 2 were slightly less than at pretest. Unusual pharmacologic or toxicologic signs observed during exposure and during the 2 hour post-exposure period included closed eyes, matted coats, soft stool, red or mucoidal nasal discharge, and salivation. Except for matted coats, these signs decreased in frequency of occurrence during the 2 hour post-exposure observation period. Signs that persisted for up to 7 days after exposure included matted coats (8/10 animals) and dried brown material around facial area (2/10 animals). Dried red nasal discharge was observed in 1-3/10 animals even at 12 to 15 days after dosing. No unusual gross post-mortem signs attributable to the test substance were observed in any test animal. The test MPCA was detected at low numbers (10 to 240 CFU/g) in the lungs of all test animals at study termination, but not in any of the other tissue or body fluids examined.

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Secondary Reviewer: R. Engler, Ph.D.
Chief, MSS, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Microorganism taxonomy/characterization TOX. CHEM NO.: 66F

SPONSOR: Mycogen, Inc.

TITLE OF REPORT: Product Chemistry: vol.'s 2, 3, and 4 of registration package

AUTHORS: Wendy Gelernter

REPORT ISSUED: September 15, 1987

CONCLUSION: Information presented was sufficient to allow classication of the test organism as a variety of Bacillus thuringiensis. Beta-exotoxin was not produced by the bacterium as indicated by a housefly larvae assay. Subcutaneous injection of 10^6 spores into mice did not cause any mortality or apparent toxicity.

The purpose of this Data Evaluation Report is to provide review on that information in the Product Chemistry section of the registration package that is pertinent to Toxicology Branch issues. Primarily, the information considered is that which relates to the taxonomy/characterization of the microorganism, stability of the microorganism, presence of mammalian toxins, subcutaneous toxicity to mice, and batch analyses for microbial contaminants.

Information provided:

The bacterium designated as Bacillus thuringiensis var. san diego is a Gram-positive, catalase +, aerobic, endospore-forming rod shaped organism, that exhibits insecticidal activity. The bacterium contains a wafer-shaped crystalline inclusion. Flagellar antigen serotype analysis would allow the bacterium to be placed in the B. thuringiesis var. morrisoni group, i.e., H-serotype 8a, 8b. The bacterium was isolated originally from California. The bacterium did not produce beta-exotoxin as indicated by a standard housefly larvae assay for toxicity.

B. thuringiensis var. san diego is intended as the active ingredient of M-ONE insecticide. Under refrigerated, buffered, sterile conditions, the bacterium is stable for at least 30 days