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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

AUG 20 1990

MEMORANDUM

SUBJECT: Review of toxicology data and waiver request in support of registration of Novodor™ Technical

TO: Philip Hutton/Michael Mendelsohn (PM-17)
Registration Division (H7505C)

FROM: Flora Chow, Chemist F. Chow
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

THROUGH: Reto Engler, Chief
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Health Effects Division (H7509C)
and
Roy D. Sjoblad, Microbiologist
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I.D. No. 38998-RL
Record No. 260,904
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MRID Nos. 414400-01,02,03,04; 414127-01,02,04,05,07; 415046-01
Caswell No. 66

** This memorandum and attachments contain CBI **

BACKGROUND

The MPCA is Novodor™ Technical, a freeze-dried fermentation broth concentrate of Bacillus thuringiensis var. tenebrionis strain NB125 (Btt), submitted by Novo-Nordisk A/S (Denmark). The MPCA is a technical grade product intended for manufacture-use. The active ingredient (a.i.) is Btt, a bacterium that produces a protein (appearing as a parasporal crystal) with insecticidal properties against the early instar larvae of the Colorado potato beetle (Leptinotarsa decemlineata).

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Toxicological data (data requirements under 40 CFR 158.740) have been submitted in support of registration of Novodor™ Technical. Also submitted is a data waiver request for storage stability testing to determine the concentration of β-exotoxin (series 151A-16).

CONCLUSION

- The data submitted are sufficient to determine the MPCA's potential for toxicity, pathogenicity and infectivity.
- The MPCA is toxic to laboratory animals following exposure by the intratracheal and inhalation routes (33% and 21% mortality, respectively). Data from both studies supports the placement of the test material in Toxicity Category III.
- The MPCA does not appear to be pathogenic or infective.
- There is insufficient information to support the request for waiver of storage stability testing (to detect the presence of β-exotoxin); there is no evidence that the MPCA product does not contain β-exotoxin and that future testing would be unwarranted.

Recommendations:

(1) Physical and chemical properties that have not been provided are: odor, density, pH, stability, viscosity, miscibility, and corrosion characteristics; this information (or waiver requests) should be supplied by the submitter.
(2) No additional toxicology testing is required. A dermal toxicity/irritation study is not required for the registration of the technical grade of an MPCA. Although the submitted study has been classified unacceptable, the test material is not expected to cause any adverse health effects. Likewise, the inhalation study is not required for registration of the technical grade of an MPCA. The submitted study provided sufficient information to place the MPCA in Toxicity Category III. In addition, the effects were similar to that observed in the pulmonary (intratracheal) study at the equivalent exposure level, and the results could support the placement of the MPCA in Toxicity Category III even in the pulmonary study.
(3) With respect to the data waiver request, data on the genetic stability of the MPCA organism during fermentation or direct evidence that the fermentation product does not contain β-exotoxin should be provided in support of the waiver request.
(4) As with any MPCA products, workers should use protective equipment, such as a dust mask, to minimize potential adverse effects from inhalation exposure.

BASIS FOR CONCLUSION

Testing for potential toxicity, pathogenicity and infectivity was conducted on Novodor™ Technical, a technical grade material. In general, testing followed the Testing Guidelines for MPCA.

The test material for all studies, except for the acute intravenous study (see footnote 3 on the following page), was SP 408, batch no. PPQ 2585; PPQ 2585 was obtained by the consolidation of material from four separate fermentation batches (after standard analysis for potency and contaminants). The material was quantified for Btt, and appropriate amounts for testing were used. All studies were performed with doses ranging from 10^8 to 10^9 CFU of Btt.

The following toxicology studies (Tier I Testing) were submitted in support of registration of the MPCA. Results are summarized, and a SACB classification* (A = acceptable, U = unacceptable, S = supplementary) is given for each study.

Study	Dose[1]	Results	Class*
Acute oral, rat	$2x10^8$ CFU	not toxic	A
Acute dermal, rabbit	10^8-10^9 CFU	non-irritant	U
Acute pulmonary, rat	$7x10^7$ CFU	33% mortality	A
Acute inhalation, rat	4h, $8x10^7$ CFU/L[2]	(TOX CAT III)	S
Acute intravenous, rat	$4x10^8$ CFU[3]	not toxic	A
Eye irritation, rabbit	10^9 CFU	TOX CAT III	A

In general, the MPCA does not appear to be pathogenic or infective.

The MPCA is toxic when administered by the intratracheal and inhalation route; it does not appear to be toxic when administered by the oral or the intravenous route. The acute pulmonary (intratracheal) study showed a 33% mortality. Microbial evaluation of the dead animals revealed the presence of

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[1]A gram of the MPCA contains 3 x 10^{10} CFU of Btt and a potency of 58,200 BTTU.

[2]Test animals receiving a lower dose (1.7 x 10^7 CFU/L) showed 0% mortality.

[3]It was technically problematic to use batch no. PPQ 2585 for this study because of the amount of large particles. Therefore, large particles were removed from the culture broth of a separate batch (batch no. Btt 33) by filtration and centrifugation before administration to test animals. The resultant test material contained 2 x 10^9 CFU Btt/g and has a potency of 1300 BTTU/g.

approximately 10^6 CFU/g lung, evidence that lung tissue had been exposed to a high number of Btt. The acute inhalation study showed a 21% mortality following a 4-hour exposure at an atmospheric concentration of 2.72 mg/L (8 x 10^7 CFU/L). The amounts of Btt (ca. 10^6 CFU/g) found in the lungs of the dead animals were comparable to that found in the acute pulmonary study. Histopathological examination of the lung tissue revealed focal alveolitis, interstitial pneumonitis and bronchiolitis. Both studies indicate that the MPCA organism is poorly eliminated from the lungs (29-day observation period). The acute inhalation study has been classified supplementary because an LC50 value was not established. The study would have been classified acceptable had the submitter provided justification for the dose levels used. Nevertheless, because the data indicates an LC50 of greater than 2 mg, the test material may be placed in Toxicity Category III.

The MPCA was found to be a mild eye irritant. Based on the eye irritation study, the MPCA may also be a mild dermal irritant. However, it is not expected to produce systemic effects because the MPCA is the water-insoluble portion of the fermentation broth, and it is unlikely to be absorbed dermally. A conclusion cannot be drawn from the dermal study that was submitted because the test material was not properly administered to the animal skin.

The submitter claims that it has no knowledge of any hypersensitivity incidents attributable to the MPCA.

Attachments:
- Product analysis
- Data waiver request
- Acute oral data evaluation
- Acute dermal data evaluation
- Acute pulmonary data evaluation
- Acute inhalation data evaluation
- Acute intravenous data evaluation
- Eye irritation data evaluation

cc: A. Kocialski (H7509C)

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PRODUCT ANALYSIS (Series 151A)

PRODUCT IDENTITY AND DISCLOSURE OF INGREDIENT (151A-10)

Product identity. (1) Product name: freeze-dried fermentation broth concentrate of Bacillus thuringiensis var. tenebrionis strain NB125. (2) Trade name: Novodor™ Technical. (3) Company code number: SP 408.

Confidential statement of formula. The submitter has provided a Confidential Statement of Formula (CSF, Form 8570-4) with the necessary information for the MPCA product as required by 40 CFR 158.740.

Information on ingredients. The MPCA organism was identified by F.G. Priest (Heriot-Watt University, UK) by two methods, a computerized numerical identification system and the standard testing as specified in "Bergey's Manual of Systematic Bacteriology, Vol. 2 (1986)" The organism was classified as Bacillus thuringiensis var. tenebrionis (abbreviated Btt).

The morphology of the vegetative cell, spores and crystals were examined by light and scanning electron microscopy. The MPCA organism was shown to be a gram positive, rod-shaped bacterium containing a central, oval spore and flat, plate-like crystals which are quadrangular to rhomboidal in outline.

Flagellar antigen serotyping was performed by H. de Barjac (Institut Pasteur, France); the MPCA organism was identified as B. thuringiensis, serotype H-8a, 8b. Host range comparison identifies the MPCA organism as belonging to pathotype C; i.e., the organism is Btt.

The plasmid profile was determined using the methods of Kronstad, Schnepf and Whiteley (J. Bact. 1983. 154:419). Nine plasmid DNA bands were detected; they were estimated to be 105, 88, 72, 35, 26, 23, 20, 17 and 10 MDa. The 88 MDa band has been reported to contain the gene for the Coleoptera-active toxin.

The MPCA organism is active against the early instar larvae of Colorado potato beetle (Leptinotarsa decemlineata). The target insect ingests the spores and crystals (i.e., the crystalline protein) during feeding, and the protein is activated under the alkaline gut conditions. The mode of action is paralysis of the gut, whereupon feeding ceases, and the insect dies within 2 to 5 days. The CSF stated potency of the MPCA is 58,400 BTTU/g, the actual potency varying with the percentage of protein crystals within the limits as certified in the CSF.

The insecticidal toxins produced by the MPCA organism was determined by the Mancini immunodiffusion technique (protocol

provided). The results indicate that the MPCA organism produces the Btt type toxins, not the Btk type (lepidopteran) or the Bti type (dipteran).

Btt, as most Bacillus species, are widely distributed in nature. The MPCA organism has been used for fermentation at the company since March 1988. The MPCA organism (Btt strain NB125) is a subculture of Btt, strain DSM 2803 (identical to isolate BI 256-82). The MPCA organism was selected because it does not produce β-exotoxin, which is reported to be produced by some strains of B. thuringiensis.

Other ingredients in the MPCA product, besides the active ingredient (a.i.), are provide in the CSF.

[redacted]

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

MANUFACTURING PROCESS (151A-11)

[redacted]

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

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Quality control of product. The potency (BTTU/g) of all fermentation batches is determined by photometric immunoassay (protocol provided). The potency may also be expressed as NBU/g (Novo BioKontrol units of toxicity per gram, protocol provided), the value derived from a bioassay using the second instar larvae of the Colorado beetle (Leptinotarsa decemlineata).

DISCUSSION OF FORMATION OF UNINTENTIONAL INGREDIENTS (151A-12)

Manufacturing control.

Microbial purity.

ANALYSIS OF SAMPLES (151A-13)

Active ingredient. The activity (potency) of the MPCA is determined for all fermentation batches. This is done by quantifying the protein crystals by photometric immunoassay (protocol provided). The potency is expressed as BTTU,

Additionally, on randomly selected batches, the potency is checked by a bioassay and a value given in NBU/g (Novo BioKontrol Units of toxicity per gram; protocol provided). In this bioassay, the test material is fed to the second instar

larvae of the Colorado beetle (Leptinotarsa decemlineata) and a potency value is determined by comparing the LC50 to a standard material of known potency.

Impurities. Residual fermentation solids (spores and cell debris) are isolated along with the protein crystals. The submitter claims that these solids are exempt, under 40 CFR 180.1001(c), from the requirement for tolerance when used in accordance with good agricultural practices, as inert ingredients in pesticide formulations applied to growing crops or to raw agriculture commodities after harvest. Accordingly, for the purposes of this submission, the submitter has considered this material an intentionally added inert ingredient, and analysis of this material was not performed.

A potential impurity in B. thuringiensis products is β-exotoxin. As part of the quality control, the submitter performs a test on the stock culture to confirm the absence of β-exotoxin (protocol provided). Only when this has been confirmed is the culture accepted for production use.

CERTIFICATION OF INGREDIENT LIMITS (151A-15)

Certified limits (as % by weight) of each component in the MPCA are provided in the CSF. For each component, the upper and lower limits are as follows:

[redacted]

The active ingredient.

[redacted]

The intentionally added inert ingredients.

[redacted]

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

QUALITY CONTROL PROCEDURE INFORMATION IS NOT INCLUDED

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PHYSICAL AND CHEMICAL PROPERTIES (151A-16)

Color: brown
Physical state: solid
Odor: not provided
Density or specific gravity: not provided
pH: not provided
Stability: not provided
Storage Stability: a waiver is requested; however, this information is not required by the testing Guidelines (July 1989)
Viscosity: not provided
Miscibility: not provided
Corrosion characteristics: not provided

Except for information on pH, items that were not provided may be argued as non-essential to the submission.

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DATA WAIVER REQUEST: Storage Stability (151A-16)

According to the Registration Standard for the reregistration of pesticide products containing Bacillus thuringiensis as the active ingredient, a storage stability study is required to determine the concentration of β-exotoxin in each end-product immediately prior to packaging and 6 months later.

The submitter claims that the MPCA organism does not produce detectable levels of β-exotoxin. The submitter tests all batches of stock culture for the presence of β-exotoxin using a company bioassay with the housefly larvae (Musca domestica); a protocol is provided. Results provided in the submission show that the bioassay can differentiate samples with β-exotoxin from those without. The submitter claims that each batch of stock culture is checked, and the batch is not accepted for production until this information is confirmed. Moreover, two batches of stock culture have also been tested by the Danish Pest Infestation Laboratory, and they were found negative in the production of β-exotoxin.

CONCLUSION

The request is reasonable considering that the MPCA organism is a strain that had been isolated for its inability to produce β-exotoxin. However, the information provided in the submission is not sufficient to assess the waiver request. (1) Although, the submitter has adequately demonstrated that the stock culture used in fermentation production does not produce β-exotoxin, the submitter has not demonstrated that the stock culture does not produce β-exotoxin under fermentation conditions (c.f. Section 180.1011 of 40 CFR wherein preparations of Bacillus thuringiensis Berliner are discussed). (2) Although, the submitter has adequately demonstrated that quality control testing for microbial contaminants maintains microbial purity during the fermentation process, there is no evidence that such testing detects changes from the characteristics of the MPCA organism. That is, there is no direct information to establish the a.i. as the MPCA production organism. Specifically, the following kinds of data would be useful: (1) genetic stability of the stock culture during storage, (2) genetic stability of the MPCA under fermentation conditions, and (3) level of β-exotoxin in the fermentation product.

Reviewed by: Flora Chow, Registration Standards and Special Review Section, Science Analysis and Coordination Branch (SACB)
Secondary reviewer: Roy D. Sjoblad, SACB RDS

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DATA EVALUATION REPORT

STUDY TYPE:	Acute oral toxicity/pathogenicity study in rats
MRID:	414400-01 CASWELL NO.: 66
TEST MATERIAL:	Novodor™ Technical (technical grade)
SYNONYMS:	Bacillus thuringiensis var. tenebrionis NB 125 (Btt); SP 408
STUDY NO.:	12488
SPONSOR:	Novo-Nordisk A/S
TESTING FACILITY:	Enzyme Toxicology Laboratory, Industrial Biotechnology, Novo Industri A/S, Denmark
TITLE OF REPORT:	SP 408 Acute oral toxicity/pathogenicity study in rats
AUTHOR(S):	Jens Bak
REPORT ISSUED:	29 June 1989
CONCLUSIONS:	A single dose of the test material containing 2 x 10^8 CFU of Bacillus thuringiensis var. tenebrionis NB 125 (Btt) was administered by gavage. The test material does not appear to be toxic, pathogenic or infective.
CLASSIFICATION:	Acceptable

I. STUDY DESIGN

Test material. SP 408, batch no. PPQ 2585, freeze-dried fermentation broth concentrate of Bacillus thuringiensis var. tenebrionis NB 125 (Btt); the concentration of Btt is 3 x 10^{10} CFU/g.

Test animals. Twenty-eight Wistar rats from Mollegards Breeding Center Ltd., Denmark, with a weight range of 125-135 g for males

and 120-134 g for females on day of treatment, were used in the study. The test group consisted of 9 animals/sex; the control group consisted of 5 animals/sex.

Methods. The test material was suspended (1:300) in sterile water to give a concentration of 2 x 10^8 CFU/mL. The test material (1 mL, 2 x 10^8 CFU) was administered to the animals by gavage. The control group of animals were untreated.

Animals were observed daily for mortality and clinical signs during the 21 days following treatment. Individual body weights of 10 test animals (5/sex) were recorded on the day of treatment and weekly thereafter.

Clearance of the test material was determined by microbial evaluation (i.e., enumeration of Btt) of feces. Fecal samples from 10 test animals (the same as the ones being weighed) were collected at 24, 48, and 72 hours, and 7, 14, and 21 days after treatment. The samples were diluted 1:10 (w/v) in a 0.1% peptone-water solution. Serial 10-fold dilutions were carried out and samples were plated on Agar 3 (peptone-yeast-beef-glucose-agar). The number of Btt colonies was counted; the limit of detection was 10 CFU/g.

Infectivity and persistence of the test material was evaluated by microbial evaluation of organs and blood of the sacrificed animals. Four animals (2/sex) were killed 7 and 14 days after treatment; all remaining animals were killed 21 days after treatment. Organ and blood samples were diluted and plated in a manner similar to the treatment of fecal samples. The number of Btt colonies was counted; the limit of detection was 10 CFU/g.

Gross necropsy was performed on all animals killed and on all animals that were found dead during the study.

II. RESULTS

None of the animals died during the study. No signs of reaction to treatment were observed.

Group mean values of body weights and body weight gains do not appear to be affected by the administration of the test material.

Gross pathological examination of the animals revealed no abnormalities.

Approximately 10^7 CFU/g was isolated from feces of all animals at 24 hours, 10^5 CFU/g at 48 hours, and 10^2 CFU/g at 72 hours. The level of Btt declined over the following weeks, and by day 15, Btt was not detected in the feces of any of the animals.

Examination of the organs and blood revealed no detectable levels of Btt, except for the liver of one animal and the lungs and liver of a second animal. Approximately 10 CFU/g tissue was found. The submitter believes these results to be an artefact.

III. DISCUSSION

The purpose of the study was to provide information on the toxicity, pathogenicity and infectivity of the test material using a single dose of 10^8 CFU administered by gavage. The observation period was 22 days.

The results showed a 0% mortality.

Microbial evaluation showed clearance of the test material from feces reached the detection limit in 14 days. Evaluation of the organs and blood showed approximately 10 CFU/g organ tissue in 3 tissue samples. Because Btt was not found in other animals, during the same sampling times, this result was likely to be caused by sample mishandling.

Reviewed by: Flora Chow, Registration Standards and Special Review Section, Science Analysis and Coordination Branch (SACB)
Secondary reviewer: Roy D. Sjoblad, SACB RDS

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DATA EVALUATION REPORT

STUDY TYPE:	Acute dermal toxicity study in rabbits
MRID:	414127-05 CASWELL NO.: 66
TEST MATERIAL:	Novodor™ Technical (technical grade)
SYNONYMS:	Bacillus thuringiensis var. tenebrionis NB 125 (Btt); SP 408
STUDY NO.:	13188
SPONSOR:	Novo-Nordisk A/S
TESTING FACILITY:	Enzyme Toxicology Laboratory, Industrial Biotechnology, Novo Industri A/S, Denmark
TITLE OF REPORT:	Acute dermal toxicity study in rabbits with SP 408, PPQ 2585
AUTHOR(S):	Ninna Berg
REPORT ISSUED:	17 January 1989
CONCLUSIONS:	A single dose of the test material containing 10^8-10^9 CFU (10 mg) of Bacillus thuringiensis var. tenebrionis NB 125 (Btt) was applied to rabbit skin. The test material produced no skin effects after 24 hours, or adverse effects of any kind after 14 days.
CLASSIFICATION:	Unacceptable. The test material (10 mg in 10 mL carrier) was applied to gauze dressing and the dressing then placed over the skin; the material should have been applied directly to the skin.

I. STUDY DESIGN

Test material. SP 408, batch no. PPQ 2585, freeze-dried fermentation broth concentrate of Bacillus thuringiensis var. tenebrionis NB 125 (Btt); the concentration of Btt is 3 x 10^{10} CFU/g.

Test animals. Ten New Zealand White rabbits (6 males and 4 females, nulliparous and non-pregnant), bred by Novo Industri A/S, Denmark, weighing 2.5 to 3.5 kg on the day before treatment, were used in the study.

Methods. The test material (10 mg, ca. 10^8-10^9 CFU) was suspended in 10 mL 0.9% saline. The back of each rabbit was clipped free of fur to expose at least 10% of the total body surface area. The suspension of test material was applied evenly to a 100 cm^2 patch of 4 layers of gauze. The gauze patch was placed over the rabbit skin and held in place with adhesives that permitted the patch to be permeable. The animals were fitted with collars to prevent disturbance to the test site. After 24 hours, the patches were removed and the test site washed with lukewarm water to remove residual material.

Animals were observed for mortality and clinical signs at 1, 2 and 4 hours after treatment, and once daily thereafter for 14 days. Primary skin irritation was evaluated 24 and 72 hours after treatment. Individual body weights were recorded the day before treatment and weekly thereafter.

II. RESULTS

Skin reactions (erythema/eschar or edema formation) to treatment were assessed at 0 value at 24 and 72 hours after treatment. The primary irritation score was calculated (PII = 0), and the test material was classified as non-irritant.

There was no evidence of treatment-related changes in body weight during the 15-day study.

III. DISCUSSION

The purpose of the study was to provide information on the potential for health hazards from a 24-hour exposure to the test material following a single dermal application.

Under the conditions of the study, no skin reactions or other clinical signs were observed in any of the animals. This result is not unexpected because the test material could have easily become imbedded into the gauze fibers and not have an opportunity to come into contact with the skin. Gross necropsy was not indicated, and therefore, was not performed.

The following procedures were deviations from the Testing Guidelines (July 1989):

1. Six male and four females rats were used instead of the recommended 5 animals/sex.
2. The test material was not applied directly to the skin as recommended.

3. The amount of test material used was 10 mg instead of 2 g per animal as recommended. A justification was provided in the document "Summary of toxicology and non-target organism and environmental expression data in support of registration of Novodor™ Technical". The submitter was following the draft guidelines dated 12/24/87, wherein a dose of at least 10^8 CFU/test site was recommended. It appears that the study was near completion before a draft of the current guidelines was available.

In summary, the study is considered inadequate because the test material was not applied properly (item #2, above). However, the test material is not expected to cause any adverse health effects, and a repeat of the dermal study is not necessary. **(Had a 2-g dose been used, signs of local effect on the skin may have been observed. Still, the test material is unlikely to produce systemic effects because the MPCA is the water-insoluble portion of the fermentation broth.)**

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Reviewed by: Flora Chow, Registration Standards and Special Review Section, Science Analysis and Coordination Branch (SACB)
Secondary reviewer: Roy D. Sjoblad, SACB RDS

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DATA EVALUATION REPORT

STUDY TYPE:	Acute pulmonary toxicity/pathogenicity study in rats
MRID:	414400-02 CASWELL NO.: 66
TEST MATERIAL:	Novodor™ Technical (technical grade)
SYNONYMS:	Bacillus thuringiensis var. tenebrionis NB 125 (Btt); SP 408
STUDY NO.:	13388
SPONSOR:	Novo-Nordisk A/S
TESTING FACILITY:	Enzyme Toxicology Laboratory, Industrial Biotechnology, Novo Industri A/S, Denmark
TITLE OF REPORT:	SP 408 Acute pulmonary toxicity/pathogenicity study in rats
AUTHOR(S):	Marete Stavnsbjerg
REPORT ISSUED:	31 August 1989
CONCLUSIONS:	A single dose of the test material containing 7×10^7 CFU of Bacillus thuringiensis var. tenebrionis NB 125 (Btt) was administered by intratracheal instillation. The test material is toxic (33% mortality). The test material does not appear to be pathogenic or infective.
CLASSIFICATION:	Acceptable

I. STUDY DESIGN

Test material. SP 408, batch no. PPQ 2585, freeze-dried fermentation broth concentrate of Bacillus thuringiensis var. tenebrionis NB 125 (Btt); the concentration of Btt is 3×10^{10} CFU/g.

Test animals. Twenty-eight Wistar rats from Mollegards Breeding Center Ltd., Denmark, with a weight range of 320-342 g for males

and 207-241 g for females on day of treatment, were used in the study. The test group consisted of 9 animals/sex; the control group consisted of 5 animals/sex.

Methods. The test material was suspended (1:300) in sterile 0.9% saline to give a concentration of 7×10^8 CFU/mL. The test animals were fasted overnight prior to treatment. They were dosed by intratracheal instillation of the suspension (0.1 mL, 7×10^7 CFU). The control group of animals were untreated.

Animals were observed daily for mortality and clinical signs during the 28 days following treatment. Individual body weights were recorded on the day of treatment and weekly thereafter.

Clearance of the test material was determined by microbial evaluation (i.e., enumeration of Btt) of feces. Fecal samples from 10 test animals (5/sex) were collected at 24, 48, and 72 hours, and 7, 14, 21, and 28 days after treatment. The samples were diluted 1:10 (w/v) in a 0.1% peptone-water solution. Serial 10-fold dilutions were carried out and samples were plated on Agar 3 (peptone-yeast-beef-glucose-agar). The number of Btt colonies was counted; the limit of detection was 10 CFU/g.

Infectivity and persistence of the test material was evaluated by microbial evaluation of organs and blood of the sacrificed animals. Four animals (2/sex) were killed 7 and 14 days after treatment; all remaining animals were killed 28 days after treatment. Organ and blood samples were diluted and plated in a manner similar to the treatment of fecal samples. The number of Btt colonies was counted; the limit of detection was 10 CFU/g.

Gross necropsy was performed on all animals killed and on all animals that were found dead during the study.

II. RESULTS

Five animals in the test group died 1 day after treatment. Four animals displayed the following clinical symptoms: decreased activity, increased respiration, and piloerection. One animal was apathetic, with discharge from the eyes and blood-stained discharge from the nose; this animal was killed 4 days after treatment. On day 8 and until the end of the study, one female rat appeared to have paresis located to the left side of the head.

Group mean values of body weights and body weight gains do not appear to be affected by the administration of the test material.

Gross pathological examination of the animals found dead showed slight cadaverousis. Two of these animals also had bleeding in the lungs. The animal that was killed on day 5 showed fibrin coagula and bleeding in the lungs. Atelectasis was observed in

animals killed 14 days after treatment. No gross pathology findings were observed in animals at the final sacrifice.

Approximately 10^6 CFU/g was isolated from feces of all animals at 24 hours, 10^5 CFU/g at 48 hours, and 10^4 CFU/g at 72 hours (two animals died at day 2). The amount of Btt declined over the following weeks, and by day 29, Btt was not detected in the feces of any of the animals.

The animals that died on day 2 were examined for the presence of Btt; only the lungs were examined and approximately 10^6 CFU/g was found. The animal killed on day 5 had approximately 10^3 CFU/g liver, 10^2 CFU/g spleen, 10^5 CFU/g lung, and 10^3 CFU/mL heart blood. The three animals killed at the first sacrifice had $\leq 10^3$ CFU/g brain, liver, kidneys, spleen, lung, mesenteric lymph node, and heart blood. One animal had 10^5 CFU/g lung. The three animals killed at the second sacrifice had 10^5 CFU/g lung. Two of the three animals had 10^2 CFU/g spleen. No Btt was isolated from the brain, liver, kidneys, mesenteric lymph node, or heart blood. Animals from the final sacrifice showed Btt only in the lungs (2/6 had 10^3 CFU/g lung).

III. DISCUSSION

The purpose of the study was to provide information on the toxicity, pathogenicity and infectivity of the test material using a single dose of 10^8 CFU administered intratracheally. The observation period was 29 days.

The results showed a mortality of 33% (6/18), with five deaths occurring 1 day after treatment and one death 4 days later. Microbial evaluation revealed the presence of approximately 10^6 CFU/g lung, evidence that lung tissue had been exposed to a high number of Btt by the intratracheal instillation of the test material. Examination of the lung tissues of the sacrificed animals showed that the test material is only slowly cleared from the lungs. The animal killed <u>in extremis</u> had sepsis (Btt was isolated from heart blood); it also had 10^5 CFU/g lung. The submitter attributed these deaths to the toxicity of the test material. Clinical symptoms were observed in 5 other animals.

Clearance of the test material from feces reached the detection limit in 28 days. The test material does not appear to be pathogenic or infective.

Although eighteen (9/sex) rats were used instead of 20 animals (10/sex), as recommended in the Testing Guidelines (July 1989), the results from this study are sufficient to demonstrate the potential for toxicity, pathogenicity and infectivity.

Reviewed by: Flora Chow, Registration Standards and Special Review Section, Science Analysis and Coordination Branch (SACB)
Secondary reviewer: Roy D. Sjoblad, SACB RDS

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DATA EVALUATION REPORT

STUDY TYPE:	Acute inhalation toxicity study in rats
MRID:	415046-01 CASWELL NO.: 66
TEST MATERIAL:	Novodor™ Technical (technical grade)
SYNONYMS:	Bacillus thuringiensis var. tenebrionis NB 125 (Btt); SP 408
STUDY NO.:	641467
SPONSOR:	Novo-Nordisk A/S
TESTING FACILITY:	Inveresk Research International, Scotland
TITLE OF REPORT:	SP 408 Acute inhalation toxicity study in rats
AUTHOR(S):	P. McDonald
REPORT ISSUED:	8 May 1989
CONCLUSIONS:	A 4-hour inhalation exposure (nose only) of the test material, at an atmospheric concentration of 2.72 mg/L (8.2×10^7 CFU/L), is toxic to rats (21% mortality). Exposure to 0.55 mg/L (1.7×10^7 CFU/L) does not appear to be toxic. The test material does not appear to be pathogenic or infective. (Toxicity Category III)
CLASSIFICATION:	Supplementary; a sufficiently high dose level was not used to establish an LC50 value. **However, the test material may be placed in Toxicity Category III because the study shows the LC50 is greater than 2 mg/L, and it may not be possible for the test animal to take in a higher dose.** (The study is not required for registration of the technical grade material.)

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I. STUDY DESIGN

Test material. SP 408, Batch No. PPQ 2585; freeze-dried fermentation broth concentrate of Bacillus thuringiensis var. tenebrionis NB 122 (Btt); the concentration of Btt is 3×10^{10} CFU/g.

Test animals. Fifty-eight Sprague-Dawley rats from Charles River (UK) Limited, England, with a weight range of 244 to 341 g for males and 187 to 239 g for females on day of treatment, were used in the study. The high dose group consisted of 14 animals/sex, and the low dose group, also 14 animals/sex.

Methods. The test material is a brown powder and was tested in its original form as a particulate dust aerosol. The test atmospheres were generated using a Wright Dust feeder (Wright, BM. 1950. J Sci Instr. 27:12). The feeder provided freely dispersed dust in a stream of clean dry air to an exposure chamber of approximately 41.5 liters. The feeder is positioned at the base of the exposure chamber. This system allowed for a single pass of the test material from the base throughout the chamber to the top, to a vacuum line fitted with a filter for sample collection. Chamber air was drawn through the filter at a measured rate of 1.0 L/min using a vacuum pump. Chamber air flow rates were monitored continuously and the values recorded at 30-minute intervals. Chamber concentration was calculated from the material collected on the filter. The particle size distribution of the test material was calculated from samples obtained with a Marple Cascade Impactor (Model 296, Anderson Samplers Inc., Atlanta, GA); two samples were taken during each exposure period. The chamber temperature and relative humidity were recorded every 30 minutes during treatment.

A high dose of 2.72 mg/L (8.2×10^7 CFU/L) and a low dose of 0.55 mg/L (1.7×10^7 CFU/L) were selected based on results from an earlier inhalation study on the test material.

The animals were restrained in tubes and the tubes fitted to the exposure chamber such that the snouts would be exposed to the test material. The length of exposure was 4 hours, during which time the animals were not allowed access to food or water. The animals were observed for clinical signs at frequent intervals during treatment and for the first 2 hours after treatment; they were observed daily for 28 days post treatment. Individual body weights were recorded on 1, 2, 3, and 7 days after treatment, and weekly thereafter.

Four animals (2/sex) from each dose group were sacrificed on 1, 2, 3, 7, 14, 21 and 28 days post treatment. Gross necropsy was performed on all animals killed and on all animals that were found dead during the study. Except for the lungs, the organs were examined in situ. The left lung lobe was used for histopathology. The right lobe was frozen and dispatched to the

company for microbial recovery (enumeration of Btt). The latter lung samples were diluted 1:10 (w/v) in a 0.1% peptone-water solution. Serial 10-fold dilutions were carried out and samples were plated on Agar 3 (peptone-yeast-beef-glucose-agar). The number of Btt colonies was counted; the limit of detection was 10 CFU/g.

II. RESULTS

Sampling of the chamber atmosphere showed that the low dose group was exposed to 0.55 mg/L of the test material (nominal = 11.11 mg/L) and the high dose group, exposed to 2.72 mg/L of the test material (nominal = 30.83 mg/L). An analysis of the particle size distribution showed that the mass mean diameter of the particles were 3.9 μm and 4.4 μm for the low and high dose groups, respectively. Particles that were < 3.5 μm were 46.3% and 36.2% by weight for the same two groups.

None of the animals in the low dose group died as a result of the treatment. In the high dose group, however, three animals were found dead at the end of exposure period; one died about 20 minutes after the exposure period, and two were found dead 1 day after exposure. Clinical signs observed in the 2 days following exposure included red staining around the head and snout, labored respiration, subdued behavior, piloerection, unkempt appearance, no reaction to stimuli (high dose group only). One low dose group animal showed damage to the snout following exposure.

Animals in both dose groups suffered a body weight loss during the first week following treatment. The effect appears to be transient, and weight gain was seen in the weeks afterwards.

Gross pathological examination revealed dark, mottled lungs and the presence of pale-colored foci on all lobes in the majority of the animals in the high dose group. Histopathological findings included focal alveolitis, interstitial pneumonitis and bronchiolitis. Focal alveolitis was more severe in the high dose group animals that were sacrificed in the 3 days post treatment. Moderate, but marked, congestion was observed in the lungs from all animals that were found dead.

Examination of animals sacrificed on day 1 showed the presence of 10^5 to 10^6 CFU/g lung in the low dose group and 10^6 CFU/g lung in the high dose group. By day 29, the low dose group showed 10^3 to 10^4 CFU/g lung, and in the high dose group, 10^4 to 10^5 CFU/g lung. These results indicate that the test material is poorly eliminated from the lungs.

III. DISCUSSION

The purpose of the study was to provide information on the toxicity, pathogenicity and persistence of the test material in the lungs of rats following a single 4-hour exposure to the snout. The observation period was 29 days.

The results showed a mortality of 21% (6/28) in the high dose group, with deaths occurring either during treatment or within 24 hours after treatment. Clinical signs such as respiratory depression were seen in all animals during the exposure period.

Large amounts of Btt were isolated from all lung samples. The concentration found in the high dose group is comparable to the 10^6 CFU/g lung found in the animals that died in the acute pulmonary (intratracheal) study. Both studies indicate that the test material was respirable and poorly eliminated from the lungs during the 28 days following exposure. Signs of lung injury were observed in histopathological examinations. Based on data from both studies, the submitter has concluded that the pathogenic potential is low.

The test material does not appear to be infective.

Reviewed by: Flora Chow, Registration Standards and Special Review Section, Science Analysis and Coordination Branch (SACB)
Secondary reviewer: Roy D. Sjoblad, SACB RDS

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DATA EVALUATION REPORT

STUDY TYPE:	Acute intravenous toxicity/pathogenicity study in rats
MRID:	414400-03 CASWELL NO.: 66
TEST MATERIAL:	Novodor™ Technical (technical grade)
SYNONYMS:	Bacillus thuringiensis var. tenebrionis NB 125 (Btt); SP 408
STUDY NO.:	13588
SPONSOR:	Novo-Nordisk A/S
TESTING FACILITY:	Enzyme Toxicology Laboratory, Industrial Biotechnology, Novo Industri A/S, Denmark
TITLE OF REPORT:	SP 408 Acute intravenous toxicity/pathogenicity study in rats
AUTHOR(S):	Anne Sietske de Boer
REPORT ISSUED:	16 August 1989
CONCLUSIONS:	A single dose of the test material containing 4×10^8 CFU of Bacillus thuringiensis var. tenebrionis NB 125 (Btt) was administered by intravenous injection. The test material does not appear to be toxic, pathogenic or infective (although one piece of data suggests a potential for infectivity).
CLASSIFICATION:	Acceptable

I. STUDY DESIGN

Test material. SP 408, batch no. Btt-33, fermentation broth concentrate of Bacillus thuringiensis var. tenebrionis NB 125 (Btt); the concentration of Btt is 10^9 to 10^{10} CFU/g.

Test animals. Twenty-eight Wistar rats from Mollegards Breeding Center Ltd., Denmark, with a weight range of 120-142 g for males

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and 117-129 g for females on day of treatment, were used in the study. The test group consisted of 9 animals/sex; the control group consisted of 5 animals/sex.

Methods. The test material was suspended (1:10) in sterile water to give a concentration of 4 x 10^8 CFU/mL. The test material (1 mL, 4 x 10^8 CFU) was administered to the animals by intravenous injection in the tail. The control group of animals were untreated.

Animals were observed daily for mortality and clinical signs during the 28 days following treatment. Individual body weights were recorded on the day of treatment and weekly thereafter.

Clearance of the test material was determined by microbial evaluation (i.e., enumeration of Btt) of feces. Fecal samples from 10 test animals (5/sex) were collected at 24, 48, and 72 hours, and 7 and 14 days after treatment. The samples were diluted 1:10 (w/v) in a 0.1% peptone-water solution. Serial 10-fold dilutions were carried out and samples were plated on Agar 3 (peptone-yeast-beef-glucose-agar). The number of Btt colonies was counted; the limit of detection was 10 CFU/g.

Infectivity and persistence of the test material was evaluated by microbial evaluation of organs and blood of the sacrificed animals. Four animals (2/sex) were killed 7 and 14 days after treatment; ten other animals (5/sex) were killed 28 days after treatment. Organ and blood samples were diluted and plated in a manner similar to the treatment of fecal samples. The number of Btt colonies was counted; the limit of detection was 10 CFU/g.

Gross necropsy was performed on all animals killed and on all animals that were found dead during the study.

II. RESULTS

None of the animals died during the study. One animal developed phlebitis of a tail vein and was killed (unscheduled) 16 days after treatment. No other signs of reaction to treatment were observed.

Group mean values of body weights and body weight gains do not appear to be affected by the administration of the test material.

Gross pathological examination of the animals killed on day 8 showed enlargement of the spleen. This was also seen for one animal killed on day 15 and one animal killed on day 29. The animal that developed phlebitis of a tail vein also showed enlarged inguinal lymph nodes.

Approximately 10^5 CFU/g was isolated from feces of all animals at 24 hours, 10^4 CFU/g at 48 hours, and 10^3 CFU/g at 72 hours. The

amount of Btt declined over the following weeks, and by day 29, Btt was not detected in the feces of any of the animals. One animal, the one that with phlebitis, showed 10^4 CFU in the feces even on days 8 and 15 (c.f. 10^2 CFU for the other animals).

Btt was isolated from all organ tissues, and except for the blood, at all sampling. In general, all tissues show a decrease in Btt over the observation period. By day 29, the brain and lung showed $\leq 10^2$ CFU/g tissue, a 10 to 100-fold decrease from day 8. By day 29, the spleen and liver showed 10^5 and 10^4 CFU/g, respectively, both a 10 to 100-fold decrease from day 8. By day 29, Btt was no longer detected in the bloodstream; on day 8, the level had been approximately 10^5 CFU/mL. The lymph nodes showed approximately 10^3 CFU/g tissue on day 29; although a slight decrease from day 8 was observed, the level of Btt was within the same order of magnitude as that on day 8 (the male rats had shown an increase in Btt levels on day 29 compared with day 15).

III. DISCUSSION

The purpose of the study was to provide information on the toxicity, pathogenicity and infectivity of the test material using a single dose of 10^8 CFU administered intravenously. The observation period was 29 days.

The results showed a mortality of 0%. The only clinical symptom observed was phlebitis in a tail vein of one animal.

Microbial evaluation showed clearance of the test material from feces reached the detection limit in 28 days. Evaluation of the organs and blood showed that the test material is slowly cleared. An increase in Btt levels from day 15 to day 29 was observed in the lymph nodes of the male rats. **Although, the submitter believes that this observation may be due to the small sampling size (2 males) of day 15, and not due to replication of Btt, the submitter has not claimed the test material is noninfective.**

The test material does not appear to be pathogenic under the testing conditions.

Reviewed by: Flora Chow, Registration Standards and Special Review Section, Science Analysis and Coordination Branch (SACB)
Secondary reviewer: Roy D. Sjoblad, SACB RDS

008066

DATA EVALUATION REPORT

STUDY TYPE:	Primary eye irritation/infection study in rabbits
MRID:	414127-07 CASWELL NO.: 66
TEST MATERIAL:	Novodor™ Technical (technical grade)
SYNONYMS:	Bacillus thuringiensis var. tenebrionis NB 125 (Btt); SP 408
STUDY NO.:	11688
SPONSOR:	Novo-Nordisk A/S
TESTING FACILITY:	Enzyme Toxicology Laboratory, Industrial Biotechnology, Novo Industri A/S, Denmark
TITLE OF REPORT:	Eye irritation/infection study in rabbits with SP 408, PPQ 2585
AUTHOR(S):	Ninna Berg
REPORT ISSUED:	28 April 1989
CONCLUSIONS:	A single dose of the test material containing 10^9 CFU of Bacillus thuringiensis var. tenebrionis NB 125 (Btt) was administered by instillation to the conjunctival sac of rabbits. The test material produced mild irritation (slight chemosis, discharge), reversible within 7 days. The test material may be placed in Toxicity Category III.
CLASSIFICATION:	Acceptable.

I. STUDY DESIGN

Test material. SP 408, batch no. PPQ 2585, freeze-dried fermentation broth concentrate of Bacillus thuringiensis var. tenebrionis NB 125 (Btt); the concentration of Btt is 3×10^{10} CFU/g.

Test animals. Six male New Zealand White rabbits, bred by Novo Industri A/S, Denmark, weighing 2.8 to 3.2 kg three days before treatment, were used in the study.Methods. A classification of pain following instillation of 0.1 g of the test material was conducted prior to the study. The pain reaction was valued at 2, and therefore, anaesthesia was not used in this study.

A swab was taken from the conjunctival sac of both eyes of each rabbit to determine the pre-test level of the test material. The lower lid of the left eye of each animal was pulled away from the eyeball, and 0.1 g (3×10^9 CFU) of the test material was administered by instillation in the conjunctival sac. The lids were held together for 1 second. The untreated eye served as control.

Animals were observed for clinical signs at 1, 24, 48, 72 hours, and at 4 and 7 days after treatment. Lesions of the conjunctiva, iris and cornea were evaluated separately. Individual body weights were recorded at 3, 16 and 21 days post treatment.

Clearance and infectivity of the test material was determined by enumeration of Btt from swab samples taken from both eyes of the animal. Samples were collected at 24, 48 and 72 hours, and 7, 15 and 21 (2 rabbits) days after treatment. The swabs were washed with 1 mL 0.1% peptone-water, and the solution plated on Agar 3 (peptone-yeast-beef-glucose-agar). The number of Btt colonies was counted.

II. RESULTS

Corneal opacities and iritis were not observed in any of the rabbits. Mild conjunctival reactions and swelling (irritation score = 1) were seen in the treated eyes of all animals. The conjunctival reactions had subsided by 48 hours, and the swelling by 24 hours. A slight discharge from the treated eyes was observed in all animals; 2 animals continued to show discharge up to 3 and 4 days after treatment. Because no reactions were observed 7 days after treatment, no further clinical observations were made.

There was no evidence of treatment-related changes in body weight during the 21-day study.

Btt colonies (< 100) were isolated from the left eye of 3 of the animals and from the right (untreated) eye of all of the animals 24 hours after treatment. The population of Btt had generally declined by 72 hours after treatment, but increased again in the samples 7-day post treatment. Swab samples taken on day 14 from the cage, the front paws and the surrounding eye area showed the presence of Btt. These findings were considered to explain the signs of eye reinfection. The cages were changed, and changed

again on day 20. Eye swab samples taken from day 16 showed the presence of Btt in 2 animals, and by day 22, Btt was no longer observed.

II. DISCUSSION

The purpose of the study was to provide information on the potential for health hazards from a single dose of 10^9 CFU to the eye. The study lasted 22 days (observation period of 8 days).

The test material elicited temporary, mild clinical reactions. The report does not discuss whether the eyes were irrigated 24 hours after treatment. There was evidence of clearance of the test material from the conjunctival sac in the few days following treatment. However, reinfection occurred and appeared to be caused by Btt populating the animal cages; reinfection was preventable by moving the animals to clean cages. (The microbial evaluation for clearance and infectivity is no longer required in the current Testing Guidelines, July 1989).