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

008347

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

APR 24 1991

MEMORANDUM

SUBJECT: Review of Toxicology and Product Analysis Data in Support of Seven Experimental User Permit (EUP) Applications of Bacillus thuringiensis (Berliner) var. aizawai.

TO: Mike Mendelsohn/Phil Hutton, PM-17
Insecticide-Rodenticide Branch
Registration Division (H7505C)

FROM: Rita Briggs, Ph.D., Chemist R.B.
Science Analysis and Coordination Branch (SACB)
Health Effects Division (H7509C)

THROUGH: Reto Engler, Ph.D., Chief
SACB/HED

DATA REVIEW RECORD

Product name: Bacillus thuringiensis subsp. aizawai
ID No: 000275-EUP-TR
Caswell No: 066
HED Project: 1-0353
MRID No: Technical Powder (ABG-6305):-
417225-01 (Disclosure of ingredients; Manufacturing process; Unintentional ingredients).
417225-03 (Taxonomy).
417225-04, 05, 06 (Host insect spectrum).
417225-07 (Intraperitoneal/subcutaneous injection)
417225-09 (Analysis of Samples)
417225-14, 16 (Physical/chemical properties)
417225-10 (HPLC assay for beta-exotoxin).
417225-11 (SDS-Page quantitation).
418089-01 (Fly larvae assay for beta-exotoxin);
418089-02 (Acute intravenous study).

Formulated End-Use Product (ABG-6314):
417225-02 (Disclosure of ingredients, Manufacturing process; Unintentional ingredients;

Certification of ingredients).
417225-08 (Analysis of Samples).
418089-03 (Acute oral toxicity/clearance study).

ACTION REQUESTED

To review toxicology and product analysis data in support of seven EUP applications for the use of Bacillus thuringiensis subsp. aizawai on various crops against lepidopteran insects. The proposed use of Bacillus thuringiensis subsp. aizawai is summarized in the following table:

CROP	TARGET PESTS	# STATES	TOTAL ACREAGE
Alfalfa	Armyworm	4	4900
Cotton	Armyworm	10	4900
Cabbage	Diamondback moth, cabbage looper, imported cabbageworm, armyworm.	10	4900
Broccoli	as above	10	4900
Cauliflower	"	8	3550
Lettuce	"	4	4900
Minor leafy/ cruciferous	"	10	4900

Note: These crops will not be destroyed at the termination of the EUP program; they are intended for food use. A food tolerance exemption has been requested (see Background section below).

BACKGROUND

Data requirements for the proposed EUP applications have been previously discussed with the registrant (see correspondence between Abbott Laboratories and Phil Hutton/Mike Mendelsohn, RD dated 8/27, 11/16, 11/21, and 11/26/90). Based on these discussions/correspondence, the registrant has requested the following EUP data waivers or exemptions:

1. Food Tolerance Exemption: The registrant believes that an exemption is justified because: (a) ABG-6314 (the production strain) is an authentic strain of Bacillus thuringiensis Berliner subsp. aizawai (ABST-1857; ATCC SD-1372) and exhibits morphological and biochemical characteristics of Bacillus thuringiensis according to Bergey's Manual of Determinative Bacteriology, 8th Edition. (b) Spore preparations are produced by pure culture fermentation methods with adequate quality control procedures to ensure a uniform product which does not deviate from the parent strain and is free of

contaminants. (c) Samples from each fermentation batch will be subcutaneously injected into laboratory mice to assess infectivity and toxicity. (d) Bacillus thuringiensis subsp. aizawai (ABTS-1857) does not produce beta-exotoxin under standard manufacturing conditions.

2. Acute Oral Toxicity/Pathogenicity Study for the technical powder (ABG-6305): It was suggested by RD, with SACB concurrence (see Phil Hutton/Mike Mendelsohn letter 11/21/90) that this study may be replaced by a modified acute oral toxicology study for the end-use product (ABG-6314).

3. Acute Pulmonary Toxicity/Pathogenicity Study for the technical powder: Abbott has requested that pulmonary data for B.t. var. kurstaki or B.t. var. israelensis be bridged to meet EUP requirements for Abbott's new B.T. var. aizawai strain providing that product characterization studies on the new strain support the request.

4. Five Lot Analysis for beta-exotoxin by Fly Bioassay: A waiver was originally requested for this assay based on the rationale that no detectable levels of beta-exotoxin were found by HPLC method. However, the registrant has since submitted data on the fly bioassay and the data are reviewed in this memorandum.

5. Storage stability: A waiver was requested because Abbott does does not anticipate that the data will be generated until after the EUP program date. In addition, the probability that beta-exotoxin production will occur during storage is unlikely since ABG-6314 (the end-use product) is a granule.

NOTE: SACB considered these issues in making its final conclusions.

SACB's CONCLUSIONS

The results of the acute oral and intravenous toxicity study, and intraperitoneal/subcutaneous injection study indicate that Bacillus thuringiensis subsp. aizawai, ABG-6314 is neither toxic nor pathogenic.

It was reported that the HPLC assay determined that five lots of Technical Powder contained no beta-exotoxin. However, data on the results were not provided. Moreover, the Housefly Bioassay did not conclusively determine that the samples were free of this toxin. For the purpose of registration, the registrant should provide data from the HPLC assay.

Both the Acute Oral Toxicity/Pathogenicity and the Acute Intravenous Toxicity/Pathogenicity studies showed incomplete clearance of the organism from the feces and persistence in the

mesenteric lymph node and spleen. The initial high dose and the possibility that the organism may be degraded in the gut, thereby escaping detection, may account for the incomplete recovery from the feces. In addition, the infectivity results are not atypical of B.t. and multiplication of the organism was not evident. Therefore, SACB believes that the organism does not pose any unreasonable risk to mammals. However, SACB recommends that future testing include a shelf control group to address the issue of possible reinfectivity .

It is SACB's opinion that B.t. var. aizawai is sufficiently similar in identity to B.t. var. kurstaki to warrant bridging of the pulmonary toxicity/pathogenicity data. It is also recommended that workers use protective equipment, such as a dust mask, to minimize inhalation.

Storage stability data, not essential for the present EUPs, should be submitted for the purpose of registration.

SUMMARY OF REVIEWS

I. PRODUCT ANALYSIS (151A-10)

Product Identity: Bacillus thuringiensis subsp. aizawai (ABTS-1857) is used to produce ABG-6305 (technical powder) and ABG-6314 (the production strain). The parent strain, ABTS-1857, is deposited at the American Type Culture Collection as ATCC-SD-1372.

Confidential Statements of Formula for ABG-6305 and ABG-6314 were submitted in accordance with 40 CFR 158.740.

History: The parent strain, ABTS-1857, is described as a Gram-positive, spore-forming, rod-shaped bacterium that produces a toxic protein contained within a parasporal crystal which, upon ingestion, is effective as a biocontrol agent for some lepidopteran larvae. The strain ABTS-1857 was isolated from a Wisconsin soil in 1987. Thirty colonies were selected, pooled as a suspension, and lyophilized for storage on 23 May 1990. Lyophilized stocks are maintained in the Fermentation Research and Development Culture Collection for archival purposes.

Biochemical and Morphological Testing: Biochemical and morphological features of the strain ABTS-1857 were analyzed according to methods specified in Bergey's Manual of Systematic Bacteriology, Vol. 2, 1986 and by non-standard methods described in Appendix A of the registrant's report. These latter methods included modified or newly developed techniques for determining: production of lecithinase, deamination of phenylalanine, requirements of sodium chloride, potassium chloride, allantoin and uric acid, and auxotrophic growth. Biochemical responses of ABTS-1857 were compared to responses of strain Btk-HD-1 [used for production of DiPel (R)], two Bacillus thuringiensis subsp. aizawai type-strains [ABTS-26(HD-133) and ABTS-1883(HD-11)], and a Bergey-type strain. The non-Abbott strains used for comparison were acquired from the U.S. Department of Agriculture Culture Collection, North Regional Research Laboratory, Peoria, Ill. and the American Type Culture Collection, Rockville, Md.

Results from the biochemical and morphology testing indicate that the majority of responses for ABTS-1857 are similar to those observed for Btk-HD-1 and for the Bergey-type strain. Similar responses include: (1) cell diameter of >1.0 mcM (2) production of catalase (3) fermentation of glucose (4) hydrolysis of casein, gelatin and starch (5) utilization of citrate (6) reduction of nitrate to nitrite (7) growth at pH 5.7 and 6.8, in 0-7% NaCl, between 15-45°C, and in the presence of lysozyme. Autotrophic growth does not occur with H_2 and CO_2 or high salt concentration (8) positive Voges-Proskauer test (9) does not degrade tyrosine, deaminate phenylalanine, or form indole or dihydroxyacetone (10) does not require NaCl, KCl, allantoin, or urate.

Strains ABTS-1857 and Btk-HD-1 were shown to differ in their ability to ferment arabinose, xylose and mannitose. While ABTS-1857 exhibits variable responses, Btk-HD-1 does not produce acid from these sugars. Several minor metabolic differences were also observed - the Bergey strain is able to grow in 7% NaCl and the ABTS-1857 and Bergey strains exhibit a trace of growth at 10°C. In addition, ABTS-1857 utilizes proprionate while Btk-HD-1 does not.

There were also minor differences between ABTS-1857 and the two Bacillus thuringiensis var. aizawai type-strains (ABTS-26 and ABTS-1883). The latter strains did not produce acid from arabinose, xylose, mannitol but exhibited a weak ability to degrade tyrosine. ABTS-26 and ABTS-1883 grow in 7% NaCl solutions in contrast to ABTS-1857 which does not.

Flagellar Antigen Serotying: ABTS-1857 has been shown to react with only the anti-H7 antibody and, therefore, is classified as serotype H-7.

Antibiotic Sensitivity Pattern: Susceptibility to antibiotics was assessed using agar disk diffusion according to standard methodology (S.O.P. 047T-12-043A; National Committee for Clinical Laboratory Standards, 1984). Results showed that ABTS-1857 is sensitive to gentamicin, erythromycin, clindamycin, vancomycin, chloramphenicol, kanamycin, and trimethoprim/sulfamethoxazole, but is resistant to penicillin, ampicillin and cephalothin. Btk-HD-1 and the two Bt. aizawai strains demonstrated similar responses.

Characterization of Crystal Proteins:
Microscopic examination of sporulated cultures of ABTS-1857 demonstrate that two crystalline inclusions are produced during routine fermentation conditions and are structurally indistinguishable from crystals produced by Btk-HD-1 (DiPel production strains). The majority of crystals are bipyramidal, ca. 0.8 x 2.0 micron; cuboidal inclusions, ca. 0.5 micron, are found at much lower levels.

SDS-PAGE analysis compared purified crystals produced by ABTS-1857 to proteins extracted from sporulated cultures of BTk-HD-1, ABTS-1857, and two Bt. subsp. aizawai strains previously described. The SDS-soluble proteins from purified crystals produced by ABTS-1857 showed as one major band corresponding to the high molecular weight protein (135 kDa) reported for the Lepidopteran active protoxin. The protein profiles for the sporulated cultures of B.t. subsp. aizawai exhibited the same major 135 kDa band. In addition, there was a background of minor bands, possibly derived from fermentation media and cellular proteins or degradation products of the 135 kDa band. SDS-Page analyses of five different lots of ABTS-1857 technical powder indicated the presence of significant amounts of the 135 kDa protein as well as numerous

minor bands. The Btk-HD-1 protein profile demonstrated the presence of two major protein bands, one corresponding to 135 kDa another corresponding to a P2 protein of approximately 65 kDa.

Plasmid Profile: A plasmid profile was derived by using a procedure adapted from two protocols - a protocol for isolation of Bacillus subtilis plasmid (Gryczan et al., J. Bacteriol. 134:318-329, 1978) and another protocol for isolation of Bacillus thuringiensis plasmids (Shivakumar et. al., J. Bacteriol. 166:194-204, 1986). Plasmid profiles of ABTS-1857, Btk-HD-1, and two B.t. subsp. aizawai type-strains [ABTS-1883 (HD-11) and ABTS-26 (HD-133)] obtained from USDA Laboratory, Peoria, Ill. The profiles show that five plasmids are of greater mass and ten plasmids are of less mass than the chromosomal fragment. The profile of ABTS-1857 is similar to ABTS-26 but not to B.t.k. HD-1 or ABTS-1883.

Biological Activity: The results from bioassays to determine the range of host insects that ABTS-1857 is active against are summarized below:

Insect	% Mortality
Coleopteran (Alphitobias diaperionus)	Bioassay - no significant difference between control and treated larvae.
Orthoptera (Acheta domestica)	Bioassay - 5-7.5% mortality among crickets from test substance; 10% among untreated controls.
Diptera (Aedes aegypti)	Bioassay - 56.6% mortality at concentration 0.05 mg/ml which is equivalent to 100x typical LC50 of Reference Substance (B.t. var. israelensis).

In addition, the biological activity of Technical Powder (Lot 42-422-BD) was determined by bioassay in which the response of 4-day-old cabbage looper (Trichoplusia ni) larvae to delta-endotoxin was assessed. Potency value was determined to be 26,644 IU/mg.

II. MANUFACTURING PROCESS (151A-11)

Basic Manufacturing Process

B.t. subsp. aizawai is maintained either on agar slants or in frozen vials. [redacted]

QUALITY CONTROL PROCEDURE INFORMATION IS NOT INCLUDED

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

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QUALITY CONTROL PROCEDURE INFORMATION IS NOT INCLUDED

[redacted]

Technical Product Formulation

[redacted]

Formation of Unintentional Ingredients (151A-12)

[redacted]

Quality control measures also included surveillance of equipment and identity and purity of raw materials according to guidelines decribed in "Basic Operating Procedures" and "Good Manufacturing Procedures, respectively.

III. ANALYSIS OF SAMPLES (151A-13)

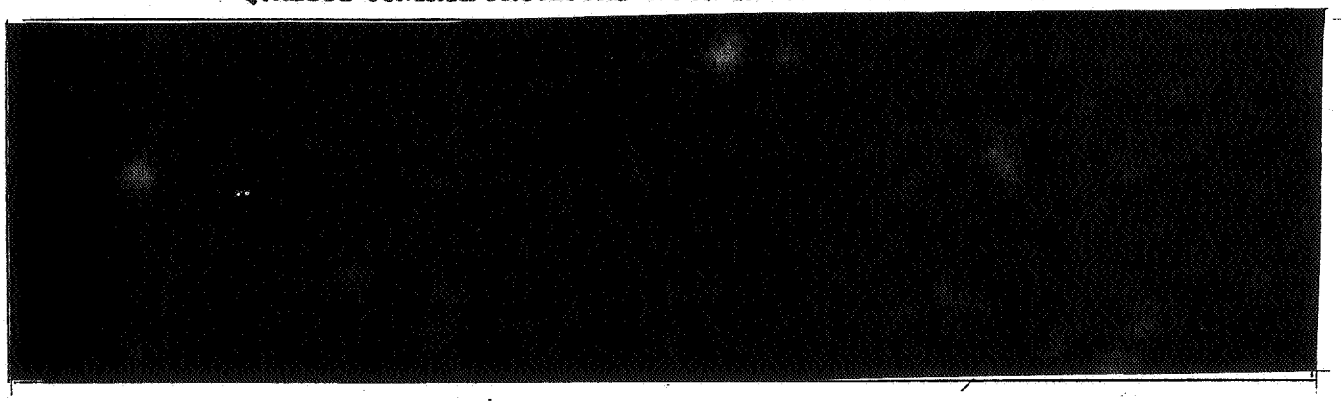
A. TECHNICAL POWDER (ABG-6305)

HPLC assay was conducted to determine the presence of beta-exotoxin in five lots of ABG-6305 Technical Powder. The lots tested are identified as 38-990-BD, 41-115-BD, 42-221-BD, 44-328-BD, 44-329-BD. The presence of beta-exotoxin was determined by comparing peak retention time in the sample with that of beta-exotoxin standard. It was reported that none of the samples contained any detectable level of beta-exotoxin (detection limit is 1 ppm) but the actual

results were not included.

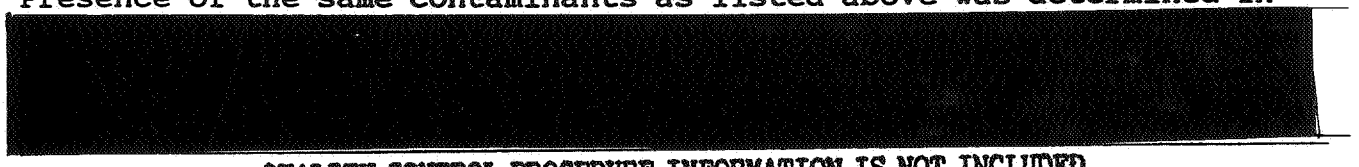
Housefly Bioassay included a ten-day exposure of 3-day-old larval Musca domestica L. to five lots of ABG-6305 Technical Powder and beta-exotoxin Reference Standard. The effects were measured as percent adult non-emergence. Test and reference substances were assayed before and after heat treatment to determine non-exotoxin related mortality. There were low levels of mortality from exposure to unautoclaved test substance (0-24%); autoclaving reduced mortality to 0-3%. Autoclaving of reference standard resulted in 28.8% loss of bioactivity. The registrant concluded from these results that fly mortality was due to an unidentified, heat-labile toxic moiety in the test substance and not to beta-exotoxin, per se.

QUALITY CONTROL PROCEDURE INFORMATION IS NOT INCLUDED



B. END-USE PRODUCT (ABG-6314)

Presence of the same contaminants as listed above was determined in



QUALITY CONTROL PROCEDURE INFORMATION IS NOT INCLUDED

IV. CERTIFICATION OF INGREDIENT LIMITS (151A-15) *CBI*

Certification of ingredients was submitted for ABG-6314, End-Use Product.

V. PHYSICAL AND CHEMICAL PROPERTIES (151A-16)
The physical and chemical properties for ABG-6305 Technical Powder and ABG-6314 (End-Use Product) are summarized in the following table:

	ABG-6305	ABG-6314
Color :	Tan	Light Brown
Physical State:	Fine powder	Fine granule
Odor:	Characteristic B.t.	Characteristic B.t.
Bulk Density:	0.504g/ml @ 23°C	0.383
pH	4.44 @ 23°C	4.36

No data on the stability of ABG-6314 are yet available. These should be submitted for the purpose of registration.

Data on the stability of ABG-6305 include:
1. Temperature - potency unchanged at 5°C; increases (14-20%) at 50°C.
2. Exposure to Air - no significant difference in biopotency.
3. Exposure to Sunlight - no significant difference in biopotency.
4. pH - potency significantly decreased at pH 7-9.
5. Stable in the presence of metal ions.

VI. SACB DISCUSSION

The biochemical and antibiotic sensitivity data suggest that the test organism, ABTS-1857, is similar enough to B.t.k. HD-1 to allow for bridging of the acute pulmonary toxicity/pathogenicity study.

SACB found the results of the housefly bioassay did not conclusively determine that beta-exotoxin was not present in the technical powder samples. Since the beta-exotoxin Reference Standard loses approximately 30% of its bioactivity upon heating, the low mortality rate from exposure to autoclaved test substances cannot be attributed solely to the presence of an unidentified, heat-labile toxic moiety. However, the reported results of the HPLC assay (no detectable beta-exotoxin in any of the samples) support the registrant's claim that the Technical Powder lots do not contain beta-exotoxin.

Storage stability data for ABG-6314 are required for registration.

The low levels of Enterococcus are considered normal since Streptacaelis (classified under Enterococcus) are routinely found at these levels.

DATA EVALUATION REPORT

Reviewed by: Rita Briggs, Ph.D., Chemist R.B.
Secondary Reviewer: Roy Sjoblad, Ph.D., Microbiologist, SACB/HED RPL

STUDY TYPE: Intraperitoneal and Subcutaneous Injection Tests - mice.
MRID NO: 417225-07
CASWELL NO: 066
TEST MATERIAL: ABG-6305 Technical Powder
SYNONYMS:
PROJECT NO: 85K-11/90
SPONSOR: Abbott Laboratories, North Chicago, Illinois
TESTING FACILITY: Abbott Laboratories
TITLE OF REPORT: Intraperitoneal and Subcutaneous Injection Tests with ABG-6305 Technical Powder
AUTHORS: E. W. Ferry
STUDY COMPLETED: November 20, 1990
CONCLUSION: No deaths or signs of toxicity were observed when animals were administered the test substance intraperitoneally at three doses - 0.005, 0.05, and 0.5 mg/animal, equivalent to 1 x 10^5, 1 x 10^7, and 1 x 10^8 CFU/animal, respectively. Similarly, a single dose of 0.063 mg (1.35 x 10^7 CFU/animal) injected subcutaneously did not result in any mortality or toxicity.
CLASSIFICATION: Acceptable

I. STUDY DESIGN

Test Material: Bacillus thuringiensis subsp. aizawai, Technical Powder (ABG-6305)

Test Animals: Three groups of five male and five female mice (weighing 18-22 g) were used for intraperitoneal injection of the test substance. Another group of five female mice (21-23 g) were dosed by the subcutaneous route.

Methods: Ten mice (5/sex) were treated by injecting the appropriate dose of ABG-6305 (prepared in 0.2 ml saline) into the peritoneal cavity of each animal. Subcutaneous doses were administered in 0.25 ml volumes. Animals were then observed for clinical signs of toxicity at intervals of 1, 2 and 4 hours after treatment, and twice daily thereafter during weekdays and once daily during weekends, for at least one week after dosing. At the termination of the study, Day 7, all animals were sacrificed.

II. RESULTS

No mortality or toxicity were observed during the 7-day observation period.

DATA EVALUATION REPORT (152A-10)

Reviewed by: Rita Briggs, Ph.D., Chemist, SACB/HED RB. RDD
Secondary Reviewer: Roy Sjoblad, Ph.D., Microbiologist, SACB/HED

STUDY TYPE:	Acute oral toxicity/pathogenic - rat
MRID NO:	418089-03
CASWELL NO:	066
TEST MATERIAL:	ABG-6314 (End-Use Product)
SYNONYMS:	
PROJECT NO:	90-0470
SPONSOR:	Abbott Laboratories, North Chicago, Ill.
TESTING FACILITY:	Ricerca Inc., Painesville, Ohio
TITLE OF REPORT:	Acute Oral Toxicity Study in Rats with ABG-6314
AUTHORS:	Mark D. Gelin
STUDY COMPLETED:	February 27, 1991
CONCLUSION:	Over the course of the 26-day study, approximately 18-54% of the test dose (a single oral dose of 1.3-1.4 x 10^{11} CFU/animal) was cleared from the feces of nine animals; one animal exhibited 85% clearance. Incomplete clearance may be attributable to initial high dose and, as reported, lack of detection of the organism in the feces due to degradation in the gut. It may also be due to reinfectivity and SACB recommends that future testing include a shelf control group. However, since no deaths or significant clinical signs of toxicity were observed, SACB does not foresee any unreasonable risk pertaining to the use of the test substance.
CLASSIFICATION:	Acceptable

I. STUDY DESIGN

Test material: Bacillus thuringiensis var. aizawai (ABG-6314, End-Use Product) Lot #46-095-BR.

Test animals: Ten young adult (5 females/5 males) Sprague-Dawley, ZML:SD (SMF) rats were obtained from Zivic-Miller Laboratories, Allison Park, Pa. and used as test animals in the study. No control (untreated) rats were included. Weight range for males and females at the beginning of the study was 245-258 g and 233-268 g, respectively.

Methods: The test dose was prepared by dispersing 1.0 gram sample in 9.0 ml sterile 0.1M phosphate buffer equivalent to a concentration of approximately 4.8 x 10^{10} CFU/gm. Following an overnight fast, a dose

volume of 10 ml/kg body weight (equivalent to approximately 1.3-1.4 x 10^{11} CFU) was administered by gavage to each animal. At 1, 21/2, and 4 hours post-treatment, and daily thereafter until the termination of the study at Day 26, the animals were observed for clinical signs of toxicity. Individual body weights also were recorded prior to fasting, shortly before dosing, and on Days 7, 14, 21 and 26 of the study. Clearance of the organism was determined by enumerating the test organism in fecal samples collected daily throughout Day 6 and on each of days 8, 10, 12, and 14. All animals were sacrificed at the termination of the study at which time a macroscopic examination was conducted to determine treatment-related abnormalities. Tissues were not evaluated for infectivity or persistence of the organism.

II. RESULTS

No mortality or significant treatment-related clinical signs of toxicity were observed during the study. Soft and decreased feces were observed in 1-2 animals but this condition did not persist beyond Day 9.

All animals gained weight over the course of the study including one female rat which lost 22 grams in body weight duuring the first week of the study.

Recovery of the test organism from the feces at the end of the study (Day 24) ranged from 2.3 x 10^{10} - 1.1 x 10^{11} CFU. This represented 18-85% recovery; most animals were in the range of 29-54%. The registrant attributed incomplete recovery to the possible destruction of the organism during passage through the gut.

III. SACB DISCUSSION

Results from the oral toxicity study demonstrated that a single oral administration of ABG-6314, at a concentration of 1.3-1.4 x 10^{11} CFU/animal, produced no deaths or significant clinical effects of toxicity. Also, all animals gained weight. However, the organism was not entirely cleared from the feces over the 26-day study. In nine of the animals, only 18-54% of the original dose was cleared; one male cleared 85%. The initial high dose, the degradation of the organism in the gut, and reinfectivity are all possible explanations for the incomplete recovery. The registrant should, in the future, include a shelf control group in this type of study to allow the issue reinfectivity to be addressed.

DATA EVALUATION REPORT (152A-13)

Reviewed by: Rita Briggs, Ph.D., Chemist, SACB/HED R.B.
Secondary Reviewer: Roy Sjoblad, Ph.D., Microbiologist, SACB/HED RDS

Study Type:	Acute intravenous toxicity/pathogenicity study in rats.
MRID:	418089-02
Caswell No:	066
Test material:	ABG-6305 Technical Powder
Synonyms:	
Project No:	901291D/ABT 143-1/AC
Sponsor:	Abbott Laboratories
Testing facility:	Huntington Research Centre Ltd., England
Authors:	John N. Carter
Title of Report:	Acute Intravenous Toxicity and Infectivity/ Pathogenicity to Rats of ABG-6305
Study completed:	February 12, 1991
Conclusion:	A single dose of at least 10^7 CFU/animal administered intravenously showed no evidence of toxicity or infectivity/pathogenicity. A pattern of clearance from the blood, cecum contents, liver, lungs, kidney, brain was established. On the other hand, viable organisms were found in the mesenteric lymph nodes and spleen at the termination of the study (Day 66). The numbers isolated, however, demonstrated a significant decline and the pattern of clearance is not unusual for B.t.
Classification:	Acceptable

I. STUDY DESIGN

Test material: Bacillus thuringiensis subsp. aizawai ABG-6305 (Lot No. 42-221-BD)

Test animals: A total of 58 CD rats (29/sex) [Crl:CD^R (SD) BR VAF Plus] obtained from Charles River U.K. Ltd., Margate, Kent, England were used in the study. They were in a weight range of 115-133 g prior to dosing on Day 1 and approximately 4-7 weeks of age. Forty-two rats (21/sex) were equally divided into 7 groups (A-G) and treated with the test substance. Sixteen (8/sex) were held as undosed controls and subdivided into Group H consisting of 12 rats (6/sex) and Group I with 4 rats (2/sex).

Methods: The test material was suspended in sterile physiological saline at a concentration of 2.94 x 10^7 CFU/ml and administered to test animals (21 males/21 females) at a volume of 3 ml/kg body weight so as to provide a single dose of at least 10^7 CFU/animal.

The animals were observed for clinical signs immediately after dosing, and at hourly intervals during Day 1. During the remainder of the study, checks for clinical symptoms were made twice daily. Individual body weights were recorded on Days 1, 4, 8, 15, 22, 29, 36, 43, 50, 57, 64, and 67.

Animals were sacrificed at the following times; Group A - 1 hour after dosing; Group B and 1 male, 1 female from Group H - at Day 4; Group C and 1 male, 1 female from Group H - at Day 8; Group D and 1 male, 1 female from Group H - at Day 15; Group E and 1 male, 1 female from Group H - at Day 22; Group F and 1 male, 1 female from Group H - at Day 36; Groups G and I and 1 male, 1 female from Group H - at day 67. A macroscopic examination was performed at the time of sacrifice and any abnormalties related to treatment recorded. In addition, samples of brain, lungs, liver, spleen, kidney, mesenteric lymph node, cecal contents were removed from each animal in each group except Group A. The test organism was enumerated in each sample to assess the degree of infectivity and persistence. Clearance of the test material also was evaluated by testing for its presence in 1 ml of blood sample taken from the orbital sinus immediately prior to sacrifice, and in cecal samples.

II. RESULTS

No mortality or clinical signs of toxicity were observed in any of the animals. Weight gains of treated groups were comparable to those of the control groups. No abnormalities during gross examination at the time of sacrifice were noted.

Clearance from the blood was established. Blood samples taken from animals at one hour after dosing contained from 10 - 1.12 x 10^2 CFU/g. At day 3, levels ranged from 46 - 5.95 x 10^2 decreasing to a range from <10 - 2.9 x 10^2 at day 7. By day 14, the number of B.T. ABG-6305 in blood samples of all animals was less than 10 CFU/g and remained low throughout the remainder of the study.

Infectivity/Persistence: At day 3, viable organisms were isolated

from all organs or tissues tested. High numbers were found in the spleen (4.12×10^5 - 1.88×10^6 CFU/g), in the lungs (1.96 - 3.79×10^4), and in the liver (3.9 - 6.68×10^4). Moderate numbers of the organism were seen in the mesenteric lymph nodes (6.4×10^2 - 2.6×10^3) and kidneys (1.2 - 3.8×10^3) while the brain contained from 43 - 3.4×10^2 CFU/g. At day 14, the brain contained less than 10 CFU/g of organism; a significant decline also was observed in the kidney. However, there were no significant changes observed in the mesenteric lymph node, lung, or liver until day 21. Relatively high numbers of the organism were still present in the spleen at the termination of the study, Day 67, although a pattern of clearance (a decline to $<10^5$) was observed in this organ with a calculated rate of clearance reported to be approximately 6.2% per day. A pattern of clearance was established for all other organs at the end of the study.

III. SACB DISCUSSION

The acute intravenous study showed no detrimental effects from treatment with ABG-6305 Technical Powder. Under the present conditions, the test material appears to be non-pathogenic. No macroscopic abnormalities were observed at the time of autopsy. All organs, except the mesenteric lymph node and spleen, were virtually free of the organism (<10 CFU) at the termination of the study. However, although the organism persisted in the mesenteric lymph node and spleen, there was, nevertheless, a reduction in the numbers of organism isolated at the termination of the study indicating a pattern of slow clearance.