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

17 APR 1992

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

Subject: Bacillus thuringiensis var. aizawaii, ABG-6305 (CenTari) Technical Powder and Wettable Dispersible Granule (WDG): Review of Data to Support Registration.

To: Mike Mendelsohn/Phil Hutton, PM-17
Insecticide-Rodenticide Branch

From: Rita Briggs, Ph.D., Chemist R.B. 4/15/92
Science Analysis and Coordination Branch (SACB)
Health Effects Division (H7509C)

Through: Reto Engler, Ph.D., Senior Scientist
SACB/HED

DATA REVIEW RECORD

Product Name : Bacillus thuringiensis (Berliner) var. aizawaii, ABG-6305
ID No : 000275-IA
Synonym: CenTari
Caswell No: 066
HED Project: 2-1243
MRID No: 421400-01: TGAI Physical Stability Study
421133-01: Analysis of Diamondback Moth Biopotency of Centari Technical Powder.
421133-02: Analysis of Diamondback Moth Biopotency of Centari Wettable Dispersible Granule.
421707-01: Analysis of Beta-exotoxin (thuringiensis) Content of Five Lots of ABG-6305 Technical Powder by Housefly Bioassay; Addendum to MRID No. 41808901.

421707-02: HPLC Assay for Beta-exotoxin in ABG-6305 (Centari) Technical Powder - Second Addendum to MRID No. 41722510 (Manufacturing Process).

BACKGROUND

In memoranda dated April 24 and December 12, 1991 from Rita Briggs to Mike Mendelsohn, SACB reported that data submitted by the registrant (Abbott Laboratories) on the HPLC and housefly bioassay studies for detection of beta-exotoxin were inadequate for registration of CenTari products. Several data gaps with respect to physical stability studies also were reported. To discuss the issues raised by SACB in these memoranda, a meeting between representatives of the registrant and the Agency (R. Briggs, M. Mendelsohn, John Kough, Tom McClintock) was held on December 16, 1991. In response to this meeting, the registrant has now submitted addendums to the HPLC and housefly assays as well as studies on physical stability in support of registration. SACB's reviews are attached.

CONCLUSIONS

1. Physical stability studies:
   There were no effects of temperature on potency values of the TGAI, and SACB believes that the registrant's rationale for not testing the effects of metal ions and pH is reasonable. However, the protocol for the sunlight stability studies is questionable. The artificial light source did not mimic natural sunlight because essentially all the UV wavelengths were filtered out. While this filtered spectrum will support proper plant growth, it is assumed that UV wavelengths play a major role in the inactivation of proteins, in this case, the Centari product containing Bacillus thuringiensis var. aizawaii. Therefore, SACB recommends that the registrant either includes a statement on the product label to protect the product from sunlight, or repeats the studies to test the full spectrum of incident sunlight on the stability of Centari.

2. Analysis of Samples:
   (a) The data provided on five-lot biopotency analysis of CenTari Technical Powder and the Wettable Dispersible Granule using the Diamondback Moth assay are adequate to satisfy the requirement for a sample analysis of bioactivity.
   (b) The questions previously raised by SACB relative to the HPLC chromatograms (see

review on page 6 have been clarified.
(c) The discussion on the method detection limit (MDL) calculations of the HPLC assay and the Housefly Bioassay for detection of beta-exotoxin indicate that the HPLC assay is 50 times more sensitive. The data from the HPLC assay also indicate that none of the five lots of technical powder tested have levels of beta-exotoxin at or above the MDL (1 mcg/g).

SUMMARY OF REVIEWS

I. Physical and Chemical Properties: (151A-16)

Study Title: TGAI Physical Stability Study of Bacillus thuringiensis subsp. aizawai
Author: Stefan T. Jaronski, Ph.D.
Testing Facility: Abbott Laboratories, Chicago, Ill.
Project ID: 91M-91-004
Study Completed: December 19, 1991
MRID No: 421400-01

The effects of temperature on bioactivity of the test substance, Bacillus thuringiensis subsp. aizawai , TGAI (Lot No. 54-870-BD) were studied (protocol submitted). Temperature regimes included 5^{o}, 25^{o}, and 50^{o} with samples assayed at the initial of the study, at 7 days and at 14 days. No changes in the physical appearance of the TGAI at any of the three temperatures were observed. At 5^{o}, the weight of the test substance increased 1.5% and 4% after 7 and 14 days, respectively. The weight gain was attributable to water absorption and had no effect on potency.

Samples of TGAI (Lot No. 54-870-BD) were also exposed to simulated sunlight for 0, 1, 2, 4, and 8 hours. The artificial light source was such that "irradiance at setting 7 is approximately equal to mid-summer sunlight at noon in Arizona" (756 W/m^2), and was filtered with "UV-Spezialglas" to omit wavelengths less than 290nm (essentially all the UV spectrum). After exposure, samples were examined for physical appearance and assayed for bioactivity. Results indicated there were no changes in the physical appearance of the TGAI at any time during the course of the study. However, potency values were statistically significantly higher after the 1 and 8 hour exposures relative to the initial sample, but not to the 2 and 4 hour samples. This finding was suggested by the registrant to be an artifact (within-sample variability) of the high initial potency, and was supported by further statistical analyses. For example, since no significant differences were determined when a comparison was made between the potencies of the initial samples from the temperature and sunlight studies, the two initial data sets were combined and compared to the test samples in the sunlight study. No statistically significant differences were found under this condition. Finally, a correlation analysis of potency versus hours of exposure indicated there was no significant correlation at the 95% probability level.

Tests to evaluate the effects of metal ions and pH were not conducted. The rationale for the omissions presented by the registrant is reportedly based on previous experience with B.t. kurstaki, B.t. israelensis, and B. spaericus . It is stated that results of bioassays using pH and metal ion solutions may be biased because microbial growth of the Test Substance occurs under these experimental conditions. For example, the registrant reports that the "Test Substance in aqueous suspension quickly begins microbial growth and fermentations so that changes (in pH) could be due to microbial degradation as much as to direct action by H^+ or OH^-". With respect to metal ions, results could be biased because several of the ions increase potencies or cause rapid fermentation of the Test Substance.

SACB Discussion: The data clearly show that temperature has no effect on the bioactivity of the TGAI. SACB also accepts the registrant's rationale for omitting the pH and metal ion studies. However, the omission of the UV spectrum from the simulated sunlight studies raises the question whether UV wavelengths play a major role in the inactivation of proteins. SACB assumes that UV light could possibly degrade the Centari product and, therefore, recommends that the registrant either (1) provides a statement on the product label that the product be protected from sunlight, or (2) repeats the studies to determine the effect of the full spectrum of incident sunlight on the stability of Centari.

II. Analysis of Samples: (151A-13)

Study Title: Analysis of Diamondback Moth Biopotency of CenTari Technical Powder.
Author: Stefan T. Jaronski, Ph.D.
Testing Facility: Abbott Laboratories, Chicago, Ill.
Project ID: 91M-91-002
Study Completed: November 22, 1991
MRID No: 421133-01

Potencies of five lots of Centari Technical Powder were determined using a Diamondback Moth bioassay. Samples tested included: Lot # 54-870-BD, 55-792-W5, 55-793-W5, 56-077-W5, 56-960-BD. Bacillus thuringiensis var. aizawai H-7 (Lot #47-419-BD) was used as a Reference Standard (STD). Biological activity of the Test Substance was determined by comparing the LD_{50} of the STD and Test Substance and multiplying by the Standard Potency (65,000 DBM units/mg). The five lots tested showed a mean potency range of 61,054 - 95,550 DBMU/mg. Only one batch, Lot #55-792-W5 which had the lowest

potency, was significantly different from the other test samples. Variability was attributed by the registrant to the manufacturing process.

SACB Discussion: The information provided on five-lot analysis of Centari Technical Powder is sufficient to satisfy the requirement for a sample analysis of bioactivity.

Study Title: Analysis of Diamondback Moth Biopotency of CenTari Wettable Dispersible Granule.
Author: Stefan T. Jaronski, Ph.D.
Testing Facility: Abbott Laboratories, Chicago, Ill.
Project ID: 91M-91-003
Study Completed: November 22, 1991
MRID No: 421133-02

Five lots (55-533-BJ, 55-574-BJ, 55-588-BJ, 56-600-BJ, and 56-604-BJ) of Centari Water dispersible granule (WDG) were assayed, using the Diamondback Moth, for bioactivity. Bacillus thuringiensis subsp. aizawai , Abbott Reference Substance (Lot #47-419-BD) was used as a Reference Standard (STD). Biological activity of the Test Substance was determined by comparing the LD_{50} of the STD and Test Substance and multiplying by the Standard Potency (65,000 DBM units/mg). The five lots tested showed a mean potency range of 45373 - 48360 DBMU/mg. There were no statistically significant differences between lots of Centari WDG.

SACB Discussion: The data on five-lot analysis of Centari WDG meet the requirements for a sample analysis of bioactivity .

Study Title: Analysis of Beta-exotoxin (thuringiensin) Content of Five Lots of ABG-6305 Technical Powder by Housefly Bioassay - Addendum to MRID No. 41808901.
Author: Stefan T. Jaronski, Ph.D.
Testing Facility: Abbott Laboratories, Chicago, Ill.
Project ID: 910-9009
Study Completed: February 27, 1991
MRID No: 421707-01

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The registrant has submitted the raw data, which SACB requested at the December 16 meeting, on the Housefly Bioassay report previously submitted (MRID 41808901). SACB's major concerns related to the 28.8% loss of bioactivity in the autoclaved reference standard and the uncertainty of whether the mortalities seen in tests using autoclaved samples were due to the presence of beta-exotoxin or perhaps to some heat-labile toxic moiety, such as alpha-toxin, which was not totally destroyed by autoclaving.

Review of Data: The data show reduced bioactivity in Reference Standard 2 (assumed to represent the autoclaved samples) which is most likely due to the impurity of the substance. The percent purity (80.7% active ingredient by weight) of the reference standard was not reported in the original submission but was conveyed to the Agency at the December meeting and in the HPLC Assay report (see next study review). The Housefly Bioassay results indicate the following mortalities from the five-lot test samples:

	Range of Mortalities (Corrected %)	
Lot #	Unautoclaved	Autoclaved
44328	1 - 20.4	0 - 2
44329	0 - 17.4	0 - 1.6
41115	0 - 12.9	0 - 3
42221	0 - 11.1	0 - 2
38996	0 - 9	0 - 3

SACB Discussion: Based on these results, it is still unclear to SACB whether or not the low levels of mortalities observed with the autoclaved test samples are due to the presence of beta-exotoxin or perhaps to some other heat-labile toxic substance (e.g. alpha-toxin) that was not totally eliminated by autoclaving. However, since the mortality rates from exposure to the autoclaved samples are very low, and since the HPLC data (see next review) support the lack of presence of beta-exotoxin in the test samples, SACB believes that the data are acceptable.

Study Title: HPLC Assay For Beta-exotoxin in ABG-6305 (CenTari) Technical Powder - Second Addendum to MRID No. 41722510.
Authors: J.Y. Lee, M. Short, and J. Yuan
Testing Facility: Abbott Laboratories, Chicago, Ill.
Project ID: 60-825-62
Study Completed: November 16, 1990
MRID No: 421707-02

This report was submitted by the registrant to clear up questions raised by SACB about the method detection limit (MDL) calculations of the HPLC assay previously submitted (MRID No. 41722510), the results of the HPLC analyses as displayed in the chromatograms, and the equivalency of the MDL calculations of the HPLC assay to those of the housefly assay for detection of beta-exotoxin.

SACB Discussion: The discussion in the present report satisfactorily addresses SACB's points of concern outlined in the December 12, 1991 memorandum from R. Briggs to M. Mendelsohn with respect to calculation of the MDL for the HPLC assay, the interpretation of the HPLC chromatograms particularly in regard to baseline noise, and the standard concentration of beta-exotoxin in the spiked samples. With this background, SACB believes that the results of the HPLC analyses support the registrant's claim that beta-exotoxin is not present in the five lots of technical powder tested at or above the limit of detection (1 mcg/g). Moreover, a comparison of the beta-exotoxin levels detected by the housefly bioassay to those detected by the HPLC assay indicate that the HPLC assay is 50 times more sensitive than the housefly bioassay.