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

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

5 MAR 1992

MEMORANDUM

SUBJECT: Review of Data Submitted for Product Stability to Support the Registration of the Bioinsecticide M-One

FROM: John L. Kough, Ph.D., Biologist
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John L. Kough 3-4-92

TO: Robert Richards/ Larry Schnaubelt (PM-72)
Special Review and Reregistration Division (H7508W)

THROUGH: Reto Engler, Ph.D.
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Health Effects Division (H7509C)

DATA REVIEW RECORD

Active Ingredient:	Bacillus thuringiensis var. san diego
Product Name:	M-One Bioinsecticide
ID No:	006401
Submission No:	S405933
DP Barcode:	D170460
Caswell No:	066
HED Project No:	2-0364
MRID No:	416795-01 (Product Chemistry- Stability Study) Waiver Request for HPLC Analysis for β-exotoxin

ACTION REQUESTED

To review the data submitted for end use product stability after one year's storage.

CONCLUSIONS

SACB finds that sufficient information has been presented to confirm the stability of the biological pesticide M-One after one year's (48 weeks) storage. The data presented also shows that the product is degraded at 40°C after 24 weeks storage, but retains about 85% activity by bioassay at 48 weeks or SDS-PAGE at 52 weeks. SACB notes that a container integrity study was included in this package. The high density polyethylene 5-gallon containers were not corroded by the product when examined after 1 year's storage.

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SACB also received a request for waiver from HPLC analysis of M-One Biopesticide end product for the presence of β-exotoxoin. The Agency does not specify the technique to be used to confirm the absence of β-exotoxin in Bacillus thuringiensis products. Since the company has adequately verified the absence of β-exotoxin at detectable levels using the fly bioassay, there is no need to run an additional HPLC analysis. All subsequent lots should still be analyzed for the presence of β-exotoxin by an appropriate method.

SUMMARY OF DATA REVIEWED

Storage Stability of End-use Product (151A-13)
The company has shown the product to be stable in commercial 5-gallon containers after 1-year's storage at ambient temperature conditions. The company has additionally shown product stability at 20°C after a year's storage in smaller containers and significant breakdown after 24 weeks at 40°C.

DATA EVALUATION REPORT

Reviewed by: John L. Kough, Ph.D., Biologist, SACB/HED JLK
Secondary Reviewer: Roy Sjoblad, Ph.D., Microbiologist, SACB/HED RDS

STUDY TYPE:	Product Chemistry- Stability Study
MRID NO:	416795-01
CASWELL NO:	066
TEST MATERIAL:	M-ONE Bioinsecticide
SYNONYMS:	Bacillus thuringiensis var. san diego
PROJECT NO:	MF-0103, MF-0104, MF-0105
SPONSOR:	Mycogen Corp., San Diego, CA
TESTING FACILITY:	Mycogen Corp., San Diego, CA
TITLE OF REPORT:	1989 M-One Production Stability Study
AUTHOR:	J. Glatzhofer
STUDY COMPLETED:	October 29, 1990
CONCLUSION:	The company has submitted data to adequately address the issue of product stability. The company has shown the product from 4 different lots retains approximately 85% of activity by SDS-PAGE analysis or Colorado Potato Beetle bioassay after 48 weeks storage. No β-exotoxin was detected in the lots by the fly larvae bioassay after 24 or 52 weeks storage. The product stability was also tracked for variations in pH, separation and viscosity.
CLASSIFICATION:	Acceptable.

I.STUDY DESIGN

Test Material:

All the test materials are aqueous liquid formulations of M-One produced by the IBIS Facility in Kingstree, SC and originally packaged in commercial 5 gallon containers. The lot numbers are 82009, 82810, 82171 and 82784 and were produced for 1989 sales.

Methods:

Two hundred gram samples of these lots were removed from 5 gallon commercial containers and dispensed into glass jars sealed with polyfoam lined lids. These subsamples were then placed in temperature controlled environments at 4°, 20° and 40°C and assayed for δ-endotoxin concentration by SDS-PAGE. The samples were also analyzed for viscosity by spindle resistance measurement, for % separation by visual determination of amount of fluid on top and for pH by electrode conductance measurement. These tests were run on the stored samples at 6, 12, 24, 36 and 52 weeks unless there was significant loss of activity before this period at any given temperature.

Bioassays for δ-endotoxin and β-exotoxin were performed on some samples from each lot using Colorado Potato Beetle or fly, respectively as the test animals. Protocols for these assays were not included in the submission. The bioassays were performed on subsamples of each lot stored at 4°C and samples taken from a 5

gallon container stored at ambient temperature after at least 48 weeks. No initial determinations of IU/mg for each lot were submitted so all deterioration is referenced to the subsample stored for 48 weeks at 4°C and assuming no significant product loss at 4°C.

II. RESULTS

The study examining product degradation under temperature stress showed that all 4 lots rapidly lost δ-endotoxin when stored at 40°C as measured by SDS-PAGE. By 24 weeks, the SDS-PAGE results showed the 40°C incubated samples contained 11.3 to 26.5% of the activity of the 4°C stored product. Samples stored at 20°C, however, retained 84.8 to 99.0% of the δ-endotoxin activity after a year compared to the 4°C samples. The 4°C samples showed no significant decrease in SDS-PAGE toxin concentration. SDS-PAGE results ranged from a 13.4% decrease to a 25% increase in the tested lots after 52 weeks incubation. These results give some indication of the variability of the SDS-PAGE densitometry test for δ-endotoxin (stated by the company at ± 20%).

The Colorado Potato Beetle bioassay results at 48 weeks showed a 25% decrease to a 4% increase in IU/mg of δ-endotoxin in the product stored at ambient temperature in 5 gallon containers compared to the subsample stored at 4°C for the same period of time. No initial value of IU/mg of δ-endotoxin for the 4 lots was submitted. The fly bioassay for both the 4°C stored sample and the 5 gallon container at ambient temperature showed no detectable β-exotoxin either initially or after 52 weeks.

The tests for % separation under temperature stress showed little change in the samples from the 4 lots tested. After a year at 20°C all samples were 6.25% or less, well below the 15% considered unacceptable. The viscosities of the sampled lots remained acceptable (between 10,000 and 60,000 centipoise) throughout the incubation tests irregardless of storage temperature. Product pH in the samples of the 4 lots was also stable within the test duration remaining below pH 5.5 and never being above pH 4.95 at 40°C and 4.86 at 20°C.

The data on physical stability of the product in 5 gallon containers stored at ambient temperatures showed that while no significant deterioration occurred in pH and viscosity, the product did show unacceptable % separation at 24 weeks. Lots 82009 and 82784 were 27.0 and 27.8%; both above the stated 15% separation criterion. The same lots at 52 weeks showed acceptable results of less than 1.0% separation.

III. SACB DISCUSSION

The results of tests with samples stored at various temperatures and with 5 gallon containers at ambient temperature storage show that the M-One Bioinsecticide maintained adequate physical and biological performance characteristics when tested

after at least 24 or 48 weeks storage. None of the 4 lots sampled initially or at 52 weeks showed the presence of β-exotoxin by the fly bioassay. The lots were tested by bioassay against Colorado Potato Beetle after 48 weeks and shown to have a negligable 15% average activity loss compared to a sample stored at 4°C.

All lots showed adequate levels of δ-endotoxin, defined by the company as 9200 μg/gm, by SDS-PAGE assay [except for lots 82171 (7980 μg/gm) and 82784 (8480 μg/gm) at 36 weeks and stored at 20°C]. When the samples are compared for stability, they are normalized by comparison to the 4°C values. These values are shown as "not read or tested" for the 24 week sample (12/11/89) at 4°C in the data sheets but must have been taken as the normalized values are used in the figures.