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

CONFIDENTIAL

MAR - 4 1991

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SUBJECT: SACB review of data/information submitted by Mycogen Corporation to support the registration of MVP Bioinsecticide (MYX7275) [ID No. 53219-G; Record No. 254257; MRID Nos. 411997-1, 411997-2; HED Project No. 0-0120; Caswell No. 714G].

TO: Willie Nelson/Phil Hutton (PM-17)
Insecticide/Rodenticide Branch
Registration Division (H7505C)

FROM: Roy D. Sjoblad, Ph.D., Microbiologist
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Health Effects Division (H7509C)

RD Sjoblad

THROUGH: Reto Engler, Ph.D., Chief
SACB, HED (H7509C)

Background: On 4/25/89, SACB Completed a review for an EUP submission by Mycogen to use MYX 7275 in a non-crop destruct program. Outstanding issues at that time included dermal toxicity effects of the fixed, killed genetically engineered microbial pesticide, intravenous toxicity and mortality, quantitation of the chemical fixative in the product, and determination of dietary and applicator/worker exposure to the product - with emphasis on the fixative material. On 11/3/89, SACB received a Registration package for MYX 7275. On 10/16/90, 12/28/90, and 2/4/91 SACB received additional data/information submitted by Mycogen Corporation for the purposes of resolving all remaining outstanding toxicity/product characterization issues. SACB has completed review of these data packages, and Memorandums have been forwarded to the Registration Division. In the present Memorandum is reviewed the data and information submitted with the 11/3/89 Registration package. This information includes: a Confidential Statement of Formula (which may currently be outdated, since a subsequent modified CSF has been submitted); a description of beginning materials and manufacturing process; methods for confirmation of effectiveness of the fixation procedure in killing the engineered bacteria; quantification of delta-endotoxin protein in the product (for certification of limits); physical and chemical characteristics of MYX 7275 insecticide and of the manufacturing use product; and, methods for determination of contaminating organisms.

SACB Conclusion: The information provided on the upper and lower limits, and limit of certification ingredients, description of beginning materials and the manufacturing process, quantitation of delta-endotoxin, preliminary analyses of product samples for delta-endotoxin and for microbial contaminants and physical and chemical characteristics (except for corrosion characteristics -data to be submitted when available) are sufficient to support registration of MYX 7275.

The method proposed to evaluate the effectiveness of the killing procedure is inadequate because it did not include techniques and methods of analysis to evaluate whether colonies growing on Pseudomonas isolation agar or L-agar plates - after enrichment in liquid media or after direct plating of fixed samples - might be viable Pseudomonas spp.. Also, the proposed procedure did not include methods to confirm whether bacterial colonies are the viable engineered bacterium. SACB believes that if the viable engineered bacterium is detected on any of the plates, then it should be assumed that the fixing technique was inadequate, and that these batches should not be used in the environment.

The Registrant should clarify the statement that it is "...theoretically possible that the enrichment sample could become contaminated with the Pseudomonas fluorescens containing the [redacted] during the processing of the sample" giving a false positive result.

Positive controls should be used for all batches, even those less than 750 ml.

The Registrant should discuss the rationale for the Tier II test, with respect to it being triggered because of ineffective killing of the [redacted] fixing time period.

The procedures for detecting viable engineered bacteria should be sufficiently reliable so that a "Product Standards Board" decision would not be needed.

Since there is no standard method for analyzing large scale fermentation batches for the presence of viable organisms, SACB believes that during production of early batches that more intensive analysis and sampling will be required for the large scale fermentations. As data are collected and confidence is gained in the effectiveness of the killing procedure, the number and intensity of sampling may be reduced as warranted.

[redacted]

The submitted report implied that Mycogen Corp. is considering modifying the genetic construct in the engineered bacterium to provide better control for the regulation of delta-endotoxin production. If Mycogen Corporation modifies the genetic construct or uses different constructs, the Agency should be notified prior to using these products in the environment.

The following data/information were submitted in the volumes for Registration of MYX 7275:

61-1. Product identity and disclosure of ingredients.

Mycogen states that the following should be added to previously submitted data (i.e., to MRID 40897401, submitted 11/1/88; and to Supplemental data submitted 5/10/89).

[redacted]

SACB Discussion: SACB is not sure why the registrant included this information, since no specific details were provided. Nevertheless, if Mycogen Corporation modifies the genetic construct in the recipient Pseudomonas or uses different constructs, such information must be submitted to the Agency so as to determine any potential for health effects, or for requirement of additional testing.

61-2. Description of beginning materials and manufacturing process.

SACB (in the 4/25/89 Memorandum) requested a description of the fermentation parameters (i.e., medium, pH, temperature, fermentor size and materials; details of the fixative concentration, and when it is added in the process; quality control methods for delta-endotoxin concentration; and, whether an MP is isolated, stored, or registered.

[redacted]

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

Bacillus Thuringiensis

Page _____ is not included in this copy.

Pages 4 through 9 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

✓ Description of the product manufacturing process.

✓ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

_____ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

SUMMARY OF DATA SUBMITTED BY MYCOGEN CORPORATION WITH THE APPLICATION FOR REGISTRATION OF MYX 7275 INSECTICIDE*

Product Chemistry, Guideline Nos. 61-1 through 61-3
Contains Confidential Business Information concerning product identity and disclosure of ingredients, description of beginning materials and manufacturing process. Also specificies that no unintentional chemical ingredients, impurities, side products, human or other non-target animal pathogens are present in the product.

Product Chemistry, Guideline Nos. 62-1 through 62-3
Contains Confidential Business Information concerning preliminary analysis of product, certificate of limits of active ingredient and analytical methods to verify certified limits.

Product Chemistry, Guideline Nos. 63-2 through 63-21 and 64-1
Physical and Chemical characteristics of the product which are presented are color, physical state, odor, density, pH, flammability, storage stability, viscosity and corrosion characteristics.

Acute Oral Toxicity in Rats (Technical), Guideline Nos. 81-1/152-30
The acute oral LD_{50} for MYX 7275 (technical) as indicated by the data is greater than 5050 mg/kg (4.63 ml/kg), the highest dose tested, when administered undiluted to albino rats.

Acute Oral Toxicity in Rats (Flowable), Guideline Nos. 81-1/152-30
The acute oral LD_{50} for MYX 7275 (flowable) as indicated by the data is greater than 5050 mg/kg (4.76 ml/kg), the highest dose tested, when administered undiluted to albino rats.

Acute Dermal Toxicity in Rats (Technical), Guideline Nos. 81-2/152-31
The acute dermal LD_{50} for MYX 7275 (technical) as indicated by the data is greater than 2020 mg/kg (1.85 ml/kg), the highest dose tested, when administered undiluted to albino rats.

Acute Pulmonary Toxicity in Rats (Technical), Guideline Nos. 81-3/152-32
The acute pulmonary LD_{50} for MYX 7275 (technical) as indicated by the data is greater than 0.1 ml. per animal, the highest dose tested, or approximately 10^8 nonviable cells per animal when administered undiluted to albino rats.

Acute Intravenous Toxicity in Rats (Technical), Guideline Nos. 152-33
The acute intravenous LD_{50} for MYX 7275 (technical) as indicated by the data is greater than 0.05 ml. per animal, the highest dose tested, or approximately 10^8 nonviable cells per animal when administered as a 1:100 dilution in sterile water to albino rats.

Primary Dermal Irritation in Rabbits (Technical), Guideline Nos. 81-5/152-34
MYX 7275 technical produced mild dermal irritation with a primary irritation score of 2.96 when tested in albino rabbits and is not considered a primary irritant.

Primary Dermal Irritation in Rabbits (Flowable), Guideline Nos. 81-5/152-34
MYX 7275 flowable produced mild dermal irritation with a primary irritation score of 2.00 when tested in albino rabbits and is not considered a primary irritant.

Eye Irritation in Rabbits (Technical), Guideline Nos. 81-4/152-35
MYX 7275 technical was rated as minimally irritating in nonwashed eyes of albino rabbits when administered as 0.1 ml. of a 50% v/v solution of the test material in sterile water. The maximum average irration score was 3.7 and the test material was categorized in Toxicity Category III.

Eye Irritation in Rabbits (Flowable), Guideline Nos. 81-4/152-35
MYX 7275 flowable was rated as minimally irritating in nonwashed eyes of albino rabbits when administered as 0.1 ml. of undiluted test material. The maximum average irritation score was 1.3 and the test material was categorized in Toxicity Category III.

Dermal Sensitization in Guinea Pigs (Flowable), Guideline Nos. 81-6/152-36
MYX 7275 was categorized as a mild sensitizer when administered undiluted to albino guinea pigs. Average skin reaction scores ranged from 0 to 0.6 on a scale of 0 to 4.0.

Avian Oral Toxicity and Pathogenicity in Mallards (Technical), Guideline Nos. 71-1/154-16
MYX 7275 showed no apparent pathogenicity or effect upon survivial of young mallards when administered by oral gavage at 1.0% (v/w) of body weight for five days.

Acute Oral Toxicity Study in Bobwhite Quail (Technical), Guideline Nos. 71-1/154-16
The acute oral LD_{50} value for MYX 7275 in bobwhite quail was determined to be greater than 2500 mg/kg, the highest dose tested. The no-observed-effect dosage was 2500 mg/kg.

Dietary LC_{50} Study with the Mallard (Technical), Guideline No. 71-2
The dietary LC_{50} value for MYX 7275 in the mallard was determined to be greater than 5620 ppm, the highest dose tested. The no-observed-effect dosage was 5620 ppm.

Acute Toxicity Study in Rainbow Trout (Technical), Guideline Nos. 72-1/154-19
The 96-hour LC_{50} value for MYX 7275 in rainbow trout was empirically estimated to be 100 mg/1, the highest dose tested. The no observed-effect concentration was 100 mg/1.

Acute Toxicity Study in Bluegill (Technical), Guideline Nos. 72-1/154-19
The 96-hour LC_{50} value of MYX 7275 in bluegill was empirically estimated to be greater than 100 mg/1, the highest dose tested. The no-observed-effect concentration was 100 mg/1.

Acute Toxicity Study in Daphnids (Technical), Guideline Nos. 72-2/154-20
The 48-hour EC_{50} value of MYX 7275 in daphnids was empirically estimated to be greater than 100 mg/1, the highest dose tested. The no-observed effect concentration was 100 mg/1.

Acute Contact Toxicity Study in Ladybird Beetles (Technical), Guideline Nos. 143-1/154-23
The contact LD_{50} value of MYX 7275 technical in the ladybird beetle was determined to be greater than 511 micrograms/beetle.

Acute Contact Toxicity Study in Green Lacewing Larvae (Technical), Guideline Nos. 143-1/154-23
The contact LD_{50} value of MYX 7275 technical in the green lacewing larva was determined to be greater than 511 micrograms/larva.

Acute Contact Toxicity Study in Parasitic Hymenoptera (Technical) Guideline Nos. 143-1/154-23
The contact LD_{50} value of MYX 7275 technical in the parasitic wasp was determined to be greater than 511 micrograms/wasp.

Acute Contact Toxicity Study in the Honey Bee (Technical) Guideline Nos. 141-1/154-24
The contact LD_{50} value of MYX 7275 technical in the honey bee was determined to be greater than 511 micrograms/bee.

Non-target Plant Germination/Emergence Study (Technical) Guideline Nos. 122-1/154-22
MYX 7275 has no adverse effect on plant vigor when applied as a seed or pre-emergence treatment. There were no observable effects on seedling germination or emergence of field corn, cabbage, ryegrass, onion, cucumber, lettuce, oats, soybeans, tomatoes and carrots.

Non-target Plant Vigor Study (Technical), Guideline Nos. 122-1/154-22
Treatment of emerged seedlings with MYX 7275 resulted in no plant mortality. MYX 7275 did not adversely affect the number of plants, the height of the seedlings or the weight of the seedlings of any test species.

* **This summary is releasable to the public after registration in accordance with 40 CFR Sec. 152.119.**