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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Review of Mycogen Engineered *Pseudomonas* Manufacturing Provisions

TO: Phillip O. Hutton, PM 17
Insecticide/Rodenticide Branch
Registration Division (H7505C)

FROM: William R. Schneider, Ph.D. 4/26/91
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Mycogen Corporation has submitted two applications for registration of a *Pseudomonas fluorescens* that has been genetically engineered to express two types of delta endotoxin genes from *Bacillus thuringiensis*. They have eliminated the risk concerns involving the exposure properties of a living microorganism by killing the bacteria. Mycogen first contacted the Agency December 11, 1986 to determine if their killed product was subject to the requirements for notification prior to conducting small scale field testing. The Office of Pesticide Programs (OPP) worked with Mycogen to develop an acceptible method for evaluating the product to determine if it was completely killed. After Mycogen demonstrated the efficacy of their kill method to the Agency's satisfaction, the Agency issued a decision that Mycogen's killed microbial product was exempt from the requirements for notification. Mycogen later applied for Experimental Use Permits. Two were granted in May, 1989, and three more in May, 1990. The current registration applications are for *Pseudomonas* containing *B.t.* delta endotoxin genes, one active against lepidopteran insects and the other active against coleopteran insects. The containment provisions apply equally to each product.

When Mycogen submitted the applications for registration of the killed *Pseudomonas* genetically engineered to contain *Bacillus thuringiensis* delta endotoxin genes, OPP decided that the greater potential exposure of commercial use warranted a reevaluation of the method developed by Mycogen for killing the bacteria and for containing the live bacteria during the manufacturing process. The microorganism that was originally produced in 1 liter fermentors could now be produced in industrial fermentors up to [redacted] volume.

Mycogen has developed a proprietary method to kill the microorganisms in the fermentation tank. HED has reviewed the data submitted for evaluation of that method and has found that the method is sufficient to kill all the *Pseudomonas*. The Health Effects Divison (HED) of OPP has required that method of killing the bacteria in the fermentation tank be modified in accordance with the data submitted (memo, Sjoblad to Hutton, April 15, 1991). This specific modification is described in the confidental attachment to this scientific review. Mycogen constantly monitors the pH of the fermentor tanks which is sufficient to show that the kill procedure is being properly implemented.

As a final check on the function of their kill procedure, Mycogen has developed a method for analysing a 1 liter sample taken from the fermentation tank. HED has recommended analysing a number of smaller samples rather than the one 1 liter sample. This would allow for a better check that the conditions within the fermentor tank were uniform and the cells were equally exposed to the components of the killing method. This would require a revision of the statistical analysis, which could be provided to us at a later date. As described above, OPP is confident that Mycogen's killing methodology is sufficient to kill the cells. This method is being monitored by recording the tank pH to ensure the proper technique was actually performed correctly. The biological monitoring is a supplementary procedure to verify cell kill and, as suggested by HED, can best be performed by taking multiple samples (measuring viability and pH) as an indicator of homogeneity within the fermentation tank. OPP believes that the solution will be homogeneous because the growth medium is very well mixed in industrial fermentation tanks which have efficient rotating agitators and are aerated by large volumes of air pumped through the tanks, but the modified monitoring method will serve as a final check. For the final record, OPP would like to have a statistical evaluation of this revised method. We recommend that this be done using actual production data.

Mycogen did not fully describe the criteria it intends to use to verify if a colony grown up from their sampling is *Pseudomonas* or not. This is not difficult but it needs to be part of their procedure for the record.

In summary, the following issues need to be addressed:

1. Mycogen must revise their kill protocol to incorporate the HED suggestions (memo, Sjoblad to Hutton, April 15, 1991). (see CBI attachment)

2. The monitoring method must be revised to include at least 10 samples,

totaling 1 liter, to be grown in enrichment media as before, but to include pH measurements of the samples, colonial descriptions, and turbidity of the enrichment broth. This revised method must be analysed statistically. In addition, Mycogen must develop detailed criteria for verifying if a colony is *Pseudomonas* or not.

It should be noted that the statistical evaluation of the revised monitoring method may require data from production and can be submitted at a later date. The revision to the cell kill method, the revisions to the monitoring method, and the increased record keeping may be specified as part of the registration. The colonial verification criteria should be submitted prior to granting the registration.

Bacillus Thuringiensis..... A.F.6409

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_____ Identity of product inert ingredients.

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✓ 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.

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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.