7-13-94

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

JUL 13 1994

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of Product Identity, Hypersensitivity Incidents and Human Cell Culture Studies with Codling Moth Granulosis Virus Submitted by the University of California and the Association for Sensible Pest Control

TO: Linda Hollis (PM-18)
Insecticide-Rodenticide Branch
Registration Division (7505C)

FROM: John L. Kough, Ph.D., Biologist
Biological Pesticides Section
Science Analysis Branch
Health Effects Division (7509C)

THROUGH: Roy D. Sjoblad, Ph.D., Section Head
Biological Pesticides Section
Science Analysis Branch
Health Effects Division (7509C)

DATA REVIEW RECORD

Active Ingredients:	Granulosis virus of *Cydia pomonella*
Product Name:	Specific T-1
ID No:	058042-R Specific-T-1
Submission No:	S462553
Chemical No:	129090 Codling moth granulosis virus
DP Barcode:	D204292
MRID:	431612-01- Analysis of samples, certification of ingredient limits
	431612-02- Cell culture tests with viral pest control agents
	431612-03- Hypersensitivity incidents with microbial pest control agents

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THIS DOCUMENT CONTAINS FIFRA CONFIDENTIAL BUSINESS INFORMATION

ACTION REQUESTED

To review the data submitted on product identity, analysis of samples and the human cell culture studies and the revised CSF to determine if this is adequate to support the registration of Specific-T-1 or *Cydia pomonella* granulosis virus (CpGV).

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BACKGROUND

Dr. Falcon has been involved with the Agency for some time registering his entomopathogenic viruses. The last review of this product (memorandum from C. Schaffer to L. Hollis, Aug. 24, 1993) indicated that acceptable acute oral, intravenous and pulmonary toxicity/pathogenicity as well as dermal toxicity and eye irritation studies were submitted. However, certain aspects of the product identity were lacking and the required cell culture study and report on hypersensitivity incidents to be submitted.

SUMMARY OF REVIEWS

431612-01- The analysis of 5 batches indicates that the dry diet ingredients makeup an average of

CLASSIFICATION: Supplementary. This study can be classified acceptable with submission of the 5 batch analyses that justify the GIB/ml value.

431612-02- Cell cultures of human lung (WI-38), skin (WS-1) and a hepatocellular carcinoma (HepG2) were challenged with 2 x 10^9 particles/ml of CpGV over a 1 hour exposure then rinsed. The treated cells showed no significant cytopathic or toxic effects from the virus treatment when examined for cellular adhesion, cellular proliferation or cellular morphology.
CLASSIFICATION: Supplementary. This assay can be upgraded to acceptable with submission of insect bioassay data for all the dilutions of the viral inoculum used to determine the dose and a summary of the historic insect bioassay data used to convert insect mortality into virus particles/ml.

431612-03- No reports of hypersensitivity have been found in the 10 years of production and handling the CpGV in Dr. Falcon's Insect Pathology Group laboratory at the University of California. Some members of the group have been exposed for 20 years without hypersensitive response. Literature references are given for the lack of hypersensitive reaction reports for baculoviruses in general.
CLASSIFICATION: Acceptable.

DATA EVALUATION REPORT

Reviewed by: John L. Kough, Ph.D., Biologist, SAB/HED

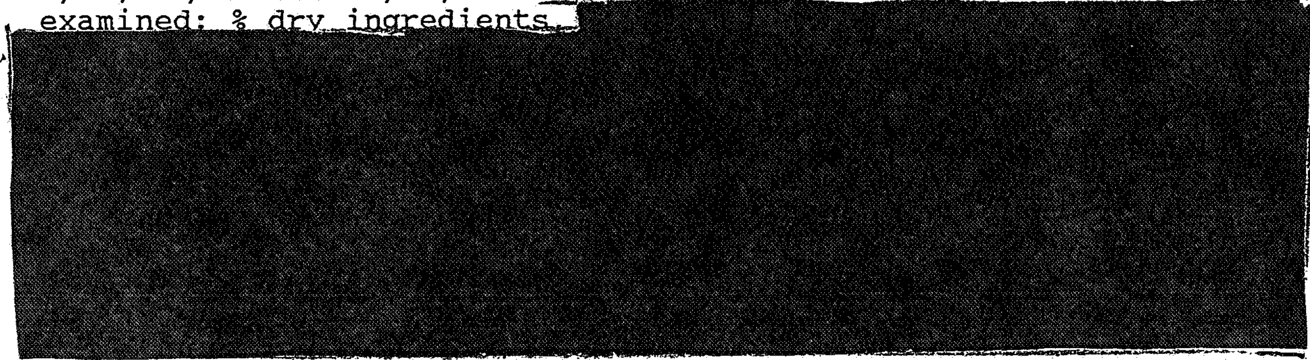
Secondary Reviewer: Roy D. Sjoblad, Ph.D., Microbiologist, SAB/HED

STUDY TYPE:	Analysis of Samples and Certification of Limits (151A-10)
MRID NO:	431612-01
CHEMICAL NO:	129090 Coddling moth granulosis virus
TEST MATERIAL:	Specific-T-1 (CpGV)
STUDY NO:	IR4 PR No. 1B, UCB: UCB-87, V3
SPONSOR:	University of California, Berkeley, CA & Association for Sensible Pest Control, Clayton, CA
TESTING FACILITY:	Insect Pathology Group, Dept of Entomological Sciences, University of California, Berkeley, CA & Consulting Diagnostic Service, Berkeley, CA
TITLE OF REPORT:	Analysis of Samples and Certification of Ingredient Limits Amendment No. 1 to MRID No. 42561102- Analysis of 5 End-Use Product Batches.
AUTHOR:	Louis A. Falcon, Ph.D. & Arthur Berlowitz, M.S.
STUDY COMPLETED:	March 7, 1994
CONCLUSION:	The analysis of 5 batches indicates that the dry diet ingredients makeup an average of

CLASSIFICATION:	Supplementary. This study can be classified acceptable with submission of the 5 batch analyses that justify the [redacted] GIB/ml value.

PRODUCT IDENTITY

Five batch analysis: The 5 batches, identified as batch 3/91; 4/91; 5/91; 6/91 and 3/93, show little variation in the endpoints examined: % dry ingredients,



A determination of bacterial contamination for the 5 batches showed the presence of *Bacillus pumilus* in all batches with a mean value

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

INERT INGREDIENT INFORMATION IS NOT INCLUDED

of 1.3 x 10^5 bacteria/ml (range=1 x 10^5 to 3.6 x 10^5) and a *Streptococcus* species (probably *S. faecalis*) at 9 x 10^5 bacteria/ml in the 3/91 batch. There was an additional letter indicating how the bacterial isolates were identified from the test results and the indication that *Serratia liquefaciens* was found in a previous batch (as reported in the memorandum from C. Schaffer to L. Hollis, June 24, 1993).

There was no reference to the amount of the active ingredient present in any of the 5 batches except to indicate that it was not less than 5.3 x 10^{10} particles per ml.

Confidential statement of formula: The indicated levels of the active ingredient are [redacted] granulosis inclusion bodies (GIB's) per ml (0.0013% by weight) with at least [redacted] GIB's/ml. The inert ingredients include [redacted]

It should be noted that either of these antibiotics can be added after processing the [redacted]

SAB COMMENTS
It is unclear why the registrant would fail to provide the range of values derived from insect bioassays to justify the active ingredient claims on the CSF. Why the registrant separated the [redacted] and dry diet ingredients in the 5 batch analysis and not in the CSF is also unclear.

SAB feels it would simplify the CSF and be more appropriate to bulk the GIB's/ml along with the [redacted] these are the source of the virus. The component would then be "CpGV and associated [redacted] since the virus is never really separated from the inerts for commercial use.

INERT INGREDIENT INFORMATION IS NOT INCLUDED

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

DATA EVALUATION REPORT

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

STUDY TYPE:	Cell culture toxicity/infectivity (152A-16)
MRID NO:	431612-02
CHEMICAL NO:	129090 Coddling moth granulosis virus
TEST MATERIAL:	Specific-T-1 (CpGV)
STUDY NO:	NVP Report NO. Z3K001G; UCB-CELL; IR-4 PR NO.1B
SPONSOR:	University of California, Berkeley, CA & Association for Sensible Pest Control, Clayton, CA
TESTING FACILITY:	Insect Pathology Group, Dept. of Entomological Sciences, University of California, Berkeley, CA & Northview Pacific Laboratories, Berkeley, CA
TITLE OF REPORT:	Cell Culture Tests with Viral Pest Control Agents (Tier I) Concurrent Dose Bioassays
AUTHORS:	Louis A. Falcon, Ph.D., Arthur Berlowitz, M.S. & Tania L. Weiss, Ph.D (NPL).
STUDY COMPLETED:	March 7, 1994
CONCLUSION:	Cell cultures of human lung (WI-38), skin (WS-1) and a hepatocellular carcinoma (HepG2) were challenged with 2 x 10^9 particles/ml of CpGV over a 1 hour exposure then rinsed. The treated cells showed no significant cytopathic or toxic effects from the virus treatment when examined for cellular adhesion, cellular proliferation or cellular morphology.
CLASSIFICATION:	Supplementary. This assay can be upgraded to acceptable with submission of insect bioassay data for all the dilutions of the viral inoculum used to determine the dose and a summary of the historic insect bioassay data used to convert insect mortality into virus particles/ml.

STUDY DESIGN

Test substance: Non-occluded preparations of the Granulosis Virus of *Cydia pomonella* (CpGV) at high dose levels (multiple insect LD_{50}'s) were used to inoculate the human cell cultures as these are the most infective form of the virus.

Test human cell lines: Three cell lines were used to examine the toxicity/ infectivity of the CpGV preparation: WI-38, a human fibroblast lung cell line that is adherent and of a normal diploid karotype of 46; WS1, a cell line derived from embryonic skin cells with a normal diploid karyotype; and HepG2 a cell line derived from a human hepatocellular carcinoma that does not produce tumors in nude mice, does not contain hepatitis B but does have an aberrant chromosome number of 55.

STUDY METHODS

Viral inoculum production: The occluded form of the virus was extracted from infected codling moth larvae probably by maceration as describe for the normal production procedure. This extract was further purified to concentrate virus by 2 sucrose density centrifugations. The first run was a slow speed (1500 rpm for 5 min.) 40% sucrose solution is remove gross cellular debris. The second run was a 40-63% sucrose gradient at 25,000 rpm for 1 hour. The virus band from each tube was removed, washed in sterile distilled water and pelleted at 15,000 rpm for 30 minutes. The pellets were resuspended in sterile distilled water.

The granule preparation of CpGV was treated with an alkaline saline solution (equal parts 1M NaCl and 1M $NaCO_3$) pH 10.3 for 1 minute to liberate the virions from the granulin occlusion. The solution was then immediately neutralized to pH 7.3 with 4 parts 1M HEPES buffer to 1 part virion suspension. The dissolved granulin protein was pelleted by centrifugation at 500 rpm for 3 minutes. The enveloped virion solution in the supernatant was then passed through a 0.45μm membrane filter to remove any gross bacterial contaminants and separated into 3 ml aliquots.

Insect bioassay to determine dose: Tenfold serial dilutions of the viral suspension were made in HEPES buffer. Two ml of each dilution were placed on the surface of insect diet in a plastic tub and allowed to dry. Thirty neonate codling moth larvae were placed with the inoculated diet in each tub and allowed to feed in the dark for 72 hours at 27°C. There were 3 tubs for each virus dilution and also controls including the HEPES buffer or distilled water.

Cell proliferation assay: Cell cultures were analyzed to determine if a colorometric assay could be used to quantify cell numbers during the assay. A CellTiter™ kit (Promega, Madison, WI) was used to determine the number of cells in the various treatments using the conversion of tetrazolium salt into a formazan crystal. The concentration of formazan crystal can be dissolved and spectrophotometrically determined by absorbance at 570nm.

The assay was tested for each cell line and found to have good linear correlation for color development and growth with initial cell concentrations between 25,000 to 50,000 cells/ml for WI-38, between 37,500 and 50,000 for WS1 and 50,000 and 85,000 for HepG2. These starting ranges were chosen because they represent the range where the colorometric assay could be used to detect effects of the virus on cell proliferation.

The cells were inoculated with a range of viral concentrations: 2 x 10^7/ml, 2 x 10^8/ml or 2 x 10^9/ml. In addition to the untreated controls, other cell cultures were treated with media or with 5 or 100mM sodium butyrate which is known to inhibit cell growth. Each treatment was done in at least 8 replicate wells.

Cell adherence assay: Another assay was done with cells inoculated

at 2 x 10^9 particles/ml after one days growth to allow vigorous growth and attachment. The inoculum remained for 1 hour then was replaced with fresh medium. The inoculated cells were incubated for 3 days then analyzed with an adherence assay. The adherence assay was done to determine if viral infection would affect the ability of the cells to attach to the culture plates. The cells were washed with phosphate buffered saline to remove unattached cells, then removed from the plates with a trypsin treatment and enumerated with a hemacytometer.

Cell morphology assay: This assay was done with 1 day old cells growing on cover slips and exposed to suspensions of 2 x 10^9 virus particles/ml. The inoculum remained for 1 hour then was replaced with fresh medium. The cells were allowed to proliferate for 3 more days then fixed in formalin for 10 minutes and stained with eosin-Y and hematoxylin. The cells were then observed under a microscope at 25X and 100X.

RESULTS

Insect bioassay of viral inoculum:
According to the registrant, insect bioassays previously done (not provided here) indicate a 1:100,000 dilution provides 50% mortality at 72 hours and contain 1 x 10^6 virus particles/ml. These non-occluded CpGV preparations show similar values (~50% mortality) at the 1:100,000 dilution and 100% mortality for the 1:10 dilution. Since the non-occluded virion preparation at a 1:100,000 dilution has 1 x 10^6 virus particles/ml, the registrant claims the undiluted solution has a concentration of 2 x 10^{10} virus particles/ml. A bioassay with the same inoculum suspension used to do the cell culture studies (and returned to the Insect Pathology Group laboratory after the experiment's termination) did not significantly decrease in potency over the course of the experiment compared to bioassays done at the start.

It should be noted that the human cell cultures inoculated and followed during the study were also returned but were not insect bioassayed since the registrant believed there was the possibility of obtaining a false positive result with these samples.

None of the results for the other viral dilutions (1:100, 1:1000 or higher) were included in the reported results. There was also no inclusion of or reference to the data used to generate the historical standard curve for CpGV which gave the LD_{50} dose equivalents.

Cell proliferation assay: There was no difference between the virus inoculated cells and the untreated controls for any of the 3 cell lines.The sodium butyrate treatment decreased cellular proliferation and was detectable by the colorometric assay as expected. While different assays were performed with similar doses of viral inocula, the cell seeding rates differed. Therefore the absorbance readings cannot be summed across assays. However, the

mean absorbance values (± the standard deviation) for the HEPES control overlapped the means for the virus treated cells in all the assays with WI-38 cells and the majority of assays with WS-1 and HepG2 cells. Two assays, one WS-1 and one HepG2, inoculated with 2 x 10^7 virus had treatment means that did not overlap with the HEPES control. In the WS-1 assay the HEPES control had a lower growth value than the virus treatment and in the HepG2 assay the HEPES control was higher. The coefficient of variation of the HEPES control in the HepG2 assay was over 35% so the usefulness of this control may be questionable.

Cell adherence assay: There was no difference between the HEPES treated and virus treated cells in this assay which compared the number of cells released from the culture plates after trypsin treatment. In the WS-1 assay the untreated control had twice the number of cells released compared to the other treatments.

Cell morphology assay: No differences were observed between the virus inoculated and untreated control cell cultures when examined for pathological effects at 25X or 100X. Photomicrographs were submitted that indicate similar gross growth characteristics of the cell cultures on the plastic cover slips.

SAB COMMENTS

This series of assays demonstrates that viral inoculation does not affect the growth or morphology of the exposed cell cultures. In the cell proliferation assay there were occasional assays that indicated a possible effect but considering the overall variation of the assay and detection system and the lack a dose response effect, it seems reasonable to conclude that no significant effects were seen.

DATA EVALUATION REPORT

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

STUDY TYPE:	Hypersensitivity Incidents Report (152A-15)
MRID NO:	431612-03
CHEMICAL NO:	129090 Coddling moth granulosis virus
TEST MATERIAL:	Specific-T-1 (CpGV)
STUDY NO:	IR-4 PR No. 1B; UCB: UCB-87
SPONSOR:	University of California, Berkeley, CA & Association for Sensible Pest Control, Clayton, CA
TESTING FACILITY:	Insect Pathology Group, Dept. of Entomological Sciences, University of California, Berkeley, CA
TITLE OF REPORT:	Baculovirus cydia pomonella Hypersensitivity Incidents with Microbial Pest Control Agents Statement of Finding No Hypersensitivity
AUTHOR:	Louis A. Falcon, Ph.D. & Arthur Berlowitz, M.S.
STUDY COMPLETED:	March 7, 1994
CONCLUSION:	No reports of hypersensitivity have been found in the 10 years of production and handling the CpGV in Dr. Falcon's Insect Pathology Group laboratory at the University of California. Some members of the group have been exposed for 20 years without hypersensitive response. Literature references are given for the lack of hypersensitive reaction reports for baculoviruses in general.
CLASSIFICATION:	Acceptable.

There have been no reports of hypersensitivity in the 10 years of production and handling the *Cydia pomonella* Granulosis Virus (CpGV) in Dr. Falcon's Insect Pathology Group laboratory at the University of California. This exposure includes the end-use product, infected larvae and diet containing the virus as well as bacteriostatic and fungistatic agents.

Citing the published literature, there have been no reports of hypersensitivity in persons involved in research with any baculovirus research although there have been positive antibody reactions in these same people to viral antigen. There has also been wide exposure to farmers and other workers under occupational conditions to natural epizootics of baculoviruses.

SAB COMMENTS

The serological reactions of workers exposed to large amounts of baculovirus is to be expected given the exposure. Cross reaction to another antigen does not explain this event. Moreover, SAB does not concur with the conclusions of Doller and Flehmig (1975) cited by the registrant that humans cannot acquire immunity to CpGV. Humans are apparently immune due to a lack of cellular recognition sites to allow entry and replication, not due to immune response means.