6-24-93

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

MEMORANDUM

24 JUN 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

Subject: SAB Review of a Request from Association for Sensible Pest Control for Support of the Registration of Baculovirus cydia pomonella (Submission No.:S439970; ID No.:058042-R; DP Barcode No.: D191527).

To: Phillip Hutton/ Linda Hollis (PM 18)
Insecticide-Rodenticide Branch
Registration Division (H7505C)

From: Cindy Schaffer, Microbiologist C. Schaffer
Biological Pesticides Section
Science Analysis Branch
Health Effects Division (H7509C)

Through: Roy Sjoblad, Ph.D., Acting Section Head
Biological Pesticides Section
Science Analysis Branch
Health Effects Division (H7509C)

Action: SAB has been asked to review product analysis and toxicology data submitted in support of the registration of Sensible Pest Control Inc.'s, SPECIFIC-T-1, with Baculovirus cydia pomonella as the active ingredient used to control Tortricid Species.

Discussion:

SAB is unable to support the registration of SPECIFIC-T-1 due to the following deficiencies:
Product Identity: 1. A five batch analysis was not presented as part of the analysis of samples by the registrant. 2. The specific impurities should be listed separately (i.e. components of [redacted]
This data would help if the registrant asked for a waiver request for toxicology requirements on the end-use product.

Toxicology data: 1. The registrant failed to submit table #3 from the acute dermal toxicity study. 2. Clinical observations were not submitted for the primary eye irritation study. 3. The registrant must evaluate the potential of the virus to infect, replicate, transform or cause toxicity, in a mammalian cell culture assay (see Subdivision M part 152A-15). 4. Any

Product impurity information has been waived

indications of hypersensitivity must be submitted to the Agency.

SUMMARY OF DATA SUBMITTED:

Product Identity (151A-10 - 151A-16):
SAB noted the following deficiencies: 1. A five batch analysis was not presented as part of the analysis of samples by the registrant. 2. The specific impurities should be listed separately (i.e. components of [redacted]). This data would help if the registrant asked for a waiver request for toxicology requirements on the end-use product.
CLASSIFICATION: UNACCEPTABLE - may be upgraded to acceptable with the submission of the above data.

Acute Oral Toxicity (152A-10):
A distinct clearance pattern was evident in the feces and heart/lungs through day 7 when rats were dosed with 2 x 10^{12} GIBs of *Baculovirus cydia pomonella*.
CLASSIFICATION: ACCEPTABLE - TOX CATEGORY IV

Acute Dermal Toxicity (152A-12):
SAB is unable to complete the review of this study since table #3 is missing from the report.
CLASSIFICATION: UNACCEPTABLE - SAB may upgrade this study to acceptable pending the submission of table #3.

Acute Intravenous Toxicity (152A-13):
Although the test microorganism was observed in most organs evaluated for clearance and infectivity, a distinct clearance pattern was observed through the end of the study. Based on the submitted data, an intravenous injection of 2 x 10^{12} GIBs of *Baculovirus cydia pomonella* was not infectious, pathogenic or toxic to rats.
CLASSIFICATION: ACCEPTABLE

Primary Eye Irritation (152A-14):
Although clinical observations were not specified and must be submitted by the registrant; *Baculovirus cydia pomonella* was not irritating to rabbit eyes when compared to those animals treated with sterile distilled water. Ocular irritation dissipated in both control and treated eyes by day 21.
CLASSIFICATION: UNACCEPTABLE - May be upgraded to acceptable with the submission of clinical observations.

Cell Culture Assay (152A-15):
The registrant failed to evaluate the potential of the virus to infect, replicate, transform or cause toxicity, in mammalian cell culture (see Subdivision M part 152A-15).
CLASSIFICATION: UNACCEPTABLE - The registrant must provide this assay.

Hypersensitivity Incidents (152A-15):
Any indications of hypersensitivity must be submitted to the Agency.

DATA EVALUATION REPORT

Reviewed by: Cindy Schaffer, Microbiologist, SAB/HED
Secondary Reviewer: J. Thomas McClintock, Ph.D., Microbiologist, SAB/HED

JTMc

STUDY TYPE:	Product Analysis Information
MRID NO:	425611-01; 425611-02; 425172-01
TEST MATERIAL:	Baculovirus cydia pomonella
SYNONYMS:	Codling Moth Granulosis Virus, SPECIFIC-T-1
PROJECT NO:	IR-4 PR No. 1B; UCB-87, V2, V3
SPONSOR:	Agricultural Experiment Station, University of California, Berkeley, CA; Association for Sensible Pest Control, Inc., Clayton, CA
TESTING FACILITY:	Insect Pathology Group, University of California, Berkeley, CA; Consulting Diagnostic Service, Berkeley, CA
TITLE OF REPORT:	Product Identity and Disclosure of Ingredients, Manufacturing Process and Discussion of the Formation of Unintentional Ingredients.; Analysis of Samples and Certification of Ingredient Limits.; Physical and Chemical Properties.
AUTHOR(S):	Louis A. Falcon, Ph.D., Arthur Berlowitz, M.S.
STUDY COMPLETED:	23 September 1992
CONCLUSION:	SAB noted the following deficiencies: 1. A five batch analysis was not presented as part of the analysis of samples by the registrant. 2. The specific impurities should be listed separately (i.e. components of [redacted] This data would help if the registrant asked for a waiver request for toxicology requirements on the end-use product.
CLASSIFICATION:	UNACCEPTABLE - may be upgraded to acceptable with the submission of the above data.

NOTE: This material contains CBI

PRODUCT ANALYSIS

151A-10 Product Analysis and Disclosure of Ingredients

Identity: The active ingredient of SPECIFIC-T-1 is Baculovirus cydia pomonella, a granulosis virus specific to the codling moth.

Confidential Statement of Formula: has been submitted. SPECIFIC-T-1 contains 0.0013% capsules of the granulosis virus (GV) of cydia pomonella (CpGV) as an insecticide; [redacted]

INERT INGREDIENT INFORMATION IS NOT INCLUDED

Information on Active Ingredient:

History: CMGV was initially isolated from dead codling moth larvae by L.E. Caltagirone in Chihuahua, Mexico, September 1963. CMGV has since been isolated from field collections in Hungary, USSR, and England and many laboratory cultures.

General Taxonomy: The Cydia pomonella granulosis virus, Mexican isolate, is a wild type baculovirus placed in the family Baculoviridae subgroup B. The virus, containing a large supercoiled double-stranded DNA genome, is embedded within a proteinaceous inclusion body, called granulin and has a molecular weight of 7.62×10^7.

Biological: During infection, the CMGV Mexican-isolate produces abnormalities in the fat body, tracheal matrix, hypodermis and malpighian tubules of codling moth larvae. As the infection proceeds, RNA synthesis increases, followed by the degradation of the nucleus and chromatin bodies and reduction of host RNA/DNA synthesis rates to normal and subnormal levels, respectively. A "virogenic stromate" is formed which involves a second resurgence of a relatively long duration of viral RNA and increased viral DNA synthesis. This virosis is the identifying feature of a CMGV infection.

Biochemical: Immunochemical analysis distinguishes the inclusion bodies of CMGV from at least 5 other granulosis viruses (Croizier & Meynadier, 1973).

Polyacrylamide gel electrophoresis:

Displays 11 bands of protein.

Electrophoresis:

1 heavy band and 5 light bands of protein are observed and are different than all other granulosis viruses analyzed (Etzel, 1974).

Restriction Profile:

No similarity was found between CMGV Mexican-isolate and other previously published GV profiles (Harvey & Volkman, 1983).

Genetic stability was displayed in the profiles of two CMGV Mexican-isolates propagated separately over a 9 year time frame (Harvey & Volkman, 1983).

Serological: Using antiserum produced in rabbits, the following serological tests are used for the characterization of CMGV:

1. A radioimmune protein blot assay determines the similarities between three different CMGV isolates (Harvey & Volkman, 1983).

2. An ELIZA, an enzyme linked immunoassay, was utilized to detect and enumerate CMGV. CMGV was observed above background levels of 5×10^5 Granulosis Inclusion Bodies (GIBs).

Active ingredient characterization:

Inerts: [redacted] of the component formulation. The inerts consist of [redacted]

Manufacturing Process (151A-11)

[redacted]

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

INERT INGREDIENT INFORMATION IS NOT INCLUDED

Unintentional Ingredients (151A-12)

None of the ingredients present in any phase of the manufacturing process of SPECIFIC-T-1 are known irritants or toxic in nature. Baculovirus cydia pomonella has a very narrow host range restricted to a few insect species within a family. The possibility of producing mutant strains capable of infecting any other organisms is non-existant. Three bacteria are commonly present in the end-use product (Note: The registrant did not specify quantities of these microorganisms). Streptococcus fecalis and Bacillus pumilus are not pathogenic; and Serratia liquifaciens which is indigenous to water, soil, plants, animal digestive tracts, has been reported, under certain conditions, to be an opportunistic pathogen on rare occasions.

Analysis of Samples (151A-13)

Each production batch is analyzed for viable Baculovirus cydia pomonella by a standard potency bioassay. The viral particles per ml are measured by the number of serial dilutions required to obtain the larval LD_{50}, containing 1 x 10^6 GIBs/ml, at 72 hours post exposure.

The end-use product batch is evaluated for bacterial contamination by plating serial dilutions on nutrient agar and Tergitol-7 +TTC agar.

The gradient purified capsule protein is evaluated by an enzyme immunoassay.

Impurities:
The approximate composition limits of impurities are: [redacted]

Certification of Limits (151A-15)
The formulated product is reported to contain between 0.0011% and 0.0015% B. cydia pomonella: [redacted]

Physical and Chemical Properties (151A-16)

COLOR:	Brown
PHYSICAL STATE:	Semi-solid suspension
ODOR:	Musty
SPECIFIC GRAVITY:	1.06
pH:	7.0
STABILITY:	Degradation of capsule protein begins at pH 8.0 Photodegradation of DNA by ultraviolet light (254 - 390 nm)

INERT INGREDIENT INFORMATION IS NOT INCLUDED

STORAGE STABILITY:	At least 10 years at -70°C.
VISCOSITY:	1.1 cP at 20°C.
MISCIBILITY:	Miscible in water
CORROSION CHARACTERISTICS:	None

DISCUSSION: The following deficiencies were noted:

1. A five batch analysis was not presented as part of the analysis of samples by the registrant.
2. The specific impurities should be listed separately (i.e. components of [redacted]. This data would help if the registrant asked for a waiver request for toxicology requirements on the end-use product.

Product impurity information has been removed

DATA EVALUATION REPORT

Reviewed by: Cindy Schaffer, Microbiologist, SAB/HED
Secondary Reviewer: J. Thomas McClintock, Ph.D., Microbiologist, SAB/HED

J. TMC

STUDY TYPE:	Acute Oral Toxicity/Pathogenicity-Rat(152A-10)
MRID NO:	425172-02
TEST MATERIAL:	Baculovirus cydia pomonella
SYNONYMS:	Codling Moth Granulosis Virus
PROJECT NO:	UCB: ORAL-87; IR-4 PR No. 1B; UCD: 2450
SPONSOR:	Association for Sensible Pest Control, Inc.; University of California at Berkeley
TESTING FACILITY:	Insect Pathology Group UC Berkeley Dept. of Entomological Sciences, Berkeley, CA; Animal Resource Service, UC Davis, Davis, CA
TITLE OF REPORT:	Baculovirus cydia pomonella Acute Oral Toxicity/Pathogenicity Study (Tier I) in Rats.
AUTHOR(S):	Louis A. Falcon, Ph.D, Arthur Berlowitz, M.S., Regina Housley D.V.M.
STUDY COMPLETED:	23 September, 1992
CONCLUSION:	A distinct clearance pattern was evident in the feces and heart/lungs through day 7 when rats were dosed with 2 x 10^{12} GIBs of Baculovirus cydia pomonella.
CLASSIFICATION:	ACCEPTABLE- TOX CATEGORY IV

I. STUDY DESIGN

Test Material: The viral pest control agent is Baculovirus cydia pomonella Mexican Isolate 1964 in water. The potency of the dosing solution was determined to be 4 x 10^{11} (granulosis inclusion bodies) GIBs/ml. Each test animal received a 5.0 ml dose by oral gavage.

Test Animals: Eighteen male and eighteen female CD-1 rats were obtained from Charles River Laboratory Inc. The male rats weighed between 164g and 200 and the female weights ranged from 154g to 180g at the beginning of the study.

Methods: The animals were assigned to two groups of nine male and nine females each. The control group (untreated) was dosed with the "control material" (not described by the registrant) and the test substance was administered to the treatment group. The rats were randomly weighed on the initial day of dosing and weekly thereafter. Treated animals were observed for signs of toxicity daily. Feces and urine samples were collected on days 3, 4, 6, 8 and 15, and 22. An unspecified number of animals were sacrificed by exanguination on days 8, 15, and 22. The animals were examined by necropsy for any macroscopic abnormalities. Samples of the gastro-intestinal tract, blood, heart, lungs, kidney,

liver, spleen, feces and urine were evaluated for the presence of viable GIB observed in the organs by incorporating the animal tissues into the codling moth diet at a ratio of 1:10. One neonate codling moth larvae was placed in each cup (in triplicate), which contained a camel's hair paint brush and a known quantity of spiked diet, and was incubated at 27.5 C until the larvae died or reached the adult stage of development. The dead larvae were individually examined for the presence of baculovirus cydia pomonella. Positive and negative control tissues were also evaluated.

II. RESULTS

A. Body Weights:
No abnormalties were noted in body weights during the study.

B. Clinical observations:
No clinical signs of toxicity were observed.

C. Necropsy observations:
No abnormalities were noted upon necropsy.

D. Viral clearance/infectivity:
Clearance and infectivity were evaluated in the gastro-intestinal tract, blood, heart, lungs, kidney, liver, spleen, feces and urine were evaluated for the presence of viable GIB observed in the organs. The submitted data (see below) demonstrated that the organism was detected in the feces through day 5 and in the heart/lungs on day 7.

ORGAN	DAY	CFU/100 ML OF DIET
Feces	2	1×10^{10}
	3	1×10^{10}
	5	1×10^{4}
Lungs	7	1×10^{3}

III. SAB DISCUSSION:

A distinct clearance pattern was shown in the feces and heart/lungs through day 7.

DATA EVALUATION REPORT

Reviewed by: Cindy Schaffer, Microbiologist, SAB/HED
Secondary Reviewer: J. Thomas McClintock, Ph.D., Microbiologist, SAB/HED

STUDY TYPE:	Acute Dermal Toxicity-Rabbit(152A-11)
MRID NO:	425172-05
TEST MATERIAL:	Baculovirus cydia pomonella
SYNONYMS:	Codling Moth Granulosis Virus (CMGV)
PROJECT NO:	NVP Report No. XOH001g; UCB: DERMTOX; IR-4 PR No. 1B
SPONSOR:	Agricultural Experiment Station, University of California, Berkeley, CA; Association for Sensible Pest Control, Inc., Clayton, CA
TESTING FACILITY:	Insect Pathology Group, University of California, Berkeley, CA; Northview Pacific Laboratories, Inc., Berkeley, CA
TITLE OF REPORT:	Acute Dermal Toxicity Testing (Tier I) with Rabbits.
AUTHOR(S):	Louis Falcon, Ph.D., Arhtur Berlowitz, M.S., M. J. Dennihan
STUDY COMPLETED:	23 September, 1992
CONCLUSION:	SAB is unable to complete the review of this study since table #3 is missing from the report.
CLASSIFICATION:	UNACCEPTABLE- SAB may upgrade this study to acceptable pending the submission of Table #3.

I. STUDY DESIGN

Test Material: The microbial pest control agent (MPCA) is Baculovirus cydia pomonella, Mexican strain 1964. The potency of the dosing solution was determined to be 2×10^{12} GIBs by neonate larval bioassay.

Test Animals: Five male and five female New Zealand rabbits were obtained from Elkhorn Rabbitry, Watsonville, CA. The male rabbits weighed between 2.2 kg and 2.4 kg and females weights ranged from 2.2 kg to 2.4 kg the day before dosing.

Methods: Approximately 24 hours prior to testing, no less than 10% of the trunk fur was clipped. The MPCA was administered, with water, over the prepared skin followed by gauze, and surgical tape. The trunk of each animal was wrapped to prevent the animals from disturbing the test site. Approximately 24 hours following application, all wrappings were removed and the skin was washed with water. The animals were observed for signs of toxicity several times during the first day post dosing and daily thereafter. The animals were evaluated for dermal irritation using the Draize

method. Body weights were recorded on day 0, day 7 and day 14.

II. **RESULTS**

A. Body Weights:

No abnormalities were noted in body weight gain during the study.

B. Dermal Irritation Scoring:

The table (table #3) describing dermal irritation was not included with this report.

III. **SAB DISCUSSION:** SAB is unable to complete the review of this study since table #3 is missing from the report.

DATA EVALUATION REPORT

Reviewed by: Cindy Schaffer, Microbiologist, SAB/HED
Secondary Reviewer: J. Thomas McClintock, Ph.D., Microbiologist, SAB/HED

STUDY TYPE:	Acute Intravenous Toxicity/Pathogenicity-Rat (152A-13)
MRID NO:	425172-04
TEST MATERIAL:	Baculovirus cydia pomonella
SYNONYMS:	Codling Moth Granulosis Virus
PROJECT NO:	UCB: IV-87; UCD: 2541; IR-4 PR No. 1B
SPONSOR:	Agricultural Experiment Station, University of California, Berkeley, CA; Association for Sensible Pest Control, Inc., Clayton, CA
TESTING FACILITY:	Insect Pathology Group, University of California, Berkeley, CA; Animal Resources Service, University of California, Davis, CA
TITLE OF REPORT:	Baculovirus cydia pomonella Acute Intravenous Toxicity/Pathogenicity Study (Tier I) in Rats.
AUTHOR(S):	Louis A. Falcon, Ph.D.; Arthur Berlowitz, M.S.; Regina Housley, D.V.M.
STUDY COMPLETED:	23 September, 1992
CONCLUSION:	Although the test microorganism was observed in most organs evaluated for clearance and infectivity, a distinct clearance pattern was observed through the end of the study. Based on the submitted data, an intravenous injection of 2 x 10^{12} GIBs of Baculovirus cydia pomonella was not infectious, pathogenic or toxic to rats.
CLASSIFICATION:	ACCEPTABLE

I. STUDY DESIGN

Test Material: The viral pest control agent (MPCA) is Baculovirus cydia pomonella, Mexican isolate 1964. The potency of the dosing solution was determined to be 2 x 10^{12} Granulosis Inclusion Bodies (GIBs)/aliquot. Each test animal received a 0.5 ml dose intravenously.

Test Animals: Twenty three male and twenty one female CD-1 rats (age not stated) were obtained from Charles River Laboratories, Inc.. The male rats weight ranged from 212 gm to 272 gm and the female weights ranged from 160 gm to 200 gm at the beginning of the study.

Methods: The animals were assigned to four groups as follows: The Group 1 animals were sacrificed two minutes post dosing and were comprised of one male and three female control rats, and two male and two females treated with the test substance; Group 2 and 3, were comprised of two controls and two

treated rats of each sex, were sacrificed 24 hours and 7 days post dosing, respectively; and Group 4 consisted of six male control rats, six treated males, four female control rats and four treated females, and were necropsied on day 21. The rats were randomly weighed on the day of initial dosing, weekly and at scheduled sacrifice times. All animals were observed for signs of toxicity daily. Rats of each sex in the treatment groups were sacrificed by exanguination. The animals were examined by necropsy for any macroscopic abnormalities. Samples of the blood, heart/lung, liver, kidney, spleen, brain and lymph nodes were evaluated for the presence of viable GIB's by incorporating the animal tissues into the codling moth diet at a ratio of 1:10. One neonate codling moth larva was placed in each cup (in triplicate), with a known quantity of spiked diet, and was incubated at 27.5 C until the larva died. Dead larvae were individually examined for the presence of Baculovirus cydia pomonella. Positive and negative control tissues were also evaluated for the presence of B. cydia pomonella GIBs.

II. RESULTS

A. Body Weights:

No abnormalities were noted in body weights.

B. Clinical Observations:

No clinical signs of toxicity were noted during the study.

C. Viral Clearance/Infectivity:

Clearance and infectivity of the test organism were evaluated in the blood, heart/lungs, liver, kidney, spleen, brain and lymph nodes. The submitted data (see below) demonstrated that although the organism was detected in all organs except the brain and kidney during the study; a distinct clearance pattern was evident.

ORGAN	DAY	MEAN GIBs/100 ML DIET
Blood	0	5×10^7
Heart/Lungs	0	8×10^3
	1	6×10^3
Liver	0	5×10^5
	1	1×10^7
	7	5×10^5
	21	4×10^3
Spleen	0	4×10^3
	1	1×10^5
	7	5×10^4
	21	6×10^3

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Lymph nodes	0	3×10^4
	7	1×10^3

D. Necropsy Evaluations:
No abnormalities were noted upon necropsy.

III. SAB DISCUSSION:
Although the test microorganism was observed in most organs evaluated for clearance and infectivity, a distinct clearance pattern was observed through the end of the study. Based on the submitted data, when administered intravenously, Baculovirus cydia pomonella was not infectious, pathogenic or toxic to rats.

DATA EVALUATION REPORT

Reviewed by: Cindy Schaffer, Microbiologist, SAB/HED
Secondary Reviewer: J. Thomas McClintock, Ph.D., Microbiologist, SAB/HED

STUDY TYPE:	Primary Eye Irritation-Rabbit(152A-14)
MRID NO:	425172-06
TEST MATERIAL:	Baculovirus cydia pomonella
SYNONYMS:	Codling Moth Granulosis Virus
PROJECT NO:	UCD: 113087; UCB: EYE-88; IR-4 PR No. 1B
SPONSOR:	Agricultural Experimental Station, University of California, Berkeley, CA; Association for Sensible Pest Control, Inc., Clayton, CA
TESTING FACILITY:	Insect Pathology Group, University of California, Berkeley, CA; Animal Resource Service, University of California, Davis, CA
TITLE OF REPORT:	Primary Eye Irritation/Infection Study (Tier I) with Rabbits.
AUTHOR(S):	Louis Falcon, Ph.D., Arthur Berlowitz, M.S., Regina Housley, D.V.M.
STUDY COMPLETED:	23 Sepember, 1992
CONCLUSION:	Although clinical observations were not specified and must be submitted by the registrant; Baculovirus cydia pomonella was not irritating to rabbit eyes when compared to those animals treated with sterile distilled water. Ocular irritation dissipated in both control and treated eyes by day 21.
CLASSIFICATION:	UNACCEPTABLE - May be upgraded to acceptable with the submission of clinical observations.

I. STUDY DESIGN

Test Material: The microbial pest control agent (MPCA) is Baculovirus cydia pomonella, Mexican Isolate 1964. The potency of the dosing material was determined to be 1.0×10^9 Granulosis Inclusion Bodies (GIB's)/0.1 ml from bioassay results.

Test Animals: Six New Zealand White rabbits were obtained from Charles Rivers Laboratories, Incorporated.

Methods: Each rabbit was restrained in a "cat bag" during the test procedure. Three rabbits received a single 0.1 ml dose of the MPCA administered into the conjuntival sac of the lower right eyelid; and three animals were given the same dose in the lower left eyelid. The eye lids were gently held together for a second to prevent a loss of material. The contralateral eye served as the control for each animal. At 24 hours post dose administration, each eye was stained with fluorescein dye and examined. The Draize Method

was used to score ocular lesions at 24 hours, and daily thereafter until ocular reactions cleared.

II. RESULTS

A. Clinical Observations/Eye Irritation Scoring:

CONJUNCTIVAE:

Redness: Moderate to severe redness was sporatically noted in 3 treated eyes up to day 15.

Slight to moderate redness was observed in three control eyes up to day 18.

Note: Clinical observations were not specified. SAB is assuming that redness was the only clinical sign noted during the study.

III. SAB DISCUSSION:

Although clinical observations were not specified and must be submitted by the registrant; Baculovirus cydia pomonella was not irritating to rabbit eyes when compared to those rabbits treated with sterile distilled water. Ocular irritation dissipated in both control and treated eyes by day 21.