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6-1-93

Reviewed by: J. Thomas McClintock, Ph.D., Microbiologist
Science Analysis Branch
Health Effects Division (H7509C)

JTMC 5/24/93

DATA EVALUATION REPORT

STUDY TYPE: Product Identity

MRID NO.: None Assigned CASWELL NO.: None Assigned

TEST MATERIAL: Capsules of Cydia pomonella granulosis virus (CpGV)

SYNONYMS: Cyd-X

STUDY NO.: FR91-13

SPONSOR: Espro Inc.
Oakland Center
8990 Route 108
Columbia, MD 21045

TESTING FACILITIES: For Restriction Enzyme Analysis:
University of Florida
Institute of Food and Agricultural Sciences
Entomology and Nematology Department
Gainesville, FLA 32611-0740

TITLE OF REPORT: Product Chemistry

AUTHOR: D. M. Kolodny-Hirsch

REPORT ISSUED: June 28, 1991

CONCLUSION: The requirements for Series 151A-10 and 151A-11 have been satisfied. The requirements for Series 151A-16 have been partially satisfied. The data and information needed to fulfill the requirements for the remaining studies in Series 151A are incomplete.

CLASSIFICATION: Unacceptable.

151A-10. Product Identity and Disclosure of Ingredients. (a). Product Identity. The active ingredient in Cyd-X is the capsules of the Cydia pomonella granulosis virus (CpGV). Although first isolated by Tanada in 1963, this particular strain was derived from a CpGV isolate provided by R. Jaques of Agriculture Canada. The technical grade material is an aqueous preparation containing 0.2% by weight of CpGV capsules. Several articles from the open literature were submitted by the registrant supporting the identity of the active ingredient (a.i.).

Espro Inc. submitted a single restriction endonuclease (REN) profile of DNA from both CpGV isolates (i.e. the isolate provided

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by Jaque compared to the CpGV isolate to be registered). CpGV DNA was purified on sucrose gradients, the enveloped nucleocapsids liberated using alkaline conditions, the DNA purified according to standard techniques which, in turn, was digested with BamHI and analyzed by agarose gel electrophoresis. It should be noted that the registrant failed to state if the restriction analysis was performed on Espro's CpGV isolated from a commercial preparation. This information must be submitted to the Agency.

The two CpGV BamHI restriction profiles were shown to be closely related but not identical suggesting genotypic variation and that the viral preparation was devoid of contaminating baculoviruses. Additional restriction profiles of GV isolates from the open literature were submitted using various enzymes (EcoRI, SmaI, XhoI, and ApaI). Such data is extremely valuable not only for the identification of CpGV, but to distinguish this viral pesticide from other baculoviruses used as microbial pest control agents. It should be noted that NO attempt was made to identify and quantitate the individual BamHI restriction fragments generated upon digestion or to analyze the CpGV DNA with another REN. This information would have provided molecular weights of the viral genome(s) and provided further evidence of the similarities and/or differences between the two isolates and other CpGVs.

(b). Confidential Statement of Formula (CSF). A CSF was provided by the registrant in support of this application. A revised CSF stating that the product contains capsules of C. pomonella granulosis virus as the active ingredient must be submitted. The aqueous suspension containing the active ingredient (i.e CpGV capsules) [redacted] can be considered the technical grade material.

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

[redacted]

151A-11. Manufacturing Process.

[redacted]

151A-12. Discussion of Formation of Unintentional Ingredients. To monitor the manufacturing and purity of this product the registrant

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states that "causes of death in the bioassay insects other than nuclear polyhedrosis are recorded and observations on surviving larvae are carried out until pupation. This procedure enables detection of possible contaminants." Presumably, the registrant is referring to deaths due to GV infection and NOT due to infection caused by nuclear polyhedrosis viruses (NPVs). No data or discussion were presented as to the type of other contaminants which would result in the death of the bioassay insects. This information should be submitted to the Agency. In addition, the registrant should provide information as to the susceptibility of codling moth larvae to NPVs.

The registrant also states that "seven different tests are performed simultaneously for the detection and enumeration of possible microbial contaminants." Although seven types of bacteria were listed no information was presented as to the specific test used for detection, identification and enumeration. This information must be submitted to the Agency. To detect toxic substances and pathogenic microorganisms the registrant appears to include an intraperitoneal injection of the test substance into mice as a quality control procedure. However no additional information or data was submitted to substantiate this control procedure. It should be noted that the registrant states that if mammalian pathogens are detected or if the bacterial contamination exceeds 10^9 CFU/gm the batch is disposed or "cleaned using appropriate methods." These "appropriate methods" must be discussed and the protocols submitted to the Agency.

151A-13. Analysis of Samples. The registrant states that "...the fundamental difference of this product from a traditional chemical renders this requirement inappropriate." This rationale is inappropriate, especially in light of the fact that the requirements for Series 151A-12 are inadequate and unacceptable. SAB agrees that analysis and identification of the insect inert components and/or impurities would be impossible; however, the registrant should provide a discussion on the methods and a statement of the precision and accuracy of the method used to analyze each sample or batch for the quantity of capsules which, in turn, allows for the determination of the certified limits.

151A-15. Certification of Ingredient Limits. A CSF was provided by the registrant stating the certified limits of Cyd-X.

The registrant states that the "procedure for sampling and enumerating granulin inclusion bodies of Cyd-X are similar to that outlined for a nuclear polyhedrosis virus in the article by Martignoni (1978)." This reference may in fact be adequate (reference not found); however this information must be presented and summarized especially if the procedure is modified.

To determine the certified limits for the active ingredient, five different lots of Cyd-X MUST be evaluated. Although the registrant states that the "...GIBs per ml shall not be less than $1x10^{11}$ final

product." and that the "LC_{50} against codling moth shall not be more than 1800 ng ..." NO data was submitted to support these statements. The method used for enumeration and/or quantification of the number of capsules per batch was not specified. The determination of the certified limits must be supported by an acceptable analytical method. This data/information must be submitted to the Agency.

The registrant also states that "No fecal coliform bacteria, or any other bacteria or other agents pathogenic for warm-blooded vertebrates are permitted as detected by culturing on differential bacteriological media, by intraperitoneal injection in mice and by oral administration to mice." Again, the registrant implies that specific methodologies are in place to assure QC/QA procedures. However, SAB recommends that each batch be analyzed by IP injection or oral feeding studies in mice. The registrant should specify the methods used to assure that each batch is void of contaminating pathogens.

151A-16. Physical and Chemical Properties. The following physical and chemical properties were submitted for the end-use product:

Property	Characteristics
Color	Light gray to light tan
Physical State	Liquid slurry
Odor	Faint, sharp, alcohol-like
Density (liquid)	1.016 g/ml, or 8.48 lb/gal (24.5°C) and 1.017 g/ml, 8.47 lb/gal (1°C)
pH	7.7
Viscosity	Required
Miscibility	Not required

Although the end-use product is not a liquid "...under recommended storage and transportation conditions..." the data and/or information on the viscosity of the material should be submitted to the Agency. Although the technical grade material is equivalent to the end-use product the viscosity of the subject product should be similar to water (compare densities to water).

Waivers were requested for Stability (151A-16[f]) and Storage Stability (151A-16[g]) studies. The following rationale was provided for each waiver request:

151A-16[f]. Several references were submitted by the registrant to support data waivers for stability. Specifically, various parameters were evaluated for their ability to inactivate both GVs and NPVs. One of the most important environmental factors considered was sunlight; whereby inactivation occurs due to the ultraviolet portion of the spectrum. Information was submitted on pH demonstrating that infectious virions, released from the occlusion bodies of NPVs, were inactivated at high alkaline (pH 12)

and low acidic conditions (pH 1.2). The interaction between temperature and pH was also noted. When virions were suspended in an alkaline solution (pH 11) no inactivation was noted at 21°C; however at elevated temperatures (40°C) the infectious virions were inactivated. Further data from the open literature was presented demonstrating thermal inactivation of baculoviruses at various temperatures.

151A-16[g]. The technical grade of the product, Cyd-X, is produced as an aqueous homogenate preparation and packaged in a non-corrosive, plastic-lined container. The proposed recommended storage is under refrigeration. For storage over long periods (i.e. more than a year) this material should be placed in a freezer. Information was submitted from the open literature to support the waiver request for stability. The stability (or shelf-life) of a freeze-dried CpGV preparation was about 2 weeks at -20°C with half of the virus activity being lost at 40 days. The greatest stability was noted for aqueous GV preparations.

It should be noted that storage stability, color, and odor information are no longer being routinely required for pesticide registration.