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

JUN 1 1993

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: SAB Review of a New Product Registration Application for Cyd-X, an Insecticidal Virus-Based Product (DP Barcode D172945; Submission No. S407410; I.D. No. 058971-U; Shaughnessy No. 122201)

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TO: Linda Hollis/Phil Hutton (PM-18)
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ACTION REQUESTED: Espro, Inc. (recently acquired by CGI, Inc.) has submitted an application for the registration of Cyd-X, a virus-based insecticidal product containing capsules of Cydia pomonella (the codling moth) granulosis virus (CpGV). With the exception of certain Product Chemistry studies (i.e. 151A-10 through -15, -17), waivers have been requested for the remaining portion of Series 151A (151A-16f and -16g) and for all toxicology data requirements as outlined in Subdivision M of the Pesticide Assessment Guidelines.

BACKGROUND INFORMATION: Granulosis viruses (Gvs), classified in the family Baculoviridae (Subgroup B), are structurally large and complex DNA-containing viruses infecting insects. Most Gvs display a narrow, if not, specific host range. In fact, CpGV has only been shown to infect another Cydia spp. (C. nigricana, the pea moth) and three other closely related genera (the Oriental fruit moth, Grapholitha molesta; the European pine shoot moth, Rhyacionia buoliana; and the false codling moth, Cryptophebia leucotreta) in the family Tortricidae. After ingestion of the virus the occlusion body protein, called granulin, is solubilized by the alkaline conditions of the larval midgut causing the liberation of infectious virions or nucleocapsids. The nucleocapsids attach to the midgut epithelial cells, are transported through the cytoplasm to the nucleus, followed by uncoating of the viral genome and initiation of viral replication. As the disease progresses infected cells lyse, normal physiological processes are disrupted,

the target insect becomes sluggish with the integument rupturing causing death.

CONCLUSIONS/RECOMMENDATIONS: Summarized below is data and information required, but not limited to, for the registration of Cyd-X. To fulfill the data requirements for Product Characterization the following information and/or data must be submitted:

- A revised CSF stating that the product contains capsules of the granulosis virus of Cydia pomonella (codling moth) as the active ingredient (see 151A-10 [b]). If appropriate, the registrant's name and address should be changed to reflect the recent "buy-out" by Crop Genetics International. In addition, the CSF contains a statement that refers to "...the number of polyhedra..." This statement must be changed to reflect the number of GV capsules.

- SAB has focused on assuring lack of contamination with pathogenic, toxin-producing microorganisms. Because of the in vivo production method the registrant must (a) specify the specific screens for the presence of significant mammalian pathogens and (b) perform an intraperitoneal or an "abbreviated" acute oral screen for each independent batch (see 151A-15).

- Specify QA/QC procedures in place to insure a healthy or disease-free insect colony.

For the registration of the technical grade material (CpGV) in the product Cyd-X, SAB has summarized the information and data submitted by the registrant to support waiver requests for certain mammalian toxicolgy studies in Series 152A. It should be noted that the data and information submitted by the registrant supports the registration of the technical grade material which consists of [redacted] If the end-use product (EP) consists of the active ingredient (CpGV) [redacted] SAB would consider this formulation identical to the technical grade material and would not recommend additional toxicology testing.

- 152A-10. The data and information submitted are sufficient to support the waiver request for the Acute Oral Toxicity study for the active ingredient.

- 152A-11. This study is not required to support the registration of the technical grade material. It should be noted that the toxicity testing for the MP and the EP are intended to provide data on the acute toxicity of the formulated product. Waivers for these studies may be granted when it is common knowledge that, or information provided by the applicant, shows that the inert

INERT INGREDIENT INFORMATION IS NOT INCLUDED

ingredients are not likely to pose any significant health risks.

- 152A-12. Although the registrant failed to provide a clear and adequate review of data from the open literature regarding the safety of pulmonary exposure to CpGV, SAB HAS SUBSTITUTED a appropriate review of the data and information to support the waiver request for this study.

- 152A-13. The data and information submitted are adequate to support the waiver request for this study.

- 152A-14. Although the registrant failed to provide a clear and adequate review of data from the open literature regarding the safety of CpGV following exposure to the eye, this study is not required for the registration of the technical grade material. SAB recommends a primary eye irritation study on the end-use product.

- 152A-15. No cases or incidents of hypersensitivity to CpGV have been reported in the literature or observed at Espro, Inc. The applicant must report any incidents of hypersensitivy to the Agency should any incident be observed in the future.

- 152A-16. Unacceptable. SAB recommends that the applicant demonstrate the ability or inability of CpGV to replicate in mammalian cell lines since recent studies have delineated between GV replication in the cytoplasm (cytoplasmic GV) and nucleus (nuclear GV) of infected host cell lines.

Detailed deficiencies are listed below.

SUMMARY OF PRODUCT IDENTITY DATA/INFORMATION

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 Series are incomplete.

CLASSIFICATION: Unacceptable.

SUMMARY OF TOXICOLOGY STUDIES

152A-10. Acute Oral Toxicity/Pathogenicity. Based on existing data from the open literature the applicant has requested a waiver for the acute oral toxicity study. The basis for this waiver is supported by studies from the open literature. **[NOTE: Instead of relying on the inadequate and inaccurate toxicology summary provided by the registrant, SAB has summarized the mammalian toxicology studies below and have used this information to support the waiver request].** In a study performed by Krieg (1978) mice

were orally dosed with a 5 x 10^{11} granula/mouse **[NOT 3 x 10^{9} capsules/guinea pig]** as a single dose or as a multiple dose over a period of 100 days. Endpoints included feed intake, body weight, temperature, blood count, serum electrophoresis, detection of virus-specific antibodies, cytological analyses of bone marrow smears, as well as anatomical and histological findings of certain organs after necropsy. No treatment-related effects were attributed to the test material in any of the tests performed in mice **[NOT guinea pigs as stated]**.

In another study, Chinese hamsters were fed a single dose (1.5 x 10^{12} capsules) or 90 single doses (1.7 x 10^{10} capsules) of Laspeyresia pomonella GV capsules/animal and observed for 90 days. In the same feeding study, mice received 97 single doses (5.2 x 10^{9} capsules) per os (stomach tube) with insect-derived L. pomonella GV. Untreated animals received a Tris-Hcl buffer solution without virus capsules. All animals were examined daily for any signs of treatment-related toxicity and/or abnormal behavior. At the end of the study, animals were examined for chromosomal aberrations. No differences in chromosome aberrations (i.e. gaps, breaks) or sister chromatid exchange rates were observed between treated and untreated control animals. In addition, no morphological changes in the gastrointestinal tract or any of the major organs were observed upon necropsy.

In experiments designed to determine if the codling moth GV stimulated antibody production, 100 mice were each fed 5 x 10^{11} capsules and sera collected on days 14, 24, 42, and 80 post-treatment (Doller and Huber, 1983). Virus-induced antibodies were not detected in the sera from any of the treated animals. Detection of active GV capsules was also monitored by collecting the feces of treated animals for bioassay analyses against neonate codling moth larvae. The bioassays demonstrated that part of the viral inoculum (second day post-feeding; 4.7 x 10^{9} capsules or 1%) can pass the intestine without damage and can be excreted in the feces in an active form. It should be noted that this data also indirectly supports the initiation of a pattern of clearance.

Additional data from various feeding studies using different baculoviruses was also submitted to support the registration of Cyd-X. In each instance no adverse effects were reported.

CLASSIFICATION: The data and information submitted are sufficient to support the waiver request for the Acute Oral Toxicity study for the technical grade active ingredient. In addition, if the end-use product (EP) consists of the active ingredient [redacted] SAB would consider this formulation identical to the technical grade material and would not recommend additional toxicology testing.

152A-11. Acute Dermal Toxicity. Based on existing data from the open literature the applicant has requested a waiver for the acute dermal toxicity study. The basis for this waiver request is supported by two referenced German studies.

CLASSIFICATION: This study is not required to support the registration of the technical grade material. It should be noted that the toxicity testing for the MP and the EP are intended to provide data on the acute toxicity of the formulated product. Waivers for these studies may be granted when it is common knowledge that, or information provided by the applicant, shows that the inert ingredients are not likely to pose any significant health risks.

152A-12. Acute Pulmonary Toxicity. The applicant has requested a waiver for this study based on existing data from the open literature. **[NOTE: Instead of relying on the inadequate and inaccurate summary of a review provided by the registrant, SAB has summarized the pulmonary toxicology studies below and have used this information to support the waiver request. The registrant should provide summaries from the original studies and adequate references.]** In the first referenced field study (Bailey and Fujita, 1987, Annals of Appl. Biol. 111:649-660), wood mice and bank voles were collected from a mist-sprayed orchard using CpGV at a rate of $2x10^{10}$ capsules/l at 1000 l/ha. Using the indirect ELISA (enzyme-linked immunosorbent assay) technique, antibody to CpGV was detected in all sera and in the feces of trapped animals. The antibody response in the given animal population increased with repeated virus application. Although considerable "GV contamination" occurred as a result of the virus application no adverse effects to the individual animals or their population dynamics were observed. **[Since the registrant DID NOT provide the original reference (No. 1) and an appropriate citation SAB has used the 1987 study by Bailey and Fujita to support the waiver request. Interestingly, these data are in closer agreement to the 1987 reference than to the reference provided by the registrant.]** Using infectious and UV-inactivated CpGV at various doses (100 μg, 1 μg, or 10 ng), Bailey and Hunter (1982) demonstrated antibody (IgG) in the sera from all wood mice treated intranasally.

Supplemental information was provided by the registrant to further support the waiver request for the required acute inhalation study.

CLASSIFICATION: The data and information submitted are adequate to support the waiver request for this study.

152A-13. Acute Intravenous Toxicity Study. The applicant has requested a waiver for this study based on existing data from the open literature. Using the radioimmunoassay technique, Doller (1981, Naturwissenschaften 68:573-574) assayed sera collected from laboratory workers who handled CpGV and from those individuals not exposed to the virus. In most instances, the sera from those laboratory workers (who handled the virus in the laboratory) as well as those individuals with no known previous contact with the virus was positive. Doller also found that newborns, infants, and children from various age groups contained antibodies to CpGV.

For antibody production, CpGV and other GVs have been used in mice, rabbits, and guinea pigs without any noted adverse effects. For example, antisera against Choristoneura murinana GV were obtained by injecting guinea pigs intraperitoneally with 2.5-3.0 mg of capsule protein in Freund's adjuvants (Kryweinczyk and Bergold, 1961, J. Insect Pathol. 3:15-28). The GVs from Pieris brassicae and P. rapae have been used in rabbits to produce antisera against the GV capsule protein (Cunningham, 1968, J. Invertebr. Pathol. 11:132-141). In this study, rabbits were administered three injections (0.5 mg) at weekly intervals. The first two injections were given subcutaneously and the third injection was given intravenously. NO adverse response or reaction to the GV injections were noted in the rabbits throughout the course of the experiment.

Antisera has also been produced against intact CpGV and virions from which most of the capsule protein had been removed. Using GV capsules ($4x10^{10}$; approx. 0.96 mg of protein) or alkaline liberated virions (0.5-1.0 mg; total was approx. 2.1 mg), New Zealand white rabbits were injected (footpads) with subsequent inoculations in the hind thighs at 4 days post-inoculation. Following a third injection (intravenously) the antisera was collected. No adverse effects were reported (Etzel and Falcon, 1976, J. Invertebr. Pathol. 27:13-26). Additional data and information from the open literature was provided by the registrant to further support the waiver request for the Acute Intravenous Toxicity study.

CLASSIFICATION: The data and information submitted are adequate to support the waiver request for this study.

152A-14. Primary Eye Irritation Study. The applicant has requested a waiver for this study based on existing data from the open literature. Two German studies were referenced and summarized but not submitted with this application. In the first study, Krieg (1978; complete citation not provided) performed an eye irritation test in guinea pigs using $5x10^{11}$ CpGV granula/ml. No adverse effects or pathological symptoms were reported. In the second study, Groner et al. (1978, Z. Ang. Zool. 65:69-80) were unable to demonstrate any adverse response following the instillation of $2x10^{10}$ granula/ml (virus not specified) to the cornea of guinea pigs. In a review (translation from German), Doller and Huber (citation not submitted) reported on the safety of CpGV following instillation into the eyes of guinea pigs. No adverse effects were reported in any of the treated animals.

CLASSIFICATION: Although the registrant failed to provide a clear and adequate review of data from the open literature regarding the safety of CpGV following exposure to the eye, this study is not required for the registration of the technical grade material.

152A-15. Hypersensitivity Incidents. No cases or incidents of hypersensitivity to CpGV have been reported in the literature or observed at Espro, Inc. The applicant must report any incidents of

hypersensitivy to the Agency should any incident be observed in the future.

152A-16. Cell Culture. The applicant has requested a waiver for this study based on existing data from the open literature. Although no mammalian cell culture studies have been conducted with CpGV, data and information on other distantly-related GVs and their ability or inability to infect homologous and heterologous cell lines were briefly discussed.

CLASSIFICATION: Unacceptable. SAB recommends that the applicant demonstrate the ability or inability of CpGV to replicate in mammalian cell lines since recent studies have delineated between GV replication in the cytoplasm (cytoplasmic GV) and nucleus (nuclear GV) of infected host cells (Tanada and Hess, 1991, Baculoviridae. Granulosis Viruses, In: Atlas of Invertebrate Viruses, edited by J. Adams and J. Bonami, pp. 227-258. CRC Press, Boca Raton, FLA).