May 1, 2001

MEMORANDUM

Subject: Efficacy Review for NP 4.5 (D&F) Detergent/Disinfectant
EPA Reg. No. 1839-95
DP Barcode: D273012
Case No. 033962

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To: Velma Noble PM 31/Zenobia Jones
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Applicant: Stepan Company.
Northfield, IL, USA

Formulation From Label:

Active Ingredient(s)	% by wt
Alkyl dimethyl benzyl ammonium chlorides	2.25%
Alkyl dimethyl ethylbenzyl ammonium chlorides	2.25%
Inert Ingredient(s)	95.50%
Total	100.00%

BACKGROUND:

Registrant has submitted label amendment for the product NP 4.5 D&F (EPA Reg. No. 1839-95) to add additional virucidal claim for Hepatitis B Virus (HBV). To support its claim, the registrant submitted for review basic and confirmatory virucidal efficacy data of the product against HBV using Duck HBV assay (MRIDs # 453304-01 and 453304-02)

DATA SUMMARY:

MRID #	453304-01
Project ID #	123-121
Study Completed on:	January 30, 2001
Test Method Used:	Virucidal Effectiveness Test Using Duck Hepatitis B Virus Test.
Author:	Nicole L. Braddock
Performing Lab:	MicroBiotest, Inc. 105B Carpenter Drive, Sterling, VA 20164

BRIEF DESCRIPTION OF TEST:

0.2 ml of a stock of Duck Hepatitis B virus (DHBV) was spread on sterile glass petri dish carriers and dried for 30 - 60 minutes at room temperature. 2 ml of each of two lots (Lots No. 2383-11-7 and 2383-11-8) of NP 4.5 (diluted 2 oz test agent/1 gallon sterile deionized water) were added on two carriers for 10 minutes contact time at 20 ± 2C. After the contact time, the virus-disinfectant mixture was neutralized with 2ml of 5% FBS, scraped from the surface and loaded onto a sephacryl columns for further neutralization. Serial tenfold dilutions were prepared from the neutralized virus in cell culture medium (CCM). Serially diluted samples (tests 10^{-1} - 10^{-6} and controls 10^{-1} - 10^{-8}) were added to Microtiter plate wells containing primary duck hepatocyte (PDH) and incubated overnight at 37 ± 2C in 5% CO_2. The plates were incubated for 7 - 14 days at 37 ± 2C in 5% CO_2 with replacement of CCM on alternate days. Following incubation, medium was aspirated, wells were washed with PBS, fixed in alcohol, washed and stained with monoclonal anti-DHBV, incubated for 2 hrs, washed and counter stained with anti-mouse-FITC, incubated for 2 hrs, washed and scored using UV-microscopy. A quaternary ammonium compound (BTC 835) at 175, and 350 ppm was used as a positive control along with the test substances Log_{10} reduction of the virus by the test substances vs control cultures was determined using the Most Probable Number (MPN) method of statistical analysis.

RECOMMENDATIONS:

Efficacy Supported by the Data

NP 4.5 (Lots No. 2383-11-7 and 2383-11-8) diluted 2 oz test agent/1 gallon sterile deionized water and added on two glass carriers carrying Duck hepatitis B virus for a 10 minutes contact time at 20 ± 2C proved to be virucidal against DHBV when tested in an in-vitro assay using duck primary hepatocyte cultures. All controls, including neutralizer effectiveness, cytotoxicity, plate and column recovery of the virus, virus stock titer, and cell viability all met the established criteria for a valid test (FR Notice OPP-00673; FRL-6736-5 and DIS/TSS-7). A positive control (BTC 835) showed complete killing of the virus at all dilutions tested at 350 ppm and partial killing of the virus at 175 ppm concentration as expected. Lots No. 2383-11-7 and 2383-11-8 both showed a Log_{10} reduction ± SE of 4.50 ± 0.30 using the MPN method of statistical analysis.

MRID #	453304-02
Project ID #	9873
Study Completed on:	January 25, 2001
Test Method Used:	Virucidal Effectiveness Confirmatory Test Using Duck Hepatitis B Virus Test.
Author:	Karen M. Ramm
Performing Lab:	ViroMed Biosafety Laboratories, 6101 Blue Circle Dr., Minneapolis, MN 55343

BRIEF DESCRIPTION OF TEST:

0.2 ml of a stock of Duck Hepatitis B virus (DHBV) was spread on sterile glass petri dish carriers and dried for 30 minutes at room temperature. 2 ml of lot no. 2383-11-7 of NP 4.5 (diluted 2 oz test agent/1 gallon sterile deionized water) was added on two carriers for 10 minutes at 20 ± 2C. After the contact time, 2.0 ml of virus-disinfectant mixture was immediately passed through a Sephadex gel column to reduce cytotoxicity. Serial tenfold dilutions were prepared from the neutralized virus in cell culture medium (CCM). Serially diluted samples (tests 10^{-1} - 10^{-6} and controls 10^{-1} - 10^{-8}) were added to Microtiter plate wells containing primary duck hepatocyte (PDH) and incubated overnight at 37 ± 2C in 5% CO_2. The plates were incubated for 11 days at 37 ± 2C in 5% CO_2 with replacement of CCM as needed. Following incubation, media was aspirated, wells were washed with PBS, fixed in ethanol. An indirect immunofluorescence assay was then performed using a monoclonal antibody specific for the envelope protein of DHBV. A quaternary ammonium compound (BTC 835) at 175, and 350 ppm was used as a positive control along with the test substances Log_{10} reduction of the virus by the test substances vs control cultures was calculated using the Most Probable Number (MPN) method of statistical analysis.

RECOMMENDATIONS:

Efficacy Supported by the Data

NP 4.5 Lot No. 2383-11-7 diluted 2 oz test agent/1 gallon sterile deionized and added on two glass carriers carrying Duck hepatitis B virus for a 10 minutes contact time at 20 ± 2C proved to be virucidal against DHBV when tested in an in-vitro assay using duck primary hepatocyte cultures. All controls, including neutralizer effectiveness, cytotoxicity, plate and column recovery of the virus, virus stock titer, and cell viability all met the established criteria for a valid confirmatory test (FR Notice OPP-00673; FRL-6736-5 and DIS/TSS-7). The log reduction ± SE calculated using MPN statistical analysis was 4.47 ± 0.09. Under these test conditions, NP4.5 lot no. 2383-11-7 demonstrated complete inactivation of DHBV.

Although, data submitted on the positive control BTC-835 demonstrated erratic results showing viral growth at 10^{-2} - 10^{-4} dilutions at both 175 and 350 ppm concentrations of the product, the Agency has as, an interim measure, decided this discrepancy will not result in a rejection of the data. The Agency is working with the testing laboratories to determine the reason for the inconsistent results with the positive control.

LABELING REVIEW:

1. Addition of virucidal efficacy against HBV is acceptable.

(A) HBV related comments:

(1) Label Claims. Featured statements/phrases, emphasized/enlarged lettering, special graphics, or any product name that employs the term "HBV", are prohibited. The terms "HBV" may be used for recognition purposes in parentheses, to accompany the official name of the virus. Featured statements/phrases, emphasized/enlarged lettering or special graphics that are employed for the primary purpose of especially promoting or drawing attention to product efficacy against hepatitis B virus, without appropriate use information, are unacceptable. For example, a featured, incomplete phrase such as "Kills HBV" will not be permitted, but the phrase "Kills HBV on pre-cleaned environmental surfaces" is acceptable.

(2) Acceptable Use Patterns. The only use patterns considered acceptable in conjunction with HBV efficacy claims are those that involve health care settings or other settings in which there is an expected likelihood of soiling of inanimate surfaces/objects with blood or body fluids, and in which the surfaces/objects likely to be soiled with blood or body fluids can be associated with the potential for the transmission of HBV.

(3) Special Directions For Use. One-step claims for HBV inactivation are not acceptable. Thorough precleaning is required for use on environmental surfaces

suspected of being contaminated by HBV. Special directions for the use of antimicrobial pesticides bearing label claims of efficacy against HBV must be provided in the following format, with each section appropriately titled and the required use information included. This information may be incorporated into the HIV specific text.

(i) The following statement must precede the special instructions for use of a virucidal agent claiming efficacy against HBV: "*KILLS HBV ON PRE-CLEANED ENVIRONMENTAL SURFACES/OBJECTS PREVIOUSLY SOILED WITH BLOOD/BODY FLUIDS in health care settings or other settings in which there is an expected likelihood of soiling of inanimate surfaces/objects with blood or body fluids, and in which the surfaces/objects likely to be soiled with blood or body fluids can be associated with the potential for transmission of Hepatitis B virus."

(ii) HEADING. Identify as "SPECIAL INSTRUCTIONS FOR CLEANING AND DECONTAMINATION AGAINST HBV OF SURFACES/OBJECTS SOILED WITH BLOOD/BODY FLUIDS."

(iii) PERSONAL PROTECTION. The specific barrier protection items to be used when handling items soiled with blood or body fluids must be identified, such as disposable gloves, gowns, masks, or eye coverings.

(iv) CLEANING PROCEDURE. The need to clean the specific surfaces/objects prior to disinfection/ sterilization must be identified (e.g., "Blood and other body fluids must be thoroughly cleaned from surfaces and objects before application of the disinfectant").

(v) DISPOSAL OF INFECTIOUS MATERIALS. The use directions must state how and where to dispose of blood, body fluids, and cleaning materials that are removed from surfaces/objects, e.g., "Blood and other body fluids should be autoclaved and disposed of according to Federal, State and local regulations for infectious waste disposal."

(vi) CONTACT TIME. When a contact time for HBV is specified that is shorter/longer than the contact time specified for other pathogens, then it must be qualified with a prominent statement such as "this contact time will not control other common types of viruses and bacteria."

(C:\copies of reviews\ EPA Reg. No. 1839-95, DP Barcode D273012, NP 4.5 (D&F)