VIRUCIDAL EFFICACY ASSAY

REPORT TITLE

10 Minute Inactivation of Influenza A_2 by Novicil 2ADPHQ

DATA REQUIREMENTS

U.S. EPA 40 CFR Part 158
"Data Requirements for Registration"

PRODUCT IDENTITY

Novicil 2ADPHQ

PROJECT NUMBER

1844

AUTHOR

C. Sue Brady, M.T.
Study Director

FINAL REPORT

December 6, 1995

PERFORMING LABORATORY

ViroMed Laboratories, Inc.
6101 Blue Circle Dr.
Minneapolis, MN 55343

SPONSOR

American Hyperform
433 Bain Bridge Street
Philadelphia, PA 19147

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10 (d) (1) (A), (B), or (C).

Company:_____

Company Agent:_____Date:_____

GOOD LABORATORY PRACTICE STATEMENT

The study referenced in this report was conducted in compliance with U.S. Environmental Protection Agency Good Laboratory Practice (GLP) regulations set forth in 40 CFR part 160.

The studies not performed by or under the direction of ViroMed Laboratories, Inc. are exempt from this Good Laboratory Practice statement and include: characterization and stability of the compound(s).

Study Director: _____ 12/16/95
C. Sue Brady, MT — Date

Submitter: _____ _____
Date

Sponsor: _____ _____
Date

QUALITY ASSURANCE UNIT SUMMARY

Study: Virucidal Efficacy

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. These studies have been performed under Good Laboratory Practice regulations (40 CFR Part 160) and in accordance to standard operating procedures and standard protocols. The Quality Assurance Unit maintains copies of study protocols and standard operating procedures and has inspected this study on the date(s) listed below. Studies are inspected at time intervals to assure the integrity of the study. The findings of these inspections have been reported to management and the Study Director.

Phase Inspected:	Project Set-up	Date: November 16, 1995
	In Process	Date: November 20, 1995
	Final Reading	Date: November 24, 1995
	Final Report	Date: December 6, 1995
	Study Director Review	Date: December 6, 1995
	Management Review	Date: December 6, 1995

Professional personnel involved:

Bonita L. Baskin, Ph.D.	- Laboratory Director
C. Sue Brady, M.T.	- Study Director
Karen M. Ramm, B.A.	- Research Assistant II
Katherine A. Paulson, C.L.A.	- Research Assistant I
Rebecca A. Grachek, M.S.	- Research Assistant I
Joyce A. Nelson	- Quality Assurance Director

Documentation of the above Quality Assurance audits have been reviewed.

Quality Assurance Director: Joan M. Kramber for Joyce A. Nelson Date: 12-6-95

Study Director: C. Sue Brady Date: 12-6-95

REPORT

EVALUATION OF VIRUCIDAL EFFICACY OF NOVICIL 2ADPHQ IN AN INANIMATE SURFACE ASSAY AGAINST INFLUENZA A_2.

TEST OBJECTIVE

The test objective is to determine the virucidal efficacy of this product on an inanimate environmental surface when in contact with Influenza A_2 for a 10 minute exposure time.

SPONSOR: American Hyperform
433 Bain Bridge Street
Philadelphia, PA 19147

SAMPLE NAME OR CODE: Novicil 2ADPHQ Sample 1 and Sample 2

DATE SAMPLE RECEIVED: November 15, 1995

TEST ARTICLE CHARACTERIZATION

The identity, strength, purity, stability, and chemical composition was not provided by Sponsor to ViroMed Laboratories, Inc.

TEST FACILITY: ViroMed Laboratories, Inc.
6101 Blue Circle Dr.
Minneapolis, MN 55343

DATA AND TEST SUBSTANCE RETENTION

A certified copy of this report as well as all materials and data pertinent to this study will be stored at ViroMed Laboratories, Inc., 6101 Blue Circle Dr., Minneapolis, MN 55343. As stated in the study protocol, test substance retention is the responsibility of the Sponsor. Unused test substances will be discarded following study completion.

INITIATION DATE: November 2, 1995
COMPLETION DATE: December 6, 1995

SUMMARY OF RESULTS:

Disinfectant:	Novicil 2ADPHQ Sample 1 and Sample 2
Dilution Tested:	Ready-to-use (RTU)
Virus:	Influenza A_2, ATCC VR-544, Strain A_2/Hong Kong
Exposure Time:	10 Minutes
Exposure Temp:	Room Temperature
Organic Soil Load:	5% Fetal Bovine Serum (FBS)
Efficacy Result:	Under these test conditions, the test substance demonstrated complete inactivation of Influenza A_2.

MATERIALS

1. Virus
The Influenza A_2 virus used in this study was obtained from the American Type Culture Collection, Rockville, Maryland, and is of the following strain: A_2/ Hong Kong (ATCC VR-544).

2. Test Cell Cultures
Rhesus monkey kidney (RMK) cells were obtained from ViroMed Laboratories, Inc. Cell Culture Division. Cultures were grown and used as monolayers in disposable tissue culture labware.

3. Test Media:
Test media used in this study was Eagles minimal essential medium (E-MEM) supplemented with 1% fetal bovine serum (fbs), 10 µg/ml gentamicin, 100 units/ml penicillin, and 2.5 µg/ml Fungizone.

METHODS

1. Preparation of Disinfectant
Two lots of Novicil 2ADPHQ, provided ready-to-use (RTU), were tested as received from Sponsor. The test substance was soluble upon use.

2. Preparation of Virus Films
Films of the virus were prepared by spreading 0.2ml amounts of undiluted virus suspension on the bottoms of 100 x 15 mm sterile glass petri dishes. Films were kept at room temperature (21°C) and at ambient humidity, until dry (20 minutes). The virus was further dried for 30 minutes at 37°C.

3. Sephadex Gel Filtration
To reduce the cytotoxic level of the virus-disinfectant mixture prior to assay of virus, and/or to reduce the virucidal level of the disinfectant, virus is separated from disinfectant by filtration through Sephadex gel. Columns of Sephadex LH-20-100 were equilibrated with phosphate buffered saline containing 1% albumin, centrifuged for 3 minutes to clear the void volume, loaded with 2mls of virus-disinfectant mixture and centrifuged again for 3 minutes.

4. Treatment of Virus Films with Disinfectant
Dried virus films were exposed to the use dilution of the disinfectant for 10 minutes at room temperature (21°C). The test substance was applied by holding the product 4-6 inches from the surface of the carrier and spraying until virus film was completely covered. The 10 minute contact time includes the 3 minute centrifugation time. Following exposure, the plate was scraped with a plastic cell scraper to resuspend the contents of the plate. The virus-disinfectant mixture was passed through a Sephadex column in order to detoxify the mixture. The filtrate (10^{-1} dilution) was then titered by serial dilution for infectivity. (Group B)

5. Treatment of Virus Control Films

A virus film was prepared as previously described (paragraph 2). The control film was exposed to test media (2ml) for the same amount of time as the test film was exposed to the disinfectant. The virus was then scraped and Sephadex filtered in the same manner as the test virus - paragraph 4. (Group A)

6. Cytotoxicity Controls

An aliquot of disinfectant, applied to a sterile petri dish as previously described for the treated film, was filtered through a Sephadex column and the filtrate was diluted serially in media and inoculated into RMK cell cultures. Cytotoxicity of the RMK cultures (Group C) was scored at the same time as virus-disinfectant and virus control cultures.

7. Assay of Non-Virucidal Level of Disinfectant

Each dilution of the Sephadex-filtered disinfectant (disinfectant control for cytotoxicity assay) was mixed with an aliquot of stock virus, and the resulting mixtures of dilutions were assayed for infectivity in order to determine the dilution(s) of disinfectant at which virucidal activity, if any, was retained. (Group D)

8. Assay for Virus Recovery

Dilutions of virus-disinfectant mixtures and the controls were inoculated into RMK cell cultures in quadruplicate. The RMK cells were inoculated with 0.1ml of each dilution and incubated at 36-38°C and 5-7% CO_2. The cells were observed for 8 days and Cytopathic Effect (CPE) and cytotoxicity were recorded. The CPE demonstrated was typical of Influenza on RMK cells. The test virus was also confirmed by an indirect fluorescent antibody assay.

9. Calculations

The method of Karber was used to calculate 50 percent end points.

$$\left[\frac{\text{(Sum of \% mortality at each dilution)}}{100} - 0.5 \times \text{(logarithm of dilution)}\right]$$

RESULTS AND CONCLUSION

Results of tests with two lots of disinfectant against Influenza A_2 are shown in Table 1. The titer of the virus control was 6.25 $\log_{10}$. Viral infectivity was not detected in the virus-disinfectant mixture for either lot at any dilution tested ($\leq$2.5 $\log_{10}$). Test substance cytotoxicity was observed for both lots at 1.5 $\log_{10}$. The neutralization control (non-virucidal level of the disinfectant) indicates that the disinfectant was neutralized at 1.5 $\log_{10}$. Taking the cytotoxicity and neutralization control results into consideration the reduction in virus titer was greater than or equal to 3.75 $\log_{10}$ for both batches of disinfectant. Tests on two lots of Novicil demonstrated complete inactivation of Influenza A_2 indicating **VIRUCIDAL** activity under these test conditions.

REFERENCES

1. ASTM Standards on Materials and Environmental Microbiology, 1987, E1053-85.
2. U.S. Environmental Protection Agency Pesticide assessment Guidelines, Subdivision G: Product Performance, November, 1982. U.S. Environmental Protection Agency, Registration Division, Office of Pesticide Programs, DIS/TSS-7, November 12, 1981.
3. Blackwell, J.H., and J.H.S. Chen. 1970. Effects of various germicidal chemicals on HEP-2 cell culture and Herpes simplex virus. J. AOAC 53:1229-1236.
4. Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections. Lennette, E.H. and Schmidt, N.J. editors. Fifth edition. 1979. p. 32-35.

TABLE 1: Results of Novicil 2ADPHQ Sample 1 and Sample 2 in an Inanimate Surface Assay against Influenza A_2 when Exposed for 10 Minutes

DILUTION	DRIED VIRUS CONTROL (GROUP A)	TEST: PRODUCT + Influenza A_2 Sample 1 (GROUP B)	TEST:PRODUCT + Influenza A_2 Sample 2 (GROUP B)
Cell Control	0 0 0 0	0 0 0 0	0 0 0 0
10^{-1}	+ + + +	T T T T	T T T T
10^{-2}	+ + + +	T T T T	T T T T
10^{-3}	+ + + +	0 0 0 0	0 0 0 0
10^{-4}	+ + + +	0 0 0 0	0 0 0 0
10^{-5}	+ + + +	0 0 0 0	0 0 0 0
10^{-6}	0 0 + +	0 0 0 0	0 0 0 0
10^{-7}	+ 0 0 0	0 0 0 0	0 0 0 0
10^{-8}	0 0 0 0	0 0 0 0	0 0 0 0
$TCID_{50}$/0.1ml	$10^{6.25}$	$\leq 10^{2.5}$	$\leq 10^{2.5}$

DILUTION	CYTOTOXICITY CONTROL Sample 1 (GROUP C)	CYTOTOXICITY CONTROL Sample 2 (GROUP C)
Cell Control	0 0 0 0	0 0 0 0
10^{-1}	T T T T	T T T T
10^{-2}	0 0 0 0	0 0 0 0
10^{-3}	0 0 0 0	0 0 0 0
10^{-4}	0 0 0 0	0 0 0 0
10^{-5}	0 0 0 0	0 0 0 0
10^{-6}	0 0 0 0	0 0 0 0
10^{-7}	0 0 0 0	0 0 0 0
10^{-8}	0 0 0 0	0 0 0 0
TCD_{50}/0.1ml	$10^{1.5}$	$10^{1.5}$

(+) = positive for the presence of test virus
(0) = no test virus recovered and/or no cytotoxicity present
(T) = Cytotoxicity present

Non-Virucidal Level (control)

DILUTION	Virus Control +Cyto. Control Sample 1 (GROUP D)	Virus Control +Cyto. Control Sample 2 (GROUP D)
Cell Control	0 0 0 0	0 0 0 0
10^{-1}	T T T T	T T T T
10^{-2}	+ + + +	+ + + +
10^{-3}	+ + + +	+ + + +
10^{-4}	+ + + +	+ + + +
10^{-5}	+ + + +	+ + + +
10^{-6}	+ + + +	+ + + +
10^{-7}	+ + + +	+ + + +
10^{-8}	+ + + +	+ + + +

Results of the Non-virucidal level control indicate that no virucidal activity is retained after Sephadex filtration.

(+) = positive for the presence of test virus
(0) = no test virus recovered and/or no cytotoxicity present
(T) = Cytotoxicity present