

FINAL STUDY REPORT

PROTOCOL TITLE

Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

Virus: Poliovirus type 2

DATA REQUIREMENT

U.S. EPA 40 CFR Part 158,
"Data Requirements for Registration"
Pesticide Assessment Guidelines - Subdivision G, 91-2(f)

PRODUCT IDENTITY

AXEN (EPA #72977-2), THE 30 PPM USE DILUTION OF AXENOHL (EPA #72977-1),
A 2400 PPM CONCENTRATE
LOT #2001.042.001 AND LOT #2001.005.001

PROTOCOL NUMBER

IMS01011002.POL

PROJECT NUMBER

12608

AUTHOR

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Study Director
Chemical Germicide Testing Services

STUDY COMPLETION DATE

February 12, 2002

PERFORMING LABORATORY

AppTec Laboratory Services
2540 Executive Drive
St. Paul, MN 55120

SPONSOR

Innovative Medical Services
1725 Gillespie Way
El Cajon, CA 92020

Page 1 of 14



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:

Innovative Medical Services

Company Agent:

Dolana Blount

Assistant to the President / CEO

Title

DOLANA BLOUNT

Signature

Date: 02.25.02



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 procedures not performed by or under the direction of AppTec Laboratory Services are exempt from this Good Laboratory Practice Statement and include: characterization and stability of the compound(s).

Study Director:	<u>Mary J. Miller</u> Mary J. Miller, M.T.	<u>2-12-02</u> Date
Submitter:	<u>Dolan</u>	<u>02.25.02</u> Date
Sponsor Representative:	<u>Dolan</u>	<u>02.25.02</u> Date



QUALITY ASSURANCE UNIT SUMMARY

Study: Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. This study has been performed under Good Laboratory Practice regulations (40 CFR Part 160) and in accordance to standard operating procedures and a standard protocol. 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.

Phase Inspected	Date	Study Director	Management
Critical Phase	January 25, 2002	January 28, 2002	February 12, 2002
Final Report	February 11, 2002	February 11, 2002	

The findings of these inspections have been reported to management and the Study Director.

Quality Assurance Auditor: *Scott W. Sherman*

Date: 2-17-02



TABLE OF CONTENTS

Title Page 1

Statement of No Data Confidentiality Claims 2

Good Laboratory Practice Statement..... 3

Quality Assurance Unit Summary 4

Table of Contents 5

Study Personnel 6

General Study Information 7

Test Substance Identity..... 7

Study Dates..... 7

Objective 7

Summary of Results..... 8

Test System 8

Methods 9

Protocol Changes..... 10

Data Analysis 10

Study Retention..... 11

References 11

Study Results 12

Study Conclusion 12

Table 1: Virus Control and Test Results 13

Table 2: Cytotoxicity Control Results 13

Table 3: Neutralization Control Results 14

STUDY PERSONNEL

STUDY DIRECTOR: Mary J. Miller, M.T.

Professional Personnel Involved:

William D. Smith, B.S.	- President
Karen M. Ramm, B.A.	- Division Director
Mary J. Miller, M.T.	- Research Scientist II
Katherine A. Paulson, M.L.T.	- Research Assistant II
Carrey A. Bauer, B.S.	- Research Assistant I

STUDY REPORT

GENERAL STUDY INFORMATION

Study Title: Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces
Project Number: 12608
Protocol Number: IMS01011002.POL
Sponsor: Innovative Medical Services
1725 Gillespie Way
El Cajon, CA 92020
Testing Facility: AppTec Laboratory Services
2540 Executive Drive
St. Paul, MN 55120

TEST SUBSTANCE IDENTITY

Test Substance Name: AXEN (EPA #72977-2), THE 30 PPM USE DILUTION OF AXENOHL (EPA #72977-1),
A 2400 PPM CONCENTRATE
Lots: LOT #2001.042.001 and LOT #2001.005.001

Test Substance Characterization

Test substance characterization as to content, stability, solubility, storage, etc., is the responsibility of the Sponsor.

STUDY DATES

Date Sample Received: November 20, 2001
Study Initiation Date: January 23, 2002
Experimental Start Date: January 25, 2002
Experimental End Date: February 1, 2002
Study Completion Date: February 12, 2002

OBJECTIVE

The objective of this study was to evaluate the virucidal efficacy of a disinfectant against Poliovirus type 2 according to test criteria and methods approved by the United States Environmental Protection Agency (U.S. EPA) for registration of a product as a virucide.

SUMMARY OF RESULTS

Test Substance: AXEN (EPA #72977-2), THE 30 PPM USE DILUTION OF AXENOHL (EPA #72977-1), A 2400 PPM CONCENTRATE, LOT #2001.042.001 AND LOT #2001.005.001

Dilution: No dilution required, used as received from Sponsor

Virus: Poliovirus type 2, ATCC VR-1002, Strain Lansing

Exposure Time: One minute and ten minutes

Exposure Temperature: Room temperature

Organic Soil Load: 1% Fetal bovine serum

Efficacy Result: Following a one minute exposure period, two lots of AXEN (EPA #72977-2), THE 30 PPM USE DILUTION OF AXENOHL (EPA #72977-1), A 2400 PPM CONCENTRATE (LOT #2001.042.001 and LOT #2001.005.001) **did not demonstrate complete inactivation** of Poliovirus type 2 under these test conditions as required by the U.S. EPA for claims of virucidal activity.

Following a ten minute exposure period, two lots of AXEN (EPA #72977-2), THE 30 PPM USE DILUTION OF AXENOHL (EPA #72977-1), A 2400 PPM CONCENTRATE (LOT #2001.042.001 and LOT #2001.005.001) met the test criteria specified in the study protocol. The results indicate **complete inactivation** of Poliovirus type 2 under these test conditions as required by the U.S. EPA for claims of virucidal activity.

TEST SYSTEM

- Virus**
The Lansing strain of Poliovirus type 2 used for this study was obtained from the American Type Culture Collection, Manassas, VA (ATCC VR-1002). Stock virus was prepared by collecting the supernatant culture fluid from infected culture cells. The cells were disrupted and cell debris removed by centrifugation at approximately 2000 RPM for five minutes at approximately 4°C. The supernatant was removed, aliquoted, and the high titer stock virus was stored at ≤ -70°C until the day of use. On the day of use, two aliquots of stock virus (AppTec Lot VML-P2-37) were removed, thawed, combined, and refrigerated until use in the assay. The stock virus culture contained 1% fetal bovine serum as the organic soil load. The stock virus tested demonstrated cytopathic effects (CPE) typical of Poliovirus on Vero cells.
- Test Cell Cultures**
Vero cells were obtained from ViroMed Laboratories, Inc., Minneapolis, MN, Cell Culture Division. Cultures were maintained and used as monolayers in disposable tissue culture labware. On the day of testing, cells were observed as having proper cell integrity and therefore, were acceptable for use in this study.
- Test Medium**
Test medium used in this study was Eagles minimal essential medium (E-MEM) supplemented with 5% heat-inactivated fetal bovine serum (FBS), 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL Fungizone.

The following table lists the test and control groups, the dilutions assayed, and the number of cultures used. See text for a more detailed explanation.

NUMBER OF DILUTIONS AND CULTURES FOR VIRUCIDAL EFFICACY STUDY			
Test or Control Group	Dilutions Assayed (log ₁₀)	Cultures per dilution	Total Cultures
Cell Control	N/A	4	4/group
Dried Virus Control* (Group A)	-1,-2,-3,-4,-5,-6,-7, -8	4	32
Sample lot #1 + virus* (Group B)	-1,-2,-3,-4,-5,-6,-7, -8	4	32
Sample lot #2 + virus* (Group B)	-1,-2,-3,-4,-5,-6,-7, -8	4	32
Cytotoxicity of lot #1 (Group C)	-1,-2,-3,-4,-5	4	20
Cytotoxicity of lot #2 (Group C)	-1,-2,-3,-4,-5	4	20
Non-Virucidal level - lot #1 (Group D)	-1,-2,-3,-4,-5	4	20
Non-Virucidal level - lot #2 (Group D)	-1,-2,-3,-4,-5	4	20

(*) = for each exposure time assayed

METHODS

- Preparation of Test Substance**
 The product was used, undiluted, as received from the Sponsor. The test substance was in solution as determined by visual observation.
- Preparation of Virus Films**
 Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of six separate 100 x 15mm sterile glass petri dishes. The virus films were dried at 10.0°C in a relative humidity of 50% until visibly dry (25 minutes).
- 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 was 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 three minutes to clear the void volume, loaded with 2.0 mL of virus-disinfectant mixture and immediately passed through the column utilizing the syringe plunger.
- Treatment of Virus Films with Test Substance (GROUP B, TABLE 1)**
 For each lot of disinfectant, separate dried virus films were exposed to 2.0 mL of the use dilution at room temperature (23°C), for each exposure time assayed. The exposure times assayed were one minute and ten minutes. Following each exposure time, the plates were scraped with a cell scraper to resuspend the contents of the plate and the virus-disinfectant mixture was immediately passed through a Sephadex column utilizing the syringe plunger in order to detoxify the mixture. The filtrate (10⁻¹ dilution) was then titered by serial dilution and assayed for infectivity.



5. Treatment of Virus Control Films (GROUP A, TABLE 1)
Two virus films were prepared as previously described (paragraph 2). Each control film was exposed to 2.0 mL of test medium for the same amount of time as the test films were exposed to the disinfectant. The exposure times assayed were one minute and ten minutes. The virus was then scraped and passed through a Sephadex column in the same manner as the test virus and the filtrate (10^{-1} dilution) was then titered by serial dilution and assayed for infectivity.
6. Cytotoxicity Assay (GROUP C, TABLE 2)
A 2.0 mL aliquot of the use dilution of each lot of the disinfectant was filtered through a Sephadex column and the filtrate was diluted serially in medium and inoculated into Vero cell cultures. Cytotoxicity of the Vero cell cultures was scored at the same time as the virus-disinfectant and virus control cultures.
7. Assay of Non-Virucidal Level of Test Substance (GROUP D, TABLE 3)
Each dilution of the Sephadex-filtered disinfectant (disinfectant control for cytotoxicity assay) was mixed with an aliquot of low titer 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. Dilutions that showed virucidal activity were not considered in determining the reduction in infectivity by the test substance.
8. Infectivity Assays
The Vero cell line, which exhibits CPE in the presence of Poliovirus type 2, was used as the indicator cell line in the infectivity assays. Cells in multiwell culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions prepared from test and control groups. Uninfected indicator cell cultures (cell controls) were inoculated with test medium alone. Cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO₂ in sterile disposable cell culture labware. The cultures were scored periodically for seven days for the absence or presence of CPE, cytotoxicity, and for viability.
9. Statistical Methods: N/A

PROTOCOL CHANGES

- Protocol Amendments:** No protocol amendments were required for this study.
- Protocol Deviations:** No protocol deviations occurred during this study.

DATA ANALYSIS

Calculation of Titers

Viral and cytotoxicity titers are expressed as $-\log_{10}$ of the 50 percent titration endpoint for infectivity (TCID₅₀) or cytotoxicity (TCD₅₀), respectively, as calculated by the method of Spearman Karber.

$$-1- \left[\frac{(\text{Sum of \% mortality at each dilution}) - 0.5 \times (\text{logarithm of dilution})}{100} \right]$$

STUDY RETENTION

Record Retention

All of the original raw data developed exclusively for this study shall be archived at AppTec Laboratory Services, 2540 Executive Drive, St. Paul, MN 55120. These original data include, but are not limited to, the following:

1. All handwritten raw data for control and test substances including, but not limited to, notebooks, data forms and calculations.
2. Any protocol amendments/deviation notifications.
3. All measured data used in formulating the final report.
4. Memoranda, specifications, and other study specific correspondence relating to interpretation and evaluation of data, other than those documents contained in the final study report.
5. Original signed protocol.
6. Certified copy of the final study report.
7. Study-specific SOP deviations made during the study.

Test Substance Retention

The test substance will be discarded following study completion per Sponsor approved protocol. It is the responsibility of the Sponsor to retain a sample of the test material.

REFERENCES

1. Annual Book of ASTM Standards 2000, Section 11 Water and Environmental Technology Volume 11.05 Biological Effects and Environmental Fate: Biotechnology; Pesticides, E1053-97.
2. U.S. Environmental Protection Agency Pesticide Assessment Guidelines, Subdivision G: Product Performance, 91-2(f), November 1982.
3. U.S. Environmental Protection Agency, Registration Division, Office of Pesticide Programs, DIS/TSS-7, November 12, 1981.
4. Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections. Schmidt, N.J. and Emmons, R.W. editors. Sixth edition, 1989. p. 18-20.
5. 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.

STUDY RESULTS

Cytotoxicity and Neutralization Controls

Test substance cytotoxicity was not observed in either lot at any dilution tested ($\leq 0.5 \log_{10}$). The neutralization control (non-virucidal level of the test substance) indicates that the test substance was neutralized at $\leq 0.5 \log_{10}$ for both lots.

One Minute Exposure Period

Results of tests with two lots of AXEN (EPA #72977-2), THE 30 PPM USE DILUTION OF AXENOHL (EPA #72977-1), A 2400 PPM CONCENTRATE (LOT #2001.042.001 and LOT #2001.005.001) exposed to Poliovirus type 2 for one minute are shown in Tables 1-3. All cell controls were negative for test virus infectivity. The titer of the dried virus control was $6.5 \log_{10}$. Following exposure, test virus infectivity was detected in the virus-test substance mixture for LOT #2001.042.001 at $1.25 \log_{10}$ and for LOT #2001.005.001 at $2.75 \log_{10}$. Taking the cytotoxicity and neutralization control results into consideration, the reduction in viral titer was $5.25 \log_{10}$ for LOT #2001.042.001 and $3.75 \log_{10}$ for LOT #2001.005.001, following a one minute exposure period.

Ten Minute Exposure Period

Results of tests with two lots of AXEN (EPA #72977-2), THE 30 PPM USE DILUTION OF AXENOHL (EPA #72977-1), A 2400 PPM CONCENTRATE (LOT #2001.042.001 and LOT #2001.005.001) exposed to Poliovirus type 2 for ten minutes are shown in Tables 1-3. All cell controls were negative for test virus infectivity. The titer of the dried virus control was $6.5 \log_{10}$. Following exposure, test virus infectivity was not detected in the virus-test substance mixture for either lot at any dilution tested ($\leq 0.5 \log_{10}$). Taking the cytotoxicity and neutralization control results into consideration, the reduction in viral titer was $\geq 6.0 \log_{10}$ for both lots, following a ten minute exposure period.

STUDY CONCLUSION

One Minute Exposure Period

Under the conditions of this investigation, in the presence of a 1% fetal bovine serum organic soil load, AXEN (EPA #72977-2), THE 30 PPM USE DILUTION OF AXENOHL (EPA #72977-1), A 2400 PPM CONCENTRATE (LOT #2001.042.001 and LOT #2001.005.001), undiluted, did not demonstrate complete inactivation of Poliovirus type 2 as required by the U.S. EPA for virucidal label claims, following a one minute exposure period.

Ten Minute Exposure Period

Under the conditions of this investigation, in the presence of a 1% fetal bovine serum organic soil load, AXEN (EPA #72977-2), THE 30 PPM USE DILUTION OF AXENOHL (EPA #72977-1), A 2400 PPM CONCENTRATE (LOT #2001.042.001 and LOT #2001.005.001), undiluted, demonstrated complete inactivation of Poliovirus type 2 as required by the U.S. EPA for virucidal label claims, following a ten minute exposure period.

In the opinion of the Study Director, there were no circumstances that may have adversely affected the quality or integrity of the data.

The use of the AppTec name, logo or any other representation of AppTec without the written approval of AppTec is prohibited. In addition, AppTec may not be referred to in any form of promotional materials, press releases, advertising or similar materials (whether by print, broadcast, communication or electronic means) without the express written permission of AppTec Laboratory Services.

TABLE 1: Effects of AXEN (EPA #72977-2), THE 30 PPM USE DILUTION OF AXENOHL (EPA #72977-1), A 2400 PPM CONCENTRATE (LOT #2001.042.001 and LOT #2001.005.001) Following a One Minute and Ten Minute Exposure to Poliovirus Type 2 Dried on an Inanimate Surface

Dilution	Dried Virus Control (GROUP A)		Poliovirus type 2 + LOT #2001.042.001 (GROUP B)		Poliovirus type 2 + LOT #2001.005.001 (GROUP B)	
	One Minute Exposure	Ten Minute Exposure	One Minute Exposure	Ten Minute Exposure	One Minute Exposure	Ten Minute Exposure
Cell Control	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10 ⁻¹	++++	++++	+0++	0 0 0 0	++++	0 0 0 0
10 ⁻²	++++	++++	0 0 0 0	0 0 0 0	0+0+	0 0 0 0
10 ⁻³	++++	++++	0 0 0 0	0 0 0 0	+0+	0 0 0 0
10 ⁻⁴	++++	++++	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10 ⁻⁵	++++	++++	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10 ⁻⁶	+0+	++++	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10 ⁻⁷	0+0	0 0 0 0	0 0 0 0	0 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	0 0 0 0	0 0 0 0	0 0 0 0
TCD ₅₀ /0.1 mL	10 ^{6.5}	10 ^{6.5}	10 ^{1.25}	≤10 ^{0.5}	10 ^{2.75}	≤10 ^{0.5}

TABLE 2: Cytotoxicity of AXEN (EPA #72977-2), THE 30 PPM USE DILUTION OF AXENOHL (EPA #72977-1), A 2400 PPM CONCENTRATE on Vero Cell Cultures

Dilution	Cytotoxicity Control LOT #2001.042.001 (GROUP C)	Cytotoxicity Control LOT #2001.005.001 (GROUP C)
Cell Control	0 0 0 0	0 0 0 0
10 ⁻¹	0 0 0 0	0 0 0 0
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
TCD ₅₀ /0.1 mL	≤10 ^{0.5}	≤10 ^{0.5}

(+) = Positive for the presence of test virus
 (0) = No test virus recovered and/or no cytotoxicity present

TABLE 3: Non-Virucidal Level of Test Substance (Neutralization Control)

Dilution	Test Virus + Cytotoxicity Control LOT #2001.042.001 (GROUP D)	Test Virus + Cytotoxicity Control LOT #2001.005.001 (GROUP D)
Cell Control	0 0 0 0	0 0 0 0
10 ⁻¹	++++	++++
10 ⁻²	++++	++++
10 ⁻³	++++	++++
10 ⁻⁴	++++	++++
10 ⁻⁵	++++	++++

(+) = Positive for the presence of test virus after low titer stock virus added (neutralization control)
 (0) = No test virus recovered and/or no cytotoxicity present

Results of the non-virucidal level control indicate that the test substance was neutralized at TCID₅₀ of ≤0.5 log₁₀ for both lots.