ATS LABS

FINAL STUDY REPORT

STUDY TITLE

Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

Virus: Avian Influenza A (H3N2) virus (Avian Reassortant)

PRODUCT IDENTITY

Axen 30
Lot #2006.003.001 and Lot #2006.158.001

DATA REQUIREMENTS

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

AUTHOR

Mary J. Miller, M.T.
Study Director

STUDY COMPLETION DATE

August 24, 2006

PERFORMING LABORATORY

ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

SPONSOR

Pure Bioscience
1725 Gillespie Way
El Cajon, CA 92020

PROJECT NUMBER

A04153

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: Pure Bioscience

Company Agent: DOLANA BLOUNT

DIRECTOR OF REGULATORY AFFAIRS
Title

Signature

Date: 08.31.2006

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 ATS Labs are exempt from this Good Laboratory Practice Statement and include: characterization and stability of the test substance(s).

Submitter: _____ Date: 08.31.2006

Sponsor: _____ Date: 08.31.2006

Study Director: _____ Date: 8-24-06
Mary J. Miller, M.T.

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 non-clinical 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 dates listed below. Studies are inspected at time intervals to assure the integrity of the study.

Phase Inspected	Date	Study Director	Management
Critical Phase	August 16, 2006	August 16, 2006	August 24, 2006
Final Report	August 24, 2006	August 24, 2006	

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

Quality Assurance Auditor: R. Tyde Date: 08-24-06

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STUDY PERSONNEL

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

Professional Personnel Involved:

Karen M. Ramm, B.A.	- Technical Director
Katherine A. Paulson, M.L.T.	- Research Assistant II
Matthew Cantin, B.S.	- Research Assistant II

STUDY REPORT

GENERAL STUDY INFORMATION

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

Project Number: A04153

Protocol Number: IMS01050206.AFLU

Sponsor: Pure Bioscience
1725 Gillespie Way
El Cajon, CA 92020

Testing Facility: ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

TEST SUBSTANCE IDENTITY

Test Substance Name: Axen 30

Lot/Batch(s): Lot #2006.003.001 and Lot #2006.158.001

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

STUDY DATES

Date Sample Received: July 25, 2006
Study Initiation Date: July 31, 2006
Experimental Start Date: August 16, 2006
Experimental End Date: August 23, 2006
Study Completion Date: August 24, 2006

OBJECTIVE

The objective of this study was to evaluate the virucidal efficacy of a test substance against Avian Influenza A (H3N2) virus (Avian Reassortant) 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 30, Lot #2006.003.001 and Lot #2006.158.001
Dilution:	Ready to use (RTU), trigger spray
Virus:	Avian Influenza A (H3N2) virus (Avian Reassortant), ATCC VR-2072, Strain A/Washington/897/80 X A/Mallard/New York/6750/78
Exposure Time:	Ten minutes
Exposure Temperature:	Room temperature (20.0°C)
Organic Soil Load:	1% fetal bovine serum
Efficacy Result:	Two lots of Axen 30 met the test criteria specified in the study protocol. The results indicate **complete inactivation** of Avian Influenza A (H3N2) virus (Avian Reassortant) under these test conditions as required by the U.S. EPA for claims of virucidal activity.

TEST SYSTEM

1. Virus
The A/Washington/897/80 X A/Mallard/New York/6750/78 strain of Avian Influenza A (H3N2) virus (Avian Reassortant) used for this study was obtained from the American Type Culture Collection, Manassas, VA (ATCC VR-2072). The stock virus was prepared by collecting the supernatant culture fluid from 75-100% infected culture cells. The cells were disrupted and cell debris removed by centrifugation at approximately 1500 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, an aliquot of stock virus (ATS Labs Lot IA-60) was removed, thawed 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 Influenza virus on Rhesus monkey kidney cells.

2. Test Cell Cultures
Rhesus monkey kidney (RMK) cells were obtained from ViroMed Laboratories, Inc., Cell Culture Division. The cultures were maintained and used as monolayers in disposable tissue culture labware. On the day of testing, the cells were observed as having proper cell integrity and therefore, were acceptable for use in this study.

3. Test Medium
The test medium used in this study was Minimum Essential Medium (MEM) supplemented with 1% heat-inactivated fetal bovine serum (FBS), 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B.

The following table lists the test and control groups, the dilutions assayed, and the number of cultures used. See the report 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	4	28
Sample lot #1 + virus (Group B)	-1,-2,-3,-4,-5,-6,-7	4	28
Sample lot #2 + virus (Group B)	-1,-2,-3,-4,-5,-6,-7	4	28
Cytotoxicity of lot #1 (Group C)	-1,-2,-3,-4,-5,-6,-7	4	28
Cytotoxicity of lot #2 (Group C)	-1,-2,-3,-4,-5,-6,-7	4	28
Non-Virucidal level - lot #1 (Group D)	-1,-2,-3,-4,-5,-6,-7	4	28
Non-Virucidal level - lot #2 (Group D)	-1,-2,-3,-4,-5,-6,-7	4	28

METHODS

1. Preparation of Test Substance
Two lots of Axen 30 (Lot #2006.003.001 and Lot #2006.158.001) were used undiluted as received from the Sponsor. On the day of testing, 100 mL of each lot of test substance was transferred to individual trigger spray bottles provided by the Sponsor on July 27, 2006. Following testing, the remaining test substance in the trigger spray bottles was discarded. The test substance was applied according to the use directions provided by the Sponsor. (See report section on the Treatment of Virus Films with Test Substance.)

2. Preparation of Virus Films
Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of three separate 100 x 15 mm sterile glass petri dishes. The virus films were air-dried at 20.0°C in a relative humidity of 50% until visibly dry (20 minutes).

3. Sephadex Gel Filtration
To reduce the cytotoxic level of the virus-test substance mixture prior to assay of virus and/or to reduce the virucidal level of the test substance, the virus was separated from test substance by filtration through Sephadex gel. Columns of Sephadex LH-20-100 were equilibrated with phosphate buffered saline containing 1% albumin and centrifuged for three minutes to clear the void volume. The columns were then ready to be used in the assay.

4. Treatment of Virus Films with Test Substance (GROUP B, TABLE 1)
For each lot of test substance, one dried virus film was individually exposed for ten minutes at room temperature (20.0°C) to the amount of spray released under use conditions. The carriers were sprayed until thoroughly wet with three sprays at a distance of 6-8 inches from the carrier surface. The virus films were completely covered with the test substance. Following the exposure time, the plates were individually scraped with a cell scraper to resuspend the contents and the virus-test substance 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 10-fold serial dilution and assayed for infectivity and/or cytotoxicity.

5. Treatment of Virus Control Films (GROUP A, TABLE 1)
A virus film was prepared as previously described (paragraph 2). The control film was exposed to 2.0 mL of test medium for ten minutes at room temperature (20.0°C). Following exposure, the virus control was scraped with a cell scraper and the virus mixture was immediately passed through a Sephadex column in the same manner as the test virus (paragraph 4). The filtrate (10^{-1} dilution) was then titered by 10-fold serial dilution and assayed for infectivity.

6. Cytotoxicity Assay (GROUP C, TABLE 2)
Each lot of the test substance was sprayed as previously described onto separate sterile petri dishes. The plates were individually scraped with a cell scraper, the contents were transferred to a Sephadex column and immediately passed through the column utilizing a syringe plunger. The filtrate (10^{-1} dilution) was then titered by 10-fold serial dilution and assayed for cytotoxicity. Cytotoxicity of the RMK cell cultures was scored at the same time as the virus-test substance and virus control cultures.

7. Assay of Non-Virucidal Level of Test Substance (GROUP D, TABLE 3)
Each dilution of the Sephadex-filtered test substance (cytotoxicity control dilutions) was mixed with an aliquot of low titer stock virus, and the resulting mixtures of dilutions were assayed for infectivity and/or cytotoxicity in order to determine the dilution(s) of test substance 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 RMK cell line, which exhibits CPE in the presence of Avian Influenza A (H3N2) virus (Avian Reassortant), 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 all test and control groups. Uninfected indicator cell cultures (cell controls) were inoculated with test medium alone. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 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: Not applicable

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 ($TCID_{50}$) or cytotoxicity (TCD_{50}), respectively, as calculated by the method of Spearman Karber.

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

Calculation of Log Reduction

Dried Virus Control $TCID_{50}$ – Test Substance $TCID_{50}$ = Log Reduction

STUDY ACCEPTANCE CRITERIA

A valid test requires 1) that at least 4 $\log_{10}$ of infectivity be recovered from the dried virus control film; 2) that when cytotoxicity is evident, at least a 3-log reduction in titer is demonstrated beyond the cytotoxic level; 3) that the cell controls be negative for infectivity. **Note:** An efficacious product must demonstrate complete inactivation of the virus at all dilutions.

STUDY RETENTION

Record Retention

All of the original raw data developed exclusively for this study shall be archived at ATS Labs, 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121. 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 final study report.
7. Study-specific SOP deviations made during the study.

Test Substance Retention

The test substance will be returned following study completion per Sponsor approved protocol. It is the responsibility of the Sponsor to retain a sample of the test material. (The Sponsor provided trigger spray bottles will be discarded following study completion.)

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

Results of tests with two lots of Axen 30 (Lot #2006.003.001 and Lot #2006.158.001), a trigger spray, exposed to Avian Influenza A (H3N2) virus (Avian Reassortant) in the presence of a 1% fetal bovine serum soil load at room temperature (20.0°C) 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 5.0 $\log_{10}$. Following exposure, test virus infectivity was not detected in the virus-test substance mixture for either lot at any dilution tested ($\leq$0.5 $\log_{10}$). 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. Taking the cytotoxicity and neutralization control results into consideration, the reduction in viral titer was $\geq$4.5 $\log_{10}$ for both lots.

STUDY CONCLUSION

Under the conditions of this investigation and in the presence of a 1% fetal bovine serum soil load, Axen 30 (Lot #2006.003.001 and Lot #2006.158.001), a trigger spray, demonstrated complete inactivation of Avian Influenza A (H3N2) virus (Avian Reassortant) following a ten minute exposure time at room temperature (20.0°C) as required by the U.S. EPA for virucidal label claims.

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

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TABLE 1: Virus Control and Test Results

Effects of Axen 30 (Lot #2006.003.001 and Lot #2006.158.001) Following a Ten Minute Exposure to Avian Influenza A (H3N2) Virus (Avian Reassortant) Dried on an Inanimate Surface

Dilution	Dried Virus Control (GROUP A)	Avian Influenza A (H3N2) virus (Avian Reassortant) + Lot #2006.003.001 (GROUP B)	Avian Influenza A (H3N2) virus (Avian Reassortant) + Lot #2006.158.001 (GROUP B)
Cell Control	0 0 0 0	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 0 0
10^{-6}	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
$TCID_{50}$/0.1 mL	$10^{5.0}$	$\leq 10^{0.5}$	$\leq 10^{0.5}$

TABLE 2: Cytotoxicity Control Results

Cytotoxicity of Axen 30 on RMK Cell Cultures

Dilution	Cytotoxicity Control Lot #2006.003.001 (GROUP C)	Cytotoxicity Control Lot #2006.158.001 (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
10^{-6}	0 0 0 0	0 0 0 0
10^{-7}	0 0 0 0	0 0 0 0
TCD_{50}/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$

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

TABLE 3: Neutralization Control Results

Non-Virucidal Level of the Test Substance (Neutralization Control)

Dilution	Test Virus + Cytotoxicity Control Lot #2006.003.001 (GROUP D)	Test Virus + Cytotoxicity Control Lot #2006.158.001 (GROUP D)
Cell Control	0 0 0 0	0 0 0 0
10^{-1}	+ + + +	+ + + +
10^{-2}	+ + + +	+ + + +
10^{-3}	+ + + +	+ + + +
10^{-4}	+ + + +	+ + + +
10^{-5}	+ + + +	+ + + +
10^{-6}	+ + + +	+ + + +
10^{-7}	+ + + +	+ + + +

(+) = 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 a $TCID_{50}$ of ≤ 0.5 $\log_{10}$ for both lots.