FINAL STUDY REPORT

STUDY TITLE

Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

Virus: Influenza A Virus

DATA REQUIREMENTS

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

PRODUCT IDENTITY

Jymrsa/MDF
A1 Batch # ATD07 + B1 Batch # BTH07
and
A2 Batch # ATH07 + B2 Batch # ATH07

PROTOCOL NUMBER

WBS01101905.FLUA

PROJECT NUMBER

A03454

AUTHOR

Karen M. Ramm, B.A.
Study Director

STUDY COMPLETION DATE

January 24, 2006

PERFORMING LABORATORY

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

SPONSOR

Jymrsa, Inc.
13570 Grove Drive, #281
Maple Grove, MN 55311

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: Jymrsa, Inc.

Company Agent: _____

_____
Title

_____ Date: _____
Signature

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:_____

Sponsor:_____ Date:_____

Study Director:_____ Date:_____
Karen M. Ramm, B.A.

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	December 28 2005	December 28, 2005	January 24, 2006
Final Report	January 20, 2006	January 20, 2006	

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

Quality Assurance Auditor:_____ Date:_____

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STUDY PERSONNEL

STUDY DIRECTOR: Karen M. Ramm, B.A.

Professional Personnel Involved:

Mary J. Miller, M.T.	- Research Scientist II
Kelleen Gutzmann, M.S.	- Research Scientist II
Katherine A. Paulson, M.L.T.	- Research Assistant II
Shanen Conway, 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: A03454

Protocol Number: WBS01101905.FLUA

Sponsor: Jymrsa, Inc.
13570 Grove Drive, #281
Maple Grove, MN 55311

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

TEST SUBSTANCE IDENTITY

Test Substance Name: Jymrsa/MDF

Lots: A1 Batch # ATD07 + B1 Batch # BTH07
and
A2 Batch # ATH07 + B2 Batch # ATH07

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 4, 2005
Study Initiation Date: November 17, 2005
Experimental Start Date: December 28, 2005
Experimental End Date: January 4, 2006
Study Completion Date: January 24, 2006

OBJECTIVE

The objective of this study was to evaluate the virucidal efficacy of a test substance against Influenza A virus 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:	Jymrsa/MDF, A1 Batch # ATD07 + B1 Batch # BTH07 and A2 Batch # ATH07 + B2 Batch # ATH07
Dilution:	Equal parts of A1 and B1 were mixed, and equal parts of A2 and B2 were mixed
Virus:	Influenza A virus, ATCC VR-544, Strain Hong Kong
Exposure Time:	Ten minutes
Exposure Temperature:	Room temperature (18-22°C)
Organic Soil Load:	5% fetal bovine serum
Efficacy Result:	Two batches of Jymrsa/MDF met the test criteria specified in the study protocol. The results indicate **complete inactivation** of Influenza A virus under these test conditions as required by the U.S. EPA for claims of virucidal activity.

TEST SYSTEM

1. Virus
The Hong Kong strain of Influenza A virus used for this study was obtained from the American Type Culture Collection, Manassas, VA (ATCC VR-544). The 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 2000 RPM for five minutes at 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 F66) was removed, thawed and refrigerated until use in the assay. The stock virus culture was adjusted to contain 5% 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,-8	4	32
Sample Batch #1 + virus (Group B)	-1,-2,-3,-4,-5,-6,-7,-8	4	32
Sample Batch #2 + virus (Group B)	-1,-2,-3,-4,-5,-6,-7,-8	4	32
Cytotoxicity of Batch #1 (Group C)	-1,-2,-3,-4,-5,-6,-7,-8	4	32
Cytotoxicity of Batch #2 (Group C)	-1,-2,-3,-4,-5,-6,-7,-8	4	32
Non-Virucidal level - Batch #1 (Group D)	-1,-2,-3,-4,-5,-6,-7,-8	4	32
Non-Virucidal level - Batch #2 (Group D)	-1,-2,-3,-4,-5,-6,-7,-8	4	32

METHODS

1. Preparation of Test Substance
   Two batches of Jymrsa/MDF (A1 Batch # ATD07 + B1 Batch # BTH07 and A2 Batch # ATH07 + B2 Batch # ATH07) were diluted by mixing equal parts of Batch A1 and Batch B1, and mixing equal parts of Batch A2 and Batch B2. The test substances were prepared by mixing 30.0 mL of Batch A1 with 30.0 mL of Batch B1, and mixing 30.0 mL of Batch A2 with 30.0 mL of Batch B2 on the day of testing. The prepared test substances were then transferred to individual trigger spray bottles provided by the testing facility. 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 19.9°C in a relative humidity of 57% 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 batch of test substance, one dried virus film was individually exposed for ten minutes at 19.9°C to the amount of spray released under use conditions. The carriers were sprayed until saturated (six 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 19.9°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 batch 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) 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 Influenza A virus, 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.

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 batches of Jymrsa/MDF (equal parts of A1 Batch # ATD07 mixed with equal parts of B1 Batch # BTH07 and equal parts of A2 Batch # ATH07 mixed with equal parts of B2 Batch # ATH07), exposed to Influenza A virus in the presence of a 5% fetal bovine serum soil load at 19.9°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 5.5 $\log_{10}$. Following exposure, test virus infectivity was not detected in the virus-test substance mixture for either batch at any dilution tested ($\leq$2.5 $\log_{10}$). Test substance cytotoxicity was observed in both batches at 2.5 $\log_{10}$. The neutralization control (non-virucidal level of the test substance) indicates that the test substance was neutralized at $\leq$2.5 $\log_{10}$ for both batches. Taking the cytotoxicity and neutralization control results into consideration, the reduction in viral titer was $\geq$3.0 $\log_{10}$ for both batches.

STUDY CONCLUSION

Under the conditions of this investigation, in the presence of a 5% fetal bovine serum soil load, Jymrsa/MDF (equal parts of A1 Batch # ATD07 mixed with equal parts of B1 Batch # BTH07 and equal parts of A2 Batch # ATH07 mixed with equal parts of B2 Batch # ATH07), demonstrated complete inactivation of Influenza A virus following a ten minute exposure time at 19.9 °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.

TABLE 1: Virus Control and Test Results

Effects of Jymrsa/MDF (A1 Batch # ATD07 + B1 Batch # BTH07 and A2 Batch # ATH07 + B2 Batch # ATH07) Following a Ten Minute Exposure to Influenza A Virus Dried on an Inanimate Surface

Dilution	Dried Virus Control (GROUP A)	Influenza A virus + A1 Batch ATD07 + B1 Batch BTH07 (GROUP B)	Influenza A virus + A2 Batch ATH07 + B2 Batch ATH07 (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 0 0
10^{-7}	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
$TCID_{50}$/0.1 mL	$10^{5.5}$	$\leq 10^{2.5}$	$\leq 10^{2.5}$

TABLE 2: Cytotoxicity Control Results

Cytotoxicity of Jymrsa/MDF on RMK Cell Cultures

Dilution	Cytotoxicity Control A1 Batch ATD07 + B1 Batch BTH07 (GROUP C)	Cytotoxicity Control A2 Batch ATH07 + B2 Batch ATH07 (GROUP C)
Cell Control	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
10^{-7}	0 0 0 0	0 0 0 0
10^{-8}	0 0 0 0	0 0 0 0
TCD_{50}/0.1 mL	$10^{2.5}$	$10^{2.5}$

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

TABLE 3: Neutralization Control Results

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

Dilution	Test Virus + Cytotoxicity Control A1 Batch ATD07 + B1 Batch BTH07 (GROUP D)	Test Virus + Cytotoxicity Control A2 Batch ATH07 + B2 Batch ATH07 (GROUP D)
Cell Control	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}	+ + + +	+ + + +
10^{-4}	+ + + +	+ + + +
10^{-5}	+ + + +	+ + + +
10^{-6}	+ + + +	+ + + +
10^{-7}	+ + + +	+ + + +
10^{-8}	+ + + +	+ + + +

(+) = 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
(T) = Cytotoxicity present

Results of the non-virucidal level control indicate that the test substance was neutralized at $TCID_{50}$ of $\leq 2.5_{10}$ for both batches.