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**FINAL STUDY REPORT**

PROTOCOL TITLE

Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

 **Virus: Human Coronavirus**

DATA REQUIREMENT

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

PRODUCT IDENTITY

Adantium  
06AD22.03 and 06AD23.03

PROTOCOL NUMBER

UPM02052803.COR.1

PROJECT NUMBER

A01644

AUTHOR

Mary J. Miller, M.T.  
Study Director

STUDY COMPLETION DATE

September 30, 2003

PERFORMING LABORATORY

ATS Labs  
2540 Executive Drive  
St. Paul, MN 55120

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SPONSOR REPRESENTATIVE

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## 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: IBHI srl

Company Agent:

SCIARAZZINI PhD URSO

Title

Signature

Date:

2003

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

Submitter: \_\_\_\_\_

Date: \_\_\_\_\_

Sponsor: \_\_\_\_\_

Date: \_\_\_\_\_

Study Director: Mary J. MillerDate: 9-30-03

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. 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	August 19, 2003	August 19, 2003	September 3, 2003
Draft Report	September 2, 2003	September 2, 2003	
Final Report	September 29, 2003	September 29, 2003	September 30, 2003

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

Quality Assurance Auditor:

*Rachelle L. Euxman*

Date:

*09/30/03*

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## STUDY PERSONNEL

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

Professional Personnel Involved:

Douglas G. Anderson, Ph.D.	- President
Karen M. Ramm, B.A.	- Technical Director
Mary J. Miller, M.T.	- Research Scientist II
Jennifer Palmen, B.S.	- Research Assistant I
Tonia Bevers	- Research Assistant I

## STUDY REPORT

### GENERAL STUDY INFORMATION

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

**Project Number:** A01644

**Protocol Number:** UPM02052803.COR.1

**Sponsor:** Dott. U. Sciamannini  
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**Sponsor Representative:** Dr. Cecilia Adami, IBHI Collaborator  
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**Testing Facility:** ATS Labs  
2540 Executive Drive  
St. Paul, MN 55120

### TEST SUBSTANCE IDENTITY

**Test Substance:** Adantium

**Lot/Batch(s):** 06AD22.03 and 06AD23.03

#### 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 13, 2003

**Study Initiation Date:** August 13, 2003

**Experimental Start Date:** August 19, 2003

**Experimental End Date:** August 27, 2003

**Study Completion Date:** September 30, 2003

### OBJECTIVE

The objective of this study was to evaluate the virucidal efficacy of a disinfectant against Human Coronavirus 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: Adantium , 06AD22.03 and 06AD23.03  
Dilution: 2.5% in filter sterilized deionized water  
Virus: Human Coronavirus, ATCC VR-740, Strain 229E  
Exposure Time: Three minutes  
Exposure Temperature: Room temperature  
Organic Soil Load: 5% fetal bovine serum  
Efficacy Result: Two batches of Adantium<sup>®</sup> (06AD22.03 and 06AD23.03) met the test criteria specified in the study protocol. The results indicate **complete inactivation** of Human Coronavirus under these test conditions as required by the U.S. EPA for claims of virucidal activity.

## TEST SYSTEM

1. Virus  
The 229E strain of Human Coronavirus used for this study was obtained from the American Type Culture Collection, Manassas, VA (ATCC VR-740). 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 approximately 1800 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 HCV-22) was removed, thawed and refrigerated until use in the assay. The stock virus culture contained 5% fetal bovine serum as the organic soil load. The stock virus tested demonstrated cytopathic effects (CPE) typical of Human Coronavirus on MRC-5 cells.
2. Test Cell Cultures  
MRC-5 (human embryonic lung) cells were obtained from ViroMed Laboratories, Inc., 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.
3. Test Medium  
Test medium used in this study was Eagle's minimal essential medium (E-MEM) supplemented with 2% heat-inactivated fetal bovine serum (FBS), 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B (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 <sub>10</sub> )	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 batch #1 + virus (Group B)	-1,-2,-3,-4,-5,-6,-7	4	28
Sample batch #2 + virus (Group B)	-1,-2,-3,-4,-5,-6,-7	4	28
Cytotoxicity of batch #1 (Group C)	-1,-2,-3,-4,-5,-6,-7	4	28
Cytotoxicity of batch #2 (Group C)	-1,-2,-3,-4,-5,-6,-7	4	28
Non-Virucidal level - batch #1 (Group D)	-1,-2,-3,-4,-5,-6,-7	4	28
Non-Virucidal level - batch #2 (Group D)	-1,-2,-3,-4,-5,-6,-7	4	28

## METHODS

- Preparation of Test Substance  
Adamantium<sup>389</sup> was prepared by adding 1.0 mL test substance to 39.0 mL filter sterilized deionized water (2.5% dilution) as requested by Sponsor. The test substance was in solution as determined by visual observation and used on the day of preparation.
- 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 15mm sterile glass petri dishes. The virus films were dried at 20.0°C in a relative humidity of 46% until visibly dry (20 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 batch of disinfectant, separate dried virus films were exposed to 2.0 mL of the use dilution for three minutes at room temperature (20.0°C). Following the 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<sup>-1</sup> dilution) was then titrated by 10-fold serial dilution and assayed for infectivity.

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 the same amount of time as the test film was exposed to the disinfectant. 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 titrated by 10-fold 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 MRC-5 cell cultures. Cytotoxicity of the MRC-5 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 MRC-5 cell line, which exhibits CPE in the presence of Human Coronavirus, 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 31-35°C in a humidified atmosphere of 5-7% CO<sub>2</sub> in sterile disposable cell culture labware. Due to an incubator failure, the cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO<sub>2</sub> for approximately 24 hours. (Actual temperature was 36.9°C with 6.0-6.1% CO<sub>2</sub>). The cultures were scored periodically for eight 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:**

Due to the failure of an incubator on August 20, 2003, the cultures were incubated for approximately 24 hours at 36-38°C in a humidified atmosphere of 5-7% CO<sub>2</sub> and not 31-35°C, as stated in the protocol. The titer of the dried virus control was 5.0 log<sub>10</sub>, which meets the requirement for a valid study. Therefore, incubating the cultures for approximately 24 hours at a higher temperature did not affect the quality or integrity of the data.

## **DATA ANALYSIS**

### **Calculation of Titers**

Viral and cytotoxicity titers are expressed as  $-\log_{10}$  of the 50 percent titration endpoint for infectivity (TCID<sub>50</sub>) or cytotoxicity (TCD<sub>50</sub>), 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<sub>50</sub> – Test Substance TCID<sub>50</sub> = Log Reduction

## **STUDY RETENTION**

### **Record Retention**

All of the original raw data developed exclusively for this study shall be archived at ATS Labs, 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 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

Results of tests with two batches of Adamantium<sup>389</sup> (06AD22.03 and 06AD23.03) exposed to Human Coronavirus for three 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<sub>10</sub>. Following exposure, test virus infectivity was not detected in the virus-test substance mixture for either batch at any dilution tested ( $\leq 1.5$  log<sub>10</sub>). Test substance cytotoxicity was observed in both batches at 1.5 log<sub>10</sub>. The neutralization control (non-virucidal level of the test substance) indicates that the test substance was neutralized at  $\leq 1.5$  log<sub>10</sub> for both batches. Taking the cytotoxicity and neutralization control results into consideration, the reduction in viral titer was  $\geq 3.5$  log<sub>10</sub> for both batches.

## STUDY CONCLUSION

Under the conditions of this investigation, in the presence of a 5% fetal bovine serum soil load, Adamantium<sup>389</sup> (06AD22.03 and 06AD23.03), diluted 2.5% in filter sterilized deionized water, demonstrated complete inactivation of Human Coronavirus 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: Effects of Adamantium<sup>389</sup> (06AD22.03 and 06AD23.03) Following a Three Minute Exposure to Human Coronavirus Dried on an Inanimate Surface**

Dilution	Dried Virus Control (GROUP A)	Human Coronavirus + 06AD22.03 (GROUP B)	Human Coronavirus + 06AD23.03 (GROUP B)
Cell Control	0 0 0 0	0 0 0 0	0 0 0 0
10 <sup>-1</sup>	+	T	T
10 <sup>-2</sup>	+	0	0
10 <sup>-3</sup>	+	0	0
10 <sup>-4</sup>	+	0	0
10 <sup>-5</sup>	+	0	0
10 <sup>-6</sup>	0	0	0
10 <sup>-7</sup>	0	0	0
TCID <sub>50</sub> /0.1 mL	10 <sup>5.0</sup>	≤10 <sup>1.5</sup>	≤10 <sup>1.5</sup>

**TABLE 2: Cytotoxicity of Adamantium<sup>389</sup> on MRC-5 Cell Cultures**

Dilution	Cytotoxicity Control 06AD22.03 (GROUP C)	Cytotoxicity Control 06AD23.03 (GROUP C)
Cell Control	0 0 0 0	0 0 0 0
10 <sup>-1</sup>	T	T
10 <sup>-2</sup>	0	0
10 <sup>-3</sup>	0	0
10 <sup>-4</sup>	0	0
10 <sup>-5</sup>	0	0
10 <sup>-6</sup>	0	0
10 <sup>-7</sup>	0	0
TCD <sub>50</sub> /0.1 mL	10 <sup>1.5</sup>	10 <sup>1.5</sup>

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

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

Dilution	Test Virus + Cytotoxicity Control 06AD22.03 (GROUP D)	Test Virus + Cytotoxicity Control 06AD23.03 (GROUP D)
Cell Control	0 0 0 0	0 0 0 0
10 <sup>-1</sup>	T T T T	T T T T
10 <sup>-2</sup>	+	+
10 <sup>-3</sup>	+	+
10 <sup>-4</sup>	+	+
10 <sup>-5</sup>	+	+
10 <sup>-6</sup>	+	+
10 <sup>-7</sup>	+	+

(+) = 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<sub>50</sub> of ≤1.5 log<sub>10</sub> for both batches.