FINAL STUDY REPORT

PROTOCOL TITLE

Residual Self-Sanitizing Efficacy

PRODUCT IDENTITY

2ADPHQ-03701J

DATA REQUIREMENTS

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

PROJECT NUMBER

10524

PROTOCOL NUMBER

ALP08020501.RES

AUTHOR

Brad K. Onstad, B.S.
Study Director
Chemcial Germicide Testing Services

STUDY COMPLETION DATE

April 6, 2001

PERFORMING LABORATORY

ViroMed Biosafety Laboratories
6101 Blue Circle Drive
Minneapolis, MN 55343

SPONSOR

Alphamed Pharmaceuticals
1409 N. Fort Harrison Avenue Suite A
Clearwater, FL 33755

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: Alphamed Pharmaceuticals

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).

Submitter:_____ Date:_____

Sponsor:_____ Date:_____

Study Director: R l Onstad Date: 4/6/01

Brad K. Onstad, B.S.

QUALITY ASSURANCE UNIT SUMMARY

Study: Residual Self-Sanitizing 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	Date	Study Director Review	Management Review
Critical Phase	February 27, 2001	February 27, 2001	April 6, 2001
Final Report	April 4, 2001	April 4, 2001	

Documentation of the above Quality Assurance audits have been reviewed.

Quality Assurance Auditor: Rachelle L. Torman Date: 04/06/01

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STUDY PERSONNEL

STUDY DIRECTOR: Brad K. Onstad, B.S.

Professional personnel involved:

Bonita L. Baskin, Ph.D.	- Laboratory Director
Karen M. Ramm, B.A.	- Division Director
Brad K. Onstad, B.S.	- Laboratory Supervisor
Adam W. Pitt, B.S.	- Research Assistant I
Travis M. Roth, B.A.	- Research Assistant I
Kristine E. Roth	- Research Assistant I
Josh P. Magnusson	- Research Assistant I

STUDY REPORT

GENERAL STUDY INFORMATION

Study Title: Residual Self-Sanitizing Efficacy

Project Number: 10524

Protocol Number: ALP08020501.RES

Sponsor: Alphamed Pharmaceuticals
1409 N. Fort Harrison Avenue Suite A
Clearwater, FL 33755

Test Facility: ViroMed Biosafety Laboratories
6101 Blue Circle Drive
Minneapolis, MN 55343

TEST SUBSTANCE IDENTITY

Test Substance Name: 2ADPHQ-03701J

Test Substance Characterization
Test substance characterization as to content, stability, etc., (40 CFR, Part 160, Subpart F [160.105]) is the responsibility of the Sponsor.

STUDY DATES

Date Sample Received: February 19, 2001
Study Initiation Date: February 16, 2001
Experimental Start Date: February 26, 2001
Experimental End Date: March 7, 2001
Study Completion Date: April 6, 2001

OBJECTIVE

The objective of this study was to determine the "Residual Self-Sanitizing Activity" of the Sponsor's test material.

STUDY MATERIALS

Test System/Growth Media

Test Organism	ATCC #	Growth Medium	Incubation Parameters
Salmonella choleraesuis	10708	AOAC Synthetic Broth	35-37°C, aerobic

The microorganism used in this study was obtained from the American Type Culture Collection, Manassas, Virginia.

Recovery Media

Neutralizing Subculture Medium: Letheen Broth
Agar Plate Medium: Tryptic Soy Agar with 5% sheep blood (BAP)

Reagents

Organic Soil Load Description: No organic soil was required

TEST METHOD

Carriers

Approximately 75mm x 25mm glass slides were placed in glass petri dishes and sterilized in an air oven for 2 hours at approximately 180°C. Individual sterile plastic petri dishes were matted with 9 cm filter paper. One sterile glass slide was transferred into each of the matted petri dishes.

Medication

The carriers were allowed to equilibrate to ambient temperature prior to medication. Each carrier was then treated with the test substance in the manner specified by the Sponsor. The spray bottle was held 4-6 inches from the carrier. The surface was sprayed for 3 seconds at which point the carriers were completely wet. A timer was started for the holding period.

Holding Periods

The petri dishes containing the treated carriers were held at room temperature with their lids ajar for the Sponsor specified 24 hour, 72 hour and 7 day holding periods.

Preparation of Test Organism

A 0.5 McFarland turbidity suspension was prepared in Butterfield's Buffer using a stock culture of the test organism. A lawn of growth was prepared by adding 0.2 mL of this suspension to agar plates and incubating 48 $\pm$ 4 hours at 35-37°C. A 3 mL aliquot of Butterfield's Buffer was transferred to each agar plate and cell swabbed to suspend the organisms. The suspension was filtered through sterile gauze and diluted to a 0.5 McFarland turbidity standard. Prior to use in testing procedures, the suspension was Vortex mixed and a 1:50 dilution in Butterfield's Buffer was made.

Carrier Contamination

After the specified holding times, 0.01 mL of the test organism suspension was added to the designated areas of separate replicates of previously treated carriers at staggered intervals. The test organisms were allowed to contact the product residue for a 15 minute exposure time at ambient temperature (21-22°C).

Subculture
Following the 15 minute exposure period, each carrier was transferred at identical staggered intervals to 40 mL of Letheen Broth and vortex mixed. The number of survivors from each carrier was determined by a standard spread plate method done on the above and/or serial dilutions thereof, using TSA containing 5% Sheep Blood (BAP).

Incubation and Observation
All subculture plates and jars were incubated for 48 ± 4 hours at 35-37°C. Following incubation, the subculture plates and jars were visually examined for growth. The colonies present on each plate were counted and recorded and the number of Colony Forming Units present on each carrier or in each suspension was calculated. Representative subculture plates demonstrating growth were confirmed to be the test organisms.

TEST CONTROLS

Numbers Control
The test procedure was performed as described using three sterile untreated carriers. The carriers were held for the appropriate holding periods, inoculated and held for the identical exposure period and subcultured in an identical manner to the test carriers. The subculture suspension was serially diluted and plated using standard microbiological techniques. Following incubation, the organism plates were observed to enumerate the concentration of the test organism present at the time of testing.

Initial Suspension Population Control
The number of microbes present in the initial suspension used in the contamination procedure was determined by a standard spread plate count procedure using agar identical to the test procedure

Purity Control
A "streak plate for isolation" was performed on the organism culture and following incubation examined in order to demonstrate a pure culture.

Carrier Sterility Control
A representative sterile carrier was added to the subculture media, incubated, and observed for lack of growth to confirm sterility.

Media Sterility Control
Uninoculated subculture medium was incubated and observed for lack of growth to confirm sterility.

Viability Control
The viability of a representative inoculated carrier of the test organism was confirmed by subculture.

Neutralization Confirmation
The neutralization of the test substance was confirmed by exposing sterile carriers to the test substance and transferring them to 40 mL of neutralizing broth. The neutralization jar was inoculated with 1.0 mL of an organism suspension containing low levels of organism, held for five minutes and 1.0 mL was plated in duplicate to the appropriate subculture medium.

A numbers control not containing the test material was also included and compared to the test, in which a sterile carrier was transferred to 40 mL of neutralizing broth. The neutralization jar was inoculated with 1.0 mL of an organism suspension containing low levels of organism, held for five minutes and 1.0 mL was plated in duplicate to the appropriate subculture medium.

STUDY ACCEPTANCE CRITERIA

Test Substance Performance Criteria
The EPA requires the results to show a bacterial reduction of at least 99.9% over the parallel control.

Control Acceptance Criteria
The study controls performed according to acceptance criteria.

PROTOCOL CHANGES

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

DATA ANALYSIS

Calculations

The colonies present on each plate were counted and recorded and the number of CFU present on each carrier or in each suspension was calculated.

1. CFU/Carrier = $\frac{\text{CFU (Average of Plates) x Dilution Factor x Volume of Neutralizing Broth}}{\text{Number of Carriers to be Tested x Volume Plated}}$

2. Geometric Mean of Number of Organisms Surviving on Control Carriers:

   The geometric mean of the number of organisms surviving on the three inoculated control carriers was determined by the following equation:

   $$\text{Geometric Mean} = \frac{\text{Antilog of } \text{Log}_{10}X_1 + \text{Log}_{10}X_2 + \text{Log}_{10}X_3}{3}$$

   where X equals number of organisms surviving per control carrier.

   An average of at least 4.0×10^4 organisms must have survived on the inoculated control carriers for the test to be valid.

3. Geometric Mean of Number of Organisms Surviving on Test Carriers:

   The geometric mean of the number of organisms surviving on the test carriers was determied by the following equation:

   $$\text{Geometric Mean} = \frac{\text{Antilog of } \text{Log}_{10}Y_1 + \text{Log}_{10}Y_2 + \text{Log}_{10}Y_3}{3}$$

   where Y equals number of organisms surviving per test carrier.

4. Percent Reduction: The following equation was used to calculate percent reduction:

   $$\% \text{ reduction} = [(a - b) / a] \times 100$$

   where: a = geometric mean of the number of organisms surviving on the control carriers
   b = geometric mean of the number of organisms surviving on the test carriers.

Statistical Analysis

None used.

STUDY RETENTION

Record Retention

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

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

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.

RESULTS

Control and Neutralization Results (Tables 1-3, 5)

All data measurements/controls including the numbers, viability, carrier sterility, neutralization confirmation, culture purity and media sterility controls were within acceptance criteria.

Test Results (Tables 4 and 6)

ANALYSIS

2ADPHQ-03701J, ready to use, demonstrated >99.999% reduction of *Salmonella choleraesuis* following a 24 hour hold, a ≥99.999% reduction of *Salmonella choleraesuis* following a 72 hour hold, and a >99.999% reduction of *Salmonella choleraesuis* following a 7 day hold with a 15 minute contact period.

STUDY CONCLUSION

Under the conditions of this investigation, 2ADPHQ-03701J, ready to use, is an effective residual sanitizer against *Salmonella choleraesuis* for hard nonporus surfaces after a 24 hour, 72 hour and 7 day hold with a 15 minute contact period.

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: CONTROL RESULTS

Type of Control	Result		
	24 Hour Hold	72 Hour Hold	7 day Hold
Culture Purity	Pure	Pure	Pure
Viability Control	Growth	Growth	Growth
Media Sterility Control	No Growth	No Growth	No Growth
Carrier Sterility Control	No Growth	No Growth	No Growth

TABLE 2: NEUTRALIZATION CONFIRMATION CONTROL RESULTS (CFU)

Test Substance	24 hour	72 hour	7 day
2ADPHQ-03701J	37, 57	5, 3	5, 8
Numbers Control	60, 52	5, 4	10, 7

The neutralization controls demonstrated sufficient growth, eliminating bacteriostasis as a cause of lack of growth in the test system.

TABLE 3: INITIAL SUSPENSION RESULTS (CFU/mL)

Test Organism	24 hour	72 hour	7 day
S. choleraesuis	1.1×10^8	8.0×10^6	1.36×10^7

TABLE 4: EVALUATION OF TEST CARRIER DATA (CFU/CARRIER)

Test Organism	Carrier #	Residual Holding Period		
		24 hour	72 hour	7 day
S. choleraesuis	1	< 1	< 40	< 1
	2	< 1	< 1	< 1
	3	< 1	< 1	< 1
	Average	< 1	< 14	< 1

TABLE 5: EVALUATION OF NUMBERS CONTROL CARRIER DATA (CFU/CARRIER)

Test Organism	Carrier #	Residual Holding Period		
		24 hour	72 hour	7 day
S. choleraesuis	1	2.9×10^5	4.38×10^5	3.6×10^5
	2	3.4×10^5	3.3×10^5	3.3×10^5
	3	3.9×10^5	4.40×10^5	3.4×10^5
	Average	**3.4×10^5**	**4.0×10^5**	**3.4×10^5**

TABLE 6: CALCULATED VALUES

Residual Holding Period	Control Log_{10} (Average)	Test Log_{10} (Average)	Control Geometric Mean (Average CFU/ Carrier)	Test Geometric Mean (Average CFU/ Carrier)	% Reduction
24 Hour	5.528	0	3.4×10^5	1	>99.999
72 Hour	5.601	0.534	4.0×10^5	3.4	≥99.999
7 Day	5.535	0	3.4×10^5	1	>99.999