



**NON-GLP
FINAL STUDY REPORT**

STUDY TITLE

ISO ACUTE SYSTEMIC INJECTION TEST

TEST ARTICLE IDENTIFICATION

Silverdyne
Lot # A

STUDY COMPLETION DATE

December 4, 2007

PERFORMING LABORATORY

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

SPONSOR

World Health Alliance International Inc.
3252 Wolfe Court
Fremont, CA 94555

PROJECT NUMBER

66960



ISO ACUTE SYSTEMIC INJECTION TEST RESULTS

Test Article Name: Silverdyne
Lot/Batch #: A
Other Identifier: Not Given
Stability: None
Sterilization Method: Non-Sterile
Storage Conditions: Room Temperature
Safety Precautions: Standard Precautions
Extraction Conditions: Not Applicable
Intended Use/Application: To eliminate bacteria

Date Sample Received: 09/28/07
Study Completion Date: 12/04/07

PURPOSE: The purpose of this test was to screen the test article for potential toxic effects as a result of a single-dose systemic injection in mice.

TEST SAMPLE PREPARATION: The test article was prepared for injection by adding 50 drops of the Sponsor-supplied test article to 1 liter of sterile tap water, and allowing to sit for 1 hour prior to dosing.

EXPERIMENTAL METHODS SUMMARY: Groups of five (5) Albino Swiss Mice (*Mus musculus*) were injected systemically with extracts of the test article or control vehicle at a dose rate of 50 mL extract to one kg body weight. The animals were observed for signs of toxicity immediately after injection and at 4 ± 0.75 , 24 ± 2 , 48 ± 2 and 72 ± 2 hours post-injection. According to ISO Guidelines, the test is considered negative if none of the animals injected with the test article extract show a significantly greater biological reaction than the animals treated with the control vehicle extract. A significant biological reaction is interpreted as death in two or more mice or other toxic signs such as convulsions, prostration, or body weight loss greater than 10 % in three or more mice.

RESULTS:

DATA TABLE A: MORTALITY, CLINICAL SIGNS AND WEIGHT LOSS INCIDENCE

	FATALITIES	TOXICITY CLINICAL SIGNS	ANIMALS WITH >10% BODY WEIGHT LOSS
	TEST	TEST	TEST
Neat Test	0/5	1/5 ^a	0/5
	CONTROL	CONTROL	CONTROL
Sterile Water	1/5 ^b	0/5	0/4

a = One test animal was caught in the restrainer during the dosing process, and was noted to be prostrate, lethargic, and had tremors after dosing. However, the animal recovered and was noted as normal for the remainder of the study. The clinical observations were attributed to the dosing procedure.

b = One control animal died immediately post injection.

STATISTICAL METHODS: Descriptive Statistics are presented in Data Table B.

CONCLUSION: TEST ARTICLE PASSES THE TEST.

Approval: 
 Spencer Kubo, BS – Study Director

Date: 12/14/07

TECHNICAL REFERENCES:

ISO 10993-11: 2006 Biological Evaluation of Medical Devices Part 11: Tests for Systemic Toxicity

ISO 10993-12: 2002 Standard, Biological Evaluation of Medical Devices, Part 12-Sample Preparation and Reference Materials.

United States Pharmacopeia USP 30 (2007), Section 88, Biological Reactivity Test, *In Vivo*, Systemic Injection Test, page 116-117.

**DATA TABLE B: ANIMAL WEIGHTS (g) AND
STANDARD DEVIATION CALCULATIONS**

Group	Animal #	Initial	24 Hrs	48 Hrs	72 Hrs	BW Change
Test Neat Saline	1	18.4	17.9	17.9	18.4	0.0
	2	18.7	17.7	17.3	17.5	-1.2
	3	19.3	18.1	18.4	18.8	-0.5
	4	18.4	18.2	18.0	18.3	-0.1
	5	17.0	16.6	16.8	17.4	0.4
Average Body Weight		18.4	17.7	17.7	18.1	-0.3
Standard Deviation		0.8	0.6	0.6	0.6	0.6

Group	Animal #	Initial	24 Hrs	48 Hrs	72 Hrs	BW Change
Control Sterile Water	11*	17.9	-	-	-	-
	12	18.6	18.5	18.6	18.9	0.3
	13	18.8	17.6	17.9	17.5	-1.3
	14	17.5	17.5	17.2	17.6	0.1
	15	17.9	17.6	18.7	18.6	0.7
Average Body Weight		18.1	17.8	18.1	18.2	0.0
Standard Deviation		0.5	0.5	0.7	0.7	0.9

* Animal died after dosing.