

**NELSON**  
LABORATORIES

07 Sep 2007

Charles Veach  
World Health Alliance International, Inc.  
3052 Wolfe Ct.  
Fremont, CA 94555

Dear Charles,

Enclosed is the final report for the testing we coordinated for you. The information is retained by the testing laboratory.

NELSON NUMBER: 388875

TESTING LAB: AppTec Laboratory Services

TYPE OF TEST: ISO Acute Systemic Injection Test

SAMPLE IDENTIFICATION:

Silverdyne (World Health Alliance International, Inc.)  
NutraSilver (Beneficial Solutions, LLC.)

If you have any questions, please feel free to call any of our Subcontracting personnel at 801-963-2600 or 800-826-2088. Thank you for testing with Nelson Laboratories, Inc.

*Jennifer Shaw*

Jennifer Shaw, B.S.  
Subcontracting Coordinator

*07 Sep 2007*

Sign Date





St. Paul



Report

**FINAL STUDY REPORT**

**STUDY TITLE**

**ISO Acute Systemic Injection Test**

**TEST ARTICLE IDENTIFICATION**

Silverdyne (World Health Alliance International, Inc.)  
NutraSilver (Beneficial Solutions, LLC.)

**STUDY COMPLETION DATE**

September 6, 2007

**PERFORMING LABORATORY**

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

**SPONSOR**

Nelson Laboratories, Inc.  
6280 South Redwood Road  
Salt Lake City, UT 84123

**PROTOCOL**

9017700

**PROJECT NUMBER**

63223

**NLI#**

388875

*Reference PO # APP-2007*



NEL05



63223



jshaw@nelsonlabs.com



### QUALITY ASSURANCE UNIT SUMMARY

STUDY: ISO Acute Systemic Injection Test.

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. This study has been performed under Good Laboratory Practices regulations (FDA, 21 CFR, Part 58 - Good Laboratory Practice for Nonclinical Laboratory Studies) 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 dates listed below. Studies are inspected at time intervals to assure the quality and integrity of the study.

<u>Phase Inspected</u>	<u>Date</u>	<u>Study Director</u>	<u>Management</u>
Dosing	08/29/07	08/30/07	09/06/07
Final Report	09/06/07	09/06/07	09/06/07

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

Quality Assurance Auditor:  Date: 9/6/07  
David Slotten

### GOOD LABORATORY PRACTICES STATEMENT

The study referenced in this report was conducted in compliance with U.S. Food and Drug Administration Good Laboratory Practice (GLP) regulations set forth in 21 CFR part 58.

The studies not performed by or under the direction of AppTec Laboratory Services, are exempt from this Good Laboratory Practice Statement and include characterization and stability of the test compound(s)/test article.

Study Director:  Date: 9/6/07  
Spencer Kubo

Professional Personnel Involved:

Bonita L. Baskin, Ph.D.	Chief Executive Officer
Lisa Olson, BS	Vice President St. Paul Operations
Don Palme, Ph.D.	Vice President In-Life Services
Roxanne Miller, AA, CVT	Manager, In-Life Technical Services
Spencer Kubo, BS	Study Director
Lynn Wallum, CVT	Animal Care Manager, In-Life Facility
Jean Mesarich, AA	Client Relations Manager

**PROJECT NUMBER:** 63223

**SPONSOR:** Nelson Laboratories, Inc.  
6280 South Redwood Road  
Salt Lake City, UT 84123

**RECORD RETENTION:** An exact copy of the original final report and all raw data pertinent to this study will be stored at AppTec Laboratory Services, 2540 Executive Drive, St. Paul, MN 55120. It is the responsibility of the Sponsor to retain a sample of the test article.

**SAMPLE IDENTIFICATION:** Nelson Laboratories, Inc.  
Silverdyne (World Health Alliance International, Inc.)  
NutraSilver (Beneficial Solutions, LLC.)  
NLI #: 388875

**CHARACTERIZATION:** The Sponsor was responsible for all test article characterization data as specified in the GLP regulations. The identity, strength, stability, purity, and chemical composition of the test article were solely the responsibility of the Sponsor. The Sponsor was responsible for supplying to the testing laboratory results of these determinations and any others that may have directly impacted the testing performed by the testing laboratory, prior to initiation of testing. Furthermore, it was the responsibility of the Sponsor to ensure that the test article submitted for testing was representative of the final product that was subjected to materials characterization. Any special requirements for handling or storage were arranged in advance of receipt and the test article was received in good condition.

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

**TEST FACILITY:** AppTec Laboratory Services  
2540 Executive Drive  
St. Paul, MN 55120

**DATE TEST ARTICLE RECEIVED:** 08/13/07  
**STUDY INITIATION DATE:** 08/15/07  
**STUDY COMPLETION DATE:** 09/06/07  
**IACUC APPROVAL NUMBER:** 98-03D

**METHOD:** This study was conducted in accordance with the ISO 10993-11: 2006 Biological Evaluation of Medical Devices Part 11: Tests for Systemic Toxicity.

**EXPERIMENTAL SUMMARY:** For the safety evaluation of the test article, mice were injected systemically with a solution of the test article and a control. The animals were observed for signs of toxicity immediately after injection and at 4, 24, 48, and 72 hours post-injection. The requirements of the test are met if none of the animals treated with the test article solution have a significantly greater adverse reaction than the animals treated with the control. None of the test article solution treated animals demonstrated adverse clinical signs at any of the observation periods and none of the animals lost in excess of 10 % of body weight over the course of the study. Therefore, the requirements of the ISO Acute Systemic Injection Test **have been met** by Nelson Laboratories, Inc., Silverdyne (World Health Alliance International, Inc.) NutraSilver (Beneficial Solutions, LLC.).

**DEVIATIONS/AMENDMENTS:** None.

## TEST MATERIAL AND PREPARATION

### Test Article Identification:

Test Article Name: Silverdyne (World Health Alliance International, Inc.)  
NutraSilver (Beneficial Solutions, LLC.)  
Lot/Batch #: Not Applicable  
NLI#: 388875  
Stability (Expiration): Not Applicable  
Storage Conditions: Room Temperature  
Safety Precautions: Standard Precautions

**Sterilization:** The Sponsor indicated that the test article was not sterilized prior to submitting the sample for testing.

**Test Sample Preparation:** Per the Sponsor's instructions, the test article was prepared by adding 3 drops of the liquid test article into one liter of sterile water. The mixture was allowed to stand at room temperature for 30 minutes prior to dosing. Sterile water that was also at room temperature was used as the control. USP sterile water was used, with a lot number of 702049F and an expiration date of February 2010.

## TEST SYSTEM

**Species/Strain/Sex:** Albino Swiss mouse (*Mus musculus*), ND4, naive, female

**Age:** No specific age required.

**Source:** Harlan Sprague Dawley, Indianapolis, IN

ANIMAL NUMBER AND WEIGHTS:	MOUSE #
Test Group:	1 - 5
Control Group:	11 - 15

All animals weights were within the 17 - 23 g weight limit at the start of the study.

**Animal Identification:** Individually identified by ink marking.

## HUSBANDRY

**Receipt:** Animals were received on 08/15/07. Each animal was examined for signs of disease and injury prior to entry into the research area.

**Housing:** Animals were housed in groups of 5 in polycarbonate cages with contact bedding. Housing density complied with AAALAC International recommendations and NIH guidelines.

**Environment:** Animal rooms were maintained under AAALAC International recommendations and the "Guide for Care and Use of Laboratory Animals". The laboratory and animal rooms were maintained as limited - access facilities.

**Diet:** Animals were supplied with Harlan Certified Rodent Diet. No known contaminants present in the feed were expected to interfere with the test results.



**Water:** Animals were supplied with potable water obtained from the St. Paul municipal water supply. No known contaminants present in the water were expected to interfere with the results.  
**Termination:** Following the final observations the animals were euthanized by CO<sub>2</sub> asphyxiation.

**Compliance:** The care, housing and handling of the animals were in compliance with AAALAC International guidelines as reported in the "Guide for the Care and Use of Laboratory Animals", National Research Council - ILAR, Revised 1996; (OPRR), "Public Health Service Policy on Humane Care and Use of Laboratory Animals", Health Research Extension Act of 1985 (Public Law 99-158), Revised 1986, and USDA, Department of Agriculture, Animal and Plant Health Inspection Service, 9 CFR, Parts 1, 2 and 3, Animal Welfare, Final Rule 1989.

**SELECTION OF ANIMALS:** Animals were randomly placed in cages upon receipt and were placed on study as available. Animals considered unsuitable due to poor health or outlying body weight were excluded from the study.

**TEST ARTICLE ADMINISTRATION:** Groups of 5 animals were injected with either the test article solution or the corresponding control as indicated in the table below:

TEST VEHICLE OR CONTROL	ROUTE	DOSE/KG	INJECTION RATE
Sterile Water	Intravenous	50 mL	~0.1 mL/sec

#### OBSERVATIONS

**Body Weights:** Body weight recordings, to the nearest 0.1 g, were made on the day of dosing and at 24, 48 and 72 ± 2 hours post-injection. See Data Table B for individual weight results.

**Clinical Signs:** Observations for mortality and signs of pharmacological and/or toxicological effects were made immediately post-injection and at 4 ± 0.75, 24 ± 2, 48 ± 2, and 72 ± 2 hours post-injection.

**EVALUATION CRITERIA:** According to ISO Guidelines, the test is considered negative if none of the animals injected with the test article solution show a significantly greater biological reaction than the animals treated with the control. 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 are interpreted as significant biological reactions.

**RESULTS:** None of the test animals on study were observed with abnormal clinical signs indicative of toxicity during the 72 hour test period. Body weight loss was within acceptable parameters over the course of the study for the test animals. One control animal died immediately after dosing, and one control animal was noted with convulsions immediately after dosing. Body weight loss was within acceptable parameters over the course of the study for the control animals. See Data Table A.

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

VEHICLE	FATALITIES		TOXICITY CLINICAL SIGNS		ANIMALS WITH >10% BODY WEIGHT LOSS	
	TEST	CONTROL	TEST	CONTROL	TEST	CONTROL
Sterile Water	0/5	1/5	0/5	1/5	0/5	0/5



**ANALYSIS AND CONCLUSION:** One control animal died immediately after dosing and one control animal was noted with convulsions immediately after dosing. The animal noted with convulsions later recovered and was noted as normal throughout the remainder of the study. Since not more than 2 control animals died or were observed with clinical signs of toxicity, and since no lost weight in excess of 10%, the test was considered valid. The injection of sterile water that was not isotonic was likely the cause of the reactions observed in the control animals. None of the test article solution treated animals were observed with clinical signs consistent with toxicity at any of the observation periods and body weight changes were within acceptable parameters over the course of the study. These findings indicate that the requirements of the ISO Acute Systemic Injection Test have been met by Nelson Laboratories, Inc., Silverdyne (World Health Alliance International, Inc.) NutraSilver (Beneficial Solutions, LLC.).

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

**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	1	18.6	18.6	17.9	17.8	-0.8
	2	21.7	21.5	20.8	20.1	-1.6
	3	19.1	19.1	19.2	18.8	-0.3
	4	21.5	21.8	21.0	20.5	-1.0
	5	18.3	18.1	17.7	17.7	-0.6
Average Body Weight		19.8	19.8	19.3	19.0	-0.9
Standard Deviation		1.6	1.7	1.6	1.3	0.5

Group	Animal #	Initial	24 Hrs	48 Hrs	72 Hrs	BW Change
Control Neat	11	20.7	19.8	20.0	19.4	-1.3
	12	21.1	20.1	19.8	20.1	-1.0
	13	18.6	18.4	18.3	18.0	-0.6
	14	19.1	18.3	19.0	19.1	0.0
	15	17.0	N/A	N/A	N/A	N/A
Average Body Weight		19.3	19.2	19.3	19.2	-0.7
Standard Deviation		1.7	0.9	0.8	0.9	0.6