Non-GLP
FINAL STUDY REPORT

STUDY TITLE:	**ISO Acute Systemic Injection Test**
TEST CODE:	9017700
TEST ARTICLE IDENTIFICATION:	PC-ISO (White)
PERFORMING LABORATORY:	WuXi AppTec, Inc. 2540 Executive Drive St. Paul, MN 55120
SPONSOR:	Stratasys, Inc. 7665 Commerce Way Eden Prairie, MN 55344
STUDY NUMBER:	145179
CLIENT MNEMONIC:	STR05
RESULT SUMMARY:	The requirements of the ISO Acute Systemic Injection Test **have been met** by the test article.

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

TEST FACILITY: WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

DATE TEST ARTICLE RECEIVED: 10/14/10
STUDY COMPLETION DATE: 11/08/10

TEST ARTICLE IDENTIFICATION

Test Article Name:	PC-ISO (White)
Lot/Batch #:	Not Applicable
Sterilization Method:	Non-Sterile
Physical State:	Insoluble Material
Expiration Date:	Not Applicable
Storage Conditions:	Room Temperature

CHARACTERIZATION: The Sponsor was responsible for all test article characterization data as specified in the 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.

SAMPLE STORAGE
Upon receipt by the Sample Receiving Department, the test samples were placed in a designated, controlled access storage area ensuring proper temperature conditions. Test and control article storage areas are designed to preclude the possibility of mix-ups, contamination, deterioration or damage. The samples remained in the storage area until retrieved by the technician for sample preparation and/or testing.

SAFETY
Appropriate routine safety procedures were followed in handling the test article, unless more cautious procedures were specified by the Sponsor. All applicable WuXi AppTec safety policies and procedures were observed during the performance of the test.

EXPERIMENTAL DESIGN

Experimental Summary
Animals were treated by intravenous or intraperitoneal routes to screen solutions or test article extracts for potential toxic effects as a result of a single-dose systemic injection. The animal species, number, and route of test article administration were as recommended in ISO 10993-11.

For the safety evaluation of the test article, mice were injected systemically with extracts of the test article in standard solutions (normal saline and cottonseed oil). 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 extract have a significantly greater adverse reaction than the animals treated with the vehicle control.

Justification For Selection Of The Test System
Mice were used in this study because they have historically been used in systemic safety evaluation studies and the guidelines have no alternative (non-animal) methods. Animals were treated by intravenous and intraperitoneal routes. The animal species, number, and route of test article administration were as recommended in ISO 10993-11.

Institutional Animal Care and Use Committee (IACUC)
The protocol and any amendments or procedures involving the care or use of animals on this study were reviewed and approved by the WuXi AppTec IACUC prior to the initiation of such procedures.

IACUC Protocol / Approval Date: 98-03E / May, 2010

AMENDMENTS / DEVIATIONS: None.

IDENTIFICATION OF TEST SYSTEM

Species/Strain Albino Swiss mice (*Mus musculus*), ND4, naive

Source: Harlan Laboratories

Sex: Female. Any females used were nulliparous and not pregnant.

Weight Range: All animal weights were within ± 20% of the mean body weight at the start of the study.

Age: All animals were young adults at the start of the study.

Animal Identification: The animals were identified per WuXi AppTec SOP: ILS-0112.

Animal Numbers:	**Mouse #**
0.9% Normal Saline Test Group:	1 - 5
0.9% Normal Saline Control Group:	21 - 25
Cottonseed Oil Test Group:	11 - 15
Cottonseed Oil Control Group:	31 - 35

HUSBANDRY

Receipt: Animals were received on 10/20/10 according to WuXi AppTec SOP: ILS-0092. Each animal was examined for signs of disease and injury. The animals were acclimated for a minimum of 5 days under the same conditions as the actual test.

Housing: Animals were housed in groups of five in polycarbonate cages with contact bedding. Housing density complied with AAALAC International recommendations and NIH guidelines. The test and control animals were housed separately.

Environment: The environmental conditions in the animal rooms were maintained according to WuXi AppTec SOP: ILS-0018. The temperature and photo-period were set to meet the AAALAC International recommendations for these species. The laboratory and animal rooms were maintained as limited-access facilities.

Diet: Animals were supplied with a certified commercial 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.

TEST MATERIAL PREPARATION
According to the Sponsor, the test article consisted of filament. A representative sample of the test article was cut for extraction, placed into test tubes and prepared at a ratio of 60 cm^2 to 20 mL of extraction vehicle.

TEST ARTICLE EXTRACTION
The extraction mixtures and corresponding control blanks were incubated for 24 ± 2 hours at 70 ± 2 °C. At the end of the extraction period, the vessels were shaken well and the liquid aseptically decanted into a sterile glass vessel. The test article was observed after all extractions to be intact with no macroscopically observable degradation. The extracts were maintained at room temperature and used within 24 hours of preparation.

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 five animals were injected with either the test article extract or the corresponding control vehicle as indicated in the table below:

EXTRACT OR CONTROL	ROUTE	DOSE/KG	INJECTION RATE
NS	Intravenous	50 mL	~0.1 mL/sec
CSO	Intraperitoneal	50 mL	Not Applicable

OBSERVATIONS

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

TERMINATION
Following the final observations the animals were euthanized by CO_2 asphyxiation.

EVALUATION CRITERIA
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. 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.

ASSAY VALIDITY
Final evaluation of the validity of the assay and test article results was based upon the following criteria and scientific judgment:

A control failure is defined as death in two or more control animals showing signs of toxicity such as convulsions or prostration or weight loss of more than 10% of body weight in three or more mice.

METHOD FOR CONTROL OF BIAS: Not applicable.

DATA ANALYSIS: Not applicable.

STATISTICAL METHODS: Descriptive statistics are presented in Data Table 2.

RECORD RETENTION: An exact copy of the original final report and all raw data pertinent to this study will be stored by WuXi AppTec, Inc. It was the responsibility of the Sponsor to retain a sample of the test article.

COMPLIANCE
The care, housing and handling of the animals were in compliance with:

- AAALAC International and NIH 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.
- USDA, Department of Agriculture, Animal and Plant Health Inspection Service, 9 CFR, Parts 1, 2, and 3, Animal Welfare, Final Rule 1989.

TEST ARTICLE DISPOSITION: Unused test samples remain in the storage area until all testing is completed. Once completed, the remaining samples will be saved as requested by the Sponsor.

RESULTS
None of the animals on study were observed with abnormal clinical signs indicative of toxicity during the 72 hour test period. All were alive at the end of the 72 hour test duration and body weight loss was within acceptable parameters over the course of the study. See Table 1.

TABLE 1: MORTALITY, CLINICAL SIGNS AND WEIGHT LOSS INCIDENCE

EXTRACT	FATALITIES		TOXICITY CLINICAL SIGNS		ANIMALS WITH >10% BODY WEIGHT LOSS	
	TEST	CONTROL	TEST	CONTROL	TEST	CONTROL
NS	0/5	0/5	0/5	0/5	0/5	0/5
CSO	0/5	0/5	0/5	0/5	0/5	0/5

ANALYSIS AND CONCLUSION
The vehicle control treated animals had no signs of toxicity at any of the observation periods and no animals lost weight in excess of 10%, indicating a valid test. None of the test article extract treated animals were observed with clinical signs consistent with toxicity at any of the observation periods. 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 the test article.

Approval: Michelle Dietzel Date: 11/8/10
Michelle Dietzel, BS – Senior Study Director

TABLE 2: ANIMAL WEIGHTS (g) AND STANDARD DEVIATION CALCULATIONS

GROUP	ANIMAL #	INITIAL	24 HRS	48 HRS	72 HRS	BW CHANGE
Test NS	1	21.8	22.9	22.3	22.2	0.4
	2	22.8	22.6	22.3	22.3	-0.5
	3	21.5	21.5	21.6	21.7	0.2
	4	22.2	22.3	22.6	22.9	0.7
	5	20.1	19.8	19.7	19.8	-0.3
Average Body Weight		**21.7**	**21.8**	**21.7**	**21.8**	**0.1**
Standard Deviation		**1.0**	**1.2**	**1.2**	**1.2**	**0.5**
Control NS	21	20.0	19.4	19.7	20.2	0.2
	22	20.1	20.0	20.5	21.4	1.3
	23	22.2	22.4	22.2	22.4	0.2
	24	19.3	19.2	18.7	19.1	-0.2
	25	18.8	18.6	18.8	19.2	0.4
Average Body Weight		**20.1**	**19.9**	**20.0**	**20.5**	**0.4**
Standard Deviation		**1.3**	**1.5**	**1.4**	**1.4**	**0.6**
Test CSO	11	22.6	22.6	22.9	23.2	0.6
	12	20.9	20.6	21.2	21.6	0.7
	13	21.3	21.7	22.0	22.1	0.8
	14	21.4	21.2	21.3	22.2	0.8
	15	20.8	21.3	21.5	21.7	0.9
Average Body Weight		**21.4**	**21.5**	**21.8**	**22.2**	**0.8**
Standard Deviation		**0.7**	**0.7**	**0.7**	**0.6**	**0.1**
Control CSO	31	21.6	21.0	21.3	22.0	0.4
	32	19.1	19.9	20.4	20.3	1.2
	33	20.5	20.5	20.6	21.0	0.5
	34	21.1	21.5	21.4	21.8	0.7
	35	20.1	20.2	20.7	21.3	1.2
Average Body Weight		**20.5**	**20.6**	**20.9**	**21.3**	**0.8**
Standard Deviation		**1.0**	**0.6**	**0.4**	**0.7**	**0.4**

REFERENCES

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

ISO 10993-12:2007 Biological Evaluation of Medical Devices, Part 12: Sample Preparation and Reference Materials.

U.S. Pharmacopeia, Section 88, current revision.

WuXi AppTec Reference Library Contents, Form ALS-4650-1

WuXi AppTec SOP: ILS-0018, Environmental Conditions in the Animal Facility

WuXi AppTec SOP: ILS-0092, Receiving Shipments of Animals

WuXi AppTec SOP: ILS-0112, Animal Identification

WuXi AppTec SOP: ILS-0115, ISO/USP/Japanese Acute Systemic Injection Test

WuXi AppTec SOP: ILS-0233, Proper Handling of Sick, Injured, and/or Moribund Animals

WuXi AppTec SOP ALS-0260, Sample Extraction Procedures

Material Certifications Disclaimer:

The Stratasys ABS M30i and PC-ISO filament has been independently tested and certified as a medical grade plastic according to ISO guidelines. This certification notwithstanding, *neither* Stratasys nor the raw material manufacturer can approve resins/plastics for particular medical, pharmaceutical, food or other applications. It is the responsibility of the FDM system end-user to make a determination of the part's suitability for end-user's intended purpose through appropriate testing and analysis of all the component parts and materials to be used in the finished products.

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