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TOXIKON FINAL GLP REPORT: 09–0302–G1

CLASS VI TEST – USP

Test Article
WaterSep HF Cartridge

Author
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Final Report Date
March 12, 2009

COMPLIANCE
21 CFR, Part 58
Good Laboratory Practice for Non–Clinical Laboratory Studies

MANAGEMENT OF THE STUDY

Performing Laboratory
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Sponsor
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STUDY SUMMARY

The USP 0.9% Sodium Chloride for Injection (NaCl), Cottonseed Oil (CSO), 1 in 20 Ethanol in NaCl (EtOH), and Polyethylene Glycol 400 (PEG) extracts of the test article, following Intracutaneous Injection in rabbits and Systemic Injection in mice, and the test article, following implantation in rabbits, did not produce a biological response. Therefore, the test article, WaterSep HF Cartridge, meets the requirements of the USP guidelines, for Class VI Plastics – 70 °C.

QUALITY ASSURANCE STATEMENT

This study was conducted in compliance with U.S. Food and Drug Administration regulations set forth in 21 CFR, Part 58.

The sections of the regulations not performed by or under the direction of Toxikon Corporation, exempt from this Good Laboratory Practice Statement, included characterization and stability of the test article and its mixture with carriers, 21 CFR, Parts 58.105 and 58.113.

The Quality Assurance Unit conducted inspections on the following dates. The findings were reported to the Study Director and to Toxikon's Management.

INSPECTIONS	DATE OF INSPECTION	DATE REPORTED STUDY DIRECTOR	DATE REPORTED MANAGEMENT
SACRIFICE	02/27/09	02/27/09	02/27/09
RAW DATA	03/12/09	03/12/09	03/12/09
FINAL REPORT	03/12/09	03/12/09	03/12/09

Meagan C Ruffen
Meagan C. Ruffen, B.S.
Quality Assurance

3/12/2009
Date

STUDY DIRECTOR SIGNATURE AND VERIFICATION DATES

This study meets the technical requirements of the protocol. The study also meets the requirements of the Good Laboratory Practice Regulations, 21 CFR, Part 58, with the exemptions as stated in the Quality Assurance Statement.

Protocol Number:	WRP/VIVO/001–08/000
Study Director:	Christopher Parker, M.S.
Company:	Toxikon Corporation
Signature:	
Date:	3/4/09
Study Supervisor:	Allan Sleger, A.S., LAT

VERIFICATION DATES:
The Study Initiation Date is the date the protocol is signed by the Study Director.

Test Article Receipt:	01/22/09
Project Log Date:	02/03/09
Study Initiation Date:	02/04/09
Extraction Dates:	02/12/09 – 02/13/09
Technical Initiation:	02/12/09
Technical Completion:	02/27/09

1.0 PURPOSE

The purpose of the study was to determine the biological response of animals to direct and indirect contact with the test article or injection of the test article extract.

2.0 REFERENCES

The study was conducted based upon the following references:

2.1 United States Pharmacopeia 31, National Formulary 26, 2008. <88> Biological Reactivity Tests, *In Vivo*.

2.2 ISO/IEC 17025, 2005, General Requirements for the Competence of Testing and Calibration Laboratories.

3.0 COMPLIANCE

The study conformed to the current FDA 21 CFR, Part 58 – Good Laboratory Practice for Non–Clinical Laboratory Studies.

4.0 IDENTIFICATION OF TEST AND CONTROL ARTICLES

The Sponsor supplied the following information on a test requisition form or other correspondence, wherever applicable (excluding confidential or trade secret information). The Sponsor was responsible for all test article characterization data as specified in the GLP regulations.

4.1 Test Article:

Test Article Name: WaterSep HF Cartridge
CAS/Code #: Not Supplied by Sponsor (N/S)
Lot/Batch #: Product: Investigator 24
Cartridge Lot #: 8241
Membrane MWCO/Lot#: 100K/102408A
Catalog #: WA 100 INV 24 SO
Physical State: N/S
Color: N/S
Expiration Date: N/S
Density: N/S
Stability: N/S
Solubility: N/S
pH: N/S
Storage Conditions: 4 ± 2 °C
Safety Precautions: Standard Toxikon Laboratory Safety Precautions

4.2 Control Articles (Toxikon Supplied):

4.2.1 Negative Control Article Name: USP 0.9% Sodium Chloride for Injection (NaCl)
Toxikon QC #: CSC–09–01–006–VV
Physical State: Liquid
Color: Colorless
Stability: Stable at Room Temperature
Storage Conditions: Room Temperature
Safety Precautions: Standard Laboratory Safety Precautions

4.2.2 Negative Control Article Name: Cottonseed Oil (CSO)
Toxikon QC #: CSC–08–12–006–VV
Physical State: Liquid
Color: Yellow
Stability: Stable at Room Temperature
Storage Conditions: Room Temperature
Safety Precautions: Standard Laboratory Safety Precautions

4.2.3 Negative Control Article Name: 1 in 20 Ethanol in NaCl (EtOH)
Toxikon QC #: CSC–08–12–008–VV; CSC–09–01–006–VV
Physical State: Liquid
Color: Colorless
Stability: Stable at Room Temperature
Storage Conditions: Room Temperature
Safety Precautions: Standard Laboratory Safety Precautions

4.2.4 Negative Control Article Name: Polyethylene Glycol 400 (PEG)
Toxikon QC #: CSC–08–09–001–VV
Physical State: Liquid
Color: Colorless
Stability: Stable at Room Temperature
Storage Conditions: Room Temperature
Safety Precautions: Standard Laboratory Safety Precautions

4.2.5 Negative Control Article Name: Negative Control High Density Polyethylene (Negative Control Plastic)
Toxikon QC #: CSC–04–05–009–VV
Physical State: Solid
Color: White
Storage Conditions: Room Temperature
Safety Precautions: Standard Laboratory Safety Precautions

5.0 IDENTIFICATION OF TEST SYSTEM

5.1 Animals Used in the Study:

5.1.1 Systemic Injection Test:

Number and Species: 40 Albino Swiss Mice (*Mus musculus*)

Sex: female (females were non–pregnant and nulliparous)

Weight/Age Range: 17.2 – 23.0 grams / at least 34 days old (adult)
weighed to the nearest 0.1 g

Health Status: healthy, not previously used in other experimental procedures

Animal Purchase: Harlan Laboratories, Indianapolis, IN

Animal Identification: ear punch

Acclimation: minimum 3 days, under same conditions as for the actual test

Animal Selection: selected from larger pool and examined to ensure lack of adverse clinical signs

5.1.2 Intracutaneous Injection and Implant Tests:

Number and Species: 8 New Zealand White rabbits (*Oryctolagus cuniculus*)

Sex: 3 males and 5 females (females were non–pregnant and nulliparous)

Weight/Age Range: 2.81 – 3.25 kilograms for Intracutaneous
3.28 – 3.50 kilograms for Implant Test
at least 10 weeks old (young adult)
weighed to nearest 10 g

Health Status: healthy, previously used in other experimental procedures

Animal Purchase: Millbrook Breeding Labs, Amherst, MA

Animal Identification: ear marker

Acclimation: minimum 3 days, under same conditions as for the actual test

Animal Selection: selected from larger pool and examined to ensure lack of adverse clinical signs

5.2 Animal Care and Maintenance:

5.2.1 Systemic Injection Test:

Animal Room Temperature: 68 ± 5 °F

Animal Room Relative Humidity: 30 – 70%

Air Exchanges per Hour: a minimum of 10 changes per hour

Lights: 12–hour light/dark cycle, full spectrum fluorescent lights

Housing: group housed (5 per cage of same sex)

Cages: polycarbonate

Bedding: hardwood chips, P.W.I. Industries, St–Hyacinthe, Quebec, Canada (contact)

Animal Rations: TEK 7012 Rodent Diet, Harlan Laboratories, Madison, WI, *ad libitum*

Water: tap water, *ad libitum*

There were no known contaminants present in the feed, water, or bedding expected to interfere with the test data.

The laboratory and animal rooms were maintained as limited–access facilities.

5.2.2 Intracutaneous Injection and Implant Tests:

Animal Room Temperature: 68 ± 5 °F

Animal Room Relative Humidity: 30 – 70%

Air Exchanges per Hour: a minimum of 10 changes per hour

Lights: 12–hour light/dark cycle, full spectrum fluorescent lights

Housing: individually housed

Cages: suspended stainless steel

Bedding: hardwood chips, P.W.I. Industries, St–Hyacinthe, Quebec, Canada (non–contact)

Animal Rations: TEK Hi–Fiber Rabbit Diet 2031, Harlan Laboratories, Madison, WI, *ad libitum*

Water: tap water, *ad libitum*

There were no known contaminants present in the feed, water, or bedding expected to interfere with the test data.

The laboratory and animal rooms were maintained as limited–access facilities.

6.0 JUSTIFICATION OF TEST SYSTEM AND ROUTE OF ADMINISTRATION

6.1 Albino mice and rabbits were used in this study because they have historically been used in USP Class VI tests and the guidelines have no alternative (non-animal) methods. The species and number of animals used in this study were recommended by the USP guidelines.

6.2 Systemic injection in mice, intracutaneous injection, and intramuscular implantation in rabbits are recommended by the USP guidelines for Class VI tests.

6.3 The test article was exposed to the test system directly and through solvents compatible with the test system.

7.0 EXPERIMENTAL DESIGN AND DOSAGE

7.1 Preparation of Test and Control Articles:

7.1.1 Systemic and Intracutaneous Testing Preparation:

7.1.1.1 To create a 60 cm^2 representative portion of the test article, the Sponsor specified the following amounts of each of the 3 components to be used: 70 cm length of hollow fiber, 1.47 cm × 1.00 cm piece of housing, and cube of epoxy with 0.17 cm sides. The test article (60 cm^2) was combined with 20 mL of vehicle at a ratio of 60 cm^2 per 20 mL per USP guidelines. The test article was separately extracted in NaCl, CSO, EtOH, and PEG at 70 ± 2 °C for 24 ± 2 hours for the Systemic Injection and Intracutaneous Injection tests.

7.1.1.2 Properly prepared test articles were placed in separate extraction bottles, and to each bottle the appropriate medium was added. The extraction medium completely covered the test article.

7.1.1.3 Each extracting medium (control article) was prepared for parallel treatments and comparisons. Each control article was prepared in the same manner as the test article.

7.1.1.4 The Systemic Injection and Intracutaneous tests were performed using the same extracts. The test article appeared unchanged by the extraction procedure. It was not degraded or deformed. The NaCl, EtOH, and PEG extracts were clear and free from particulates. The CSO extract appeared cloudy compared to the control article extract. Each extract was agitated vigorously prior to administration. All other test article preparation was as specified by the Sponsor.

7.1.2 Implant Testing Preparation:
The test and control articles were cut into strips measuring 1 mm × 10 mm. The test and control article strips were sterilized by dipping in 70% ethanol prior to implantation.

7.2 Pre–Dose Procedure:

7.2.1 Systemic Injection Test:

7.2.1.1 Acclimated animals were weighed prior to dosing.

7.2.1.2 For the Systemic Injection Test, the PEG test article extract and the corresponding control were diluted with NaCl to obtain PEG concentration of approximately 200 mg/mL.

7.2.2 Intracutaneous Injection Test:

7.2.2.1 On the day of the test, the animals were weighed and clipped free of fur on the dorsal side.

7.2.2.2 For the Intracutaneous Test, the PEG test article extract and the corresponding control were diluted with NaCl to obtain PEG concentration of approximately 120 mg/mL.

7.2.3 Implant Test:
Four rabbits were used for the Implantation Test. On the day of the test, the animals were weighed and the skin on both sides of the spinal column was clipped free of fur. Each animal was anesthetized to prevent muscular movement.

7.3 Dose Administration:

7.3.1 Systemic Injection Test:
Groups of 5 animals were injected with either the test article extract or the corresponding control article extract in the same amounts and by the same routes set forth below:

Extract	Route	Dose/kg	Injection Rate
NaCl	Intravenous	50 mL	0.1 mL/second
CSO	Intraperitoneal	50 mL	—
EtOH	Intravenous	50 mL	0.1 mL/second
PEG	Intraperitoneal	10 g	—

7.3.2 Intracutaneous Injection Test:

7.3.2.1 A volume of 0.2 mL of each test article extract was injected intracutaneously at five sites on one side of each of two rabbits. More than one test article extract was used per rabbit.

7.3.2.2 At five sites on the other side of each rabbit, 0.2 mL of the corresponding control article was injected.

7.3.3 Implant Test:
Portions of each of the 3 specified components were implanted. Two rabbits were implanted with the epoxy (T1), hollow fiber membrane (T2), and the control article. Two additional rabbits were implanted with the housing (T3) and the control article. Four samples of each

test article component and the control article were implanted into the paravertebral muscle on one side of the spine of each of four rabbits (2.5 to 5.0 cm from the midline, parallel to the spinal column and about 2.5 cm from each other).

7.4 Post–Dose Procedure:

7.4.1 Systemic Injection Test:

7.4.1.1 The animals were observed for clinical signs immediately after injection, 4 hours after injection, and at 24, 48, and 72 ± 2 hours after injection. Observations conducted included all clinical and toxicologic signs.

7.4.1.2 The animals were weighed at the end of the observation period.

7.4.1.3 Animals were sacrificed by carbon dioxide inhalation.

7.4.2 Intracutaneous Injection Test:

7.4.2.1 The injection sites on each animal were observed for signs of erythema and edema 24, 48, and 72 hours after injection of the test article. Observations were scored according to the Evaluation of Skin Reactions (Appendix I). Observations conducted also included all clinical signs.

7.4.2.2 All average erythema and edema scores for the test and control sites at 24, 48, and 72 hours were totaled separately and divided by 12 (2 animals × 3 scoring periods × 2 scoring categories) to determine the overall mean score for the test article versus the corresponding control article.

7.4.2.3 Animals were weighed at the end of the observation period.

7.4.2.4 The animals were returned to the general colony.

7.4.3 Implant Test:

7.4.3.1 The animals were maintained for a period of 7 days.

7.4.3.2 The animals were observed daily for this period to ensure proper healing of the implant sites and for clinical signs of toxicity. Observations included all clinical manifestations.

7.4.3.3 At the end of the observation period, the animals were weighed. Each animal was sacrificed by an injectable barbiturate.

7.4.3.4 Sufficient time was allowed to elapse for the tissue to be cut without bleeding.

7.4.3.5 The area of the tissue surrounding the center portion of each implant strip was examined macroscopically using a magnifying lens. Hemorrhaging, necrosis, discolorations, and infections were scored using the following scale:

0 = Normal
1 = Mild
2 = Moderate
3 = Severe

Encapsulation, if present, was scored by first measuring the width of the capsule (the distance from the periphery of the implant to the periphery of the capsule) rounded to the nearest 0.1 mm. The encapsulation was scored as follows:

Capsule Width	Score
None	0
Up to 0.5 mm	1
0.6 to 1.0 mm	2
1.1 to 2.0 mm	3
Greater than 2.0 mm	4

The differences between the average scores for the test article and control article implant sites were calculated.

8.0 EVALUATION CRITERIA

8.1 Systemic Injection Test:
The test is considered negative if none of the animals injected with the test article show a significantly greater biological reaction than the animals treated with the control article.

If two or more mice die, or show signs of toxicity such as convulsions or prostration, or if three or more mice lose more than 2 g of body weight, the test article does not meet the requirements of the test. If any animal treated with a test article shows only slight signs of biological reaction, and not more than one animal shows gross signs of biological reaction or dies, a repeat test is conducted using groups of 10 mice. On the repeat test, all 10 animals must not show a significantly greater biological reaction than the animals treated with the control article.

8.2 Intracutaneous Injection Test:
The requirements of the test are met if the difference between the test article and control article mean reaction scores (erythema/edema) is 1.0 or less.

If at any observation point, the average reaction to the test article sites is questionably greater than the corresponding control article sites, a repeat for the particular test article extract/solution is conducted using an additional 3 rabbits. On the repeat test, the requirements of the test is met if the difference between the test article and control article mean reaction scores (erythema/edema) is 1.0 or less.

8.3 Implant Test:
The test is considered negative if, in each rabbit, the difference between the average scores for each category of biological reaction for the test article and control article implant sites does not exceed 1.0; or if the difference between the mean scores for all categories of biological reaction

for each test article and the average score for all categories for all the control implant sites does not exceed 1.0, for not more than one of four test article strips.

8.4 Class VI Requirements:
The test article satisfies the requirements of the USP Class VI test if the requirements described above are met.

8.5 The study and its design employ methodology to minimize uncertainty of measurement and control of bias for data collection and analysis.

9.0 RESULTS

9.1 Systemic Injection Test:

9.1.1 Animal Weights:
All of the test and control animals increased in weight (Table 1).

9.1.2 Clinical Observations:
None of the test or control animals exhibited overt signs of toxicity at any of the observation points (Table 1).

9.1.3 The test is considered negative because none of the animals injected with extracts of the test article showed a significantly greater biological reaction than the animals treated with the control articles.

9.2 Intracutaneous Injection Test:

9.2.1 Animal Weights:
All of the animals increased in weight (Table 2).

9.2.2 Clinical Observations:
There were no overt signs of toxicity observed in any test or control animals (Table 2).

9.2.3 The difference between the test article and control article mean reaction scores (erythema/edema) was less than 1.0. The test article meets the requirements of the Intracutaneous Test (Table 3).

9.3 Implant Test:

9.3.1 Animal Weights:
All of the animals increased in weight (Table 2).

9.3.2 Clinical Observations:
There were no overt signs of toxicity noted in either animal. Macroscopic evaluation of any of the test article components and control article implant sites showed no significant infection, encapsulation, hemorrhage, necrosis, or discoloration (Tables 2 and 4).

9.3.3 The test is considered negative, since in each rabbit the difference between the average scores for all of the categories of biological reaction for the test article components and

control article implant sites did not exceed 1.0, and the difference between the mean scores for all categories of biological reaction for all of the test article implant sites and the average score for all categories for all the control implant sites did not exceed 1.0. The test article meets the requirements of the Implantation Test (Table 4).

10.0 CONCLUSION

The USP 0.9% Sodium Chloride for Injection (NaCl), Cottonseed Oil (CSO), 1 in 20 Ethanol in NaCl (EtOH), and Polyethylene Glycol 400 (PEG) extracts of the test article, following Intracutaneous Injection in rabbits and Systemic Injection in mice, and the test article, following implantation in rabbits, did not produce a biological response. Therefore, the test article, WaterSep HF Cartridge, meets the requirements of the USP guidelines, for Class VI Plastics – 70 °C.

11.0 RECORDS

11.1 Original raw data are archived at Toxikon Corporation.

11.2 A copy of the final report and any report amendments is archived at Toxikon Corporation.

11.3 The original final report, and a copy of any protocol amendments or deviations, is forwarded to the Sponsor.

11.4 All used and unused test article shall be disposed of by Toxikon, per Sponsor's request.

12.0 CONFIDENTIALITY AGREEMENT

Statements of confidentiality were not agreed upon prior to study initiation.

13.0 ANIMAL WELFARE STATEMENT

The Sponsor assured that, to the best of their knowledge, this study did not unnecessarily duplicate previous testing and that there were no non–animal alternatives acceptable for the evaluation of this test article as defined by the protocol.

No evidence of pain and suffering was reported to the Veterinarian and/or Study Director.

Toxikon strictly adhered to the following standards in maintaining the animal care and use program:

United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service, 9 CFR Ch. 1 (1/1/95 edition), Subchapter A–Animal Welfare.

"Guide for the Care and Use of Laboratory Animals," National Research Council, 1996. (NIH).

Office for Laboratory Animal Welfare (OLAW), "Public Health Service Policy on Humane Care and Use of Laboratory Animals," Health Research Extension Act of 1985 (Public Law 99–158 November 20, 1985), Reprinted 1996.

ISO 10993-2, 2006, Biological Evaluation of Medical Devices – Part 2: Animal Welfare Requirements.

Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) International.

14.0 PROTOCOL DEVIATION

As per Sponsor request, 3 separate components of the test article (housing, filter, and epoxy) were implanted. As a result of the increase in components, 2 additional rabbits were added, increasing the total number of rabbits in the study to 8.

This change has no impact on the integrity of the study.

TABLE 1
Systemic Injection Test:
Animal Weights and Clinical Observations

Test Article: WaterSep HF Cartridge

Lot/Batch #: Product: Investigator 24
Cartridge Lot #: 8241
Membrane MWCO/Lot#: 100K/102408A
Catalog #: WA 100 INV 24 SO

Group	Animal #	Sex	Dose (mL)	Body Weight (g)			Signs of Toxicity*
				Day 0 02/13/09	Day 3 02/16/09	Weight Change	
NaCl Test 50 mL/kg	1	Female	1.1	22.3	25.0	2.7	None
	2	Female	0.9	18.4	20.5	2.1	None
	3	Female	1.0	19.3	22.0	2.7	None
	4	Female	1.1	21.6	24.2	2.6	None
	5	Female	1.1	22.1	24.9	2.8	None
NaCl Control 50 mL/kg	6	Female	1.0	19.8	21.5	1.7	None
	7	Female	1.1	22.9	25.0	2.1	None
	8	Female	1.1	21.2	24.0	2.8	None
	9	Female	1.0	19.1	22.5	3.4	None
	10	Female	1.0	19.5	21.4	1.9	None
CSO Test 50 mL/kg	11	Female	1.1	22.6	24.7	2.1	None
	12	Female	1.1	21.5	23.5	2.0	None
	13	Female	1.1	21.1	24.5	3.4	None
	14	Female	1.0	20.7	22.8	2.1	None
	15	Female	1.0	20.2	22.5	2.3	None
CSO Control 50 mL/kg	16	Female	1.1	22.4	24.9	2.5	None
	17	Female	1.0	19.0	20.7	1.7	None
	18	Female	1.1	21.4	24.8	3.4	None
	19	Female	0.9	18.3	20.8	2.5	None
	20	Female	1.1	21.9	24.1	2.2	None
EtOH Test 50 mL/kg	21	Female	1.0	20.3	22.6	2.3	None
	22	Female	0.9	17.7	19.9	2.2	None
	23	Female	0.9	17.5	19.4	1.9	None
	24	Female	0.9	18.6	22.1	3.5	None
	25	Female	0.9	19.5	22.5	3.0	None
EtOH Control 50 mL/kg	26	Female	1.1	21.8	24.2	2.4	None
	27	Female	1.0	20.2	22.1	1.9	None
	28	Female	0.9	18.7	21.1	2.4	None
	29	Female	1.1	21.2	24.1	2.9	None
	30	Female	0.9	18.7	20.5	1.8	None
PEG Test 10 g/kg	31	Female	1.1	21.4	23.7	2.3	None
	32	Female	0.9	17.7	20.1	2.4	None
	33	Female	1.0	19.9	22.0	2.1	None
	34	Female	0.9	17.2	19.6	2.4	None
	35	Female	0.9	18.6	20.4	1.8	None
PEG Control 10 g/kg	36	Female	1.2	23.0	26.1	3.1	None
	37	Female	0.9	18.9	20.8	1.9	None
	38	Female	1.0	20.6	23.7	3.1	None
	39	Female	1.1	21.3	23.7	2.4	None
	40	Female	0.9	17.3	19.5	2.2	None

* Summary of clinical observations - Immediately, 4, 24, 48, and 72 h after injection.

TABLE 2
Intracutaneous Injection and Implant Tests: Animal Weights and Clinical Observations

Test Article: WaterSep HF Cartridge

Lot/Batch #: Product: Investigator 24
Cartridge Lot #: 8241
Membrane MWCO/Lot#: 100K/102408A
Catalog #: WA 100 INV 24 SO

Group	Animal #	Sex	Body Weight (kg)			Signs of Toxicity*
			Day 0 02/13/09	Day 3 02/16/09	Weight Change	
NaCl & CSO	90096	Female	2.83	2.93	0.10	None
	90097	Male	2.81	2.83	0.02	None
EtOH & PEG	90098	Female	3.12	3.21	0.09	None
	90099	Male	3.25	3.35	0.10	None
Group	**Animal #**	**Sex**	**Body Weight (kg)**			**Signs of Toxicity***
			Day 0 02/20/09	**Day 7 02/27/09**	**Weight Change**	
Implant	90006	Female	3.50	3.61	0.11	None
	90014	Female	3.35	3.52	0.17	None
	90029	Male	3.28	3.44	0.16	None
	90030	Female	3.31	3.50	0.19	None

* Summary of Clinical Observations, Day 0 through Day 3, excluding skin reactions for the Intracutaneous Injection Test and Day 0 through Day 7 for the Implant Test.

TABLE 3
Intracutaneous Test Skin Reaction Scores

Test Article: WaterSep HF Cartridge

Lot/Batch #: Product: Investigator 24
Cartridge Lot #: 8241
Membrane MWCO/Lot#: 100K/102408A
Catalog #: WA 100 INV 24 SO

NaCl Extract

Animal #	Vehicle	Time	Site Numbers Scoring (ER/ED)									
			T–1	T–2	T–3	T–4	T–5	C–1	C–2	C–3	C–4	C–5
90096	NaCl	24 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		48 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		72 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
90097	NaCl	24 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		48 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		72 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Total			0.0					0.0				

Overall Mean Score* for Test Article = 0.0
Overall Mean Score* for Control Article = 0.0
Difference between Test Article and Control Article Overall Mean Score = 0.0–0.0 = 0.0

CSO Extract

Animal #	Vehicle	Time	Site Numbers Scoring (ER/ED)									
			T–1	T–2	T–3	T–4	T–5	C–1	C–2	C–3	C–4	C–5
90096	CSO	24 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		48 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		72 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
90097	CSO	24 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		48 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		72 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Total			0.0					0.0				

Overall Mean Score* for Test Article = 0.0
Overall Mean Score* for Control Article = 0.0
Difference between Test Article and Control Article Overall Mean Score = 0.0–0.0 = 0.0

ER = Erythema; ED = Edema; T = Test Sites; C = Control Sites
* Overall Mean Score = Total erythema plus edema scores divided by 12
(2 animals × 3 scoring periods × 2 scoring categories)

TABLE 3
Intracutaneous Test Skin Reaction Scores (Cont.)

Test Article: WaterSep HF Cartridge

Lot/Batch #: Product: Investigator 24
Cartridge Lot #: 8241
Membrane MWCO/Lot#: 100K/102408A
Catalog #: WA 100 INV 24 SO

EtOH Extract

Animal #	Vehicle	Time	Site Numbers Scoring (ER/ED)									
			T–1	T–2	T–3	T–4	T–5	C–1	C–2	C–3	C–4	C–5
90098	EtOH	24 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		48 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		72 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
90099	EtOH	24 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		48 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		72 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Total			0.0					0.0				

Overall Mean Score* for Test Article = 0.0
Overall Mean Score* for Control Article = 0.0
Difference between Test Article and Control Article Overall Mean Score = 0.0–0.0 = 0.0

PEG Extract

Animal #	Vehicle	Time	Site Numbers Scoring (ER/ED)									
			T–1	T–2	T–3	T–4	T–5	C–1	C–2	C–3	C–4	C–5
90098	PEG	24 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		48 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		72 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
90099	PEG	24 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		48 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
		72 hours	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Total			0.0					0.0				

Overall Mean Score* for Test Article = 0.0
Overall Mean Score* for Control Article = 0.0
Difference between Test Article and Control Article Overall Mean Score = 0.0–0.0 = 0.0

ER = Erythema; ED = Edema; T = Test Sites; C = Control Sites
* Overall Mean Score = Total erythema plus edema scores divided by 12
(2 animals × 3 scoring periods × 2 scoring categories)

TABLE 4
Implant Test:
Macroscopic Observations

Test Article: WaterSep HF Cartridge

Lot/Batch #: Product: Investigator 24
Cartridge Lot #: 8241
Membrane MWCO/Lot#: 100K/102408A
Catalog #: WA 100 INV 24 SO

Animal #: 90006

Tissue Site	T1-1	T1-2	T1-3	T1-4	T1 Avg	T2-1	T2-2	T2-3	T2-4	T2 Avg	C1	C2	C3	C4	Control Avg
Infection	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Encapsulation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hemorrhage	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Necrosis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Discoloration	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	0	0	0	0		0	0	0	0		0	0	0	0	
Mean Score (total/5)	0	0	0	0		0	0	0	0		0	0	0	0	

Animal #: 90014

Tissue Site	T1-1	T1-2	T1-3	T1-4	T1 Avg	T2-1	T2-2	T2-3	T2-4	T2 Avg	C1	C2	C3	C4	Control Avg
Infection	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Encapsulation	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0
Hemorrhage	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Necrosis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Discoloration	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	1	1	1	1		0	0	0	0		0	0	0	0	
Mean Score (total/5)	0.2	0.2	0.2	0.2		0	0	0	0		0	0	0	0	

T = Test
C = Control
Avg = Average

TABLE 4
Implant Test:
Macroscopic Observations (Cont.)

Test Article: WaterSep HF Cartridge

Lot/Batch #: Product: Investigator 24
Cartridge Lot #: 8241
Membrane MWCO/Lot#: 100K/102408A
Catalog #: WA 100 INV 24 SO

Animal #: 90029

Tissue Site	T3-1	T3-2	T3-3	T3-4	Test Avg	C1	C2	C3	C4	Control Avg
Infection	0	0	0	0	0	0	0	0	0	0
Encapsulation	0	0	0	0	0	0	0	0	0	0
Hemorrhage	0	0	0	0	0	0	0	0	0	0
Necrosis	0	0	0	0	0	0	0	0	0	0
Discoloration	0	0	0	0	0	0	0	0	0	0
Total	0	0	0	0		0	0	0	0	
Mean Score (total/5)	0	0	0	0		0	0	0	0	

Animal #: 90030

Tissue Site	T3-1	T3-2	T3-3	T3-4	Test Avg	C1	C2	C3	C4	Control Avg
Infection	0	0	0	0	0	0	0	0	0	0
Encapsulation	0	0	0	0	0	0	0	0	0	0
Hemorrhage	0	0	0	0	0	0	0	0	0	0
Necrosis	0	0	0	0	0	0	0	0	0	0
Discoloration	0	0	0	0	0	0	0	0	0	0
Total	0	0	0	0		0	0	0	0	
Mean Score (total/5)	0	0	0	0		0	0	0	0	

T = Test
C = Control
Avg = Average

APPENDIX I
Evaluation of Skin Reactions

Erythema and Eschar Formation	Score
No erythema	0
Very slight erythema (barely perceptible)	1
Well–defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4

Total possible erythema score = 4

Edema Formation*

No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well–defined by definite raising)	2
Moderate edema (raised approximately l mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4

Total possible edema score = 4

* Excludes non–inflammatory (mechanical) edema from the blank or extract fluid.

APPENDIX II
Software Systems

Software	Use
Adobe Acrobat 8 Professional	Document preparation
DocuKnowledge 3.0	Lotus Domino–based document management system used for SOPs
Lotus Domino Rel. 5	Client–server application for sponsor, sample, test codes, and quotation management application databases
MS Office 2007 Small Business Suite	Business software (suite includes Word, Excel, PowerPoint, Outlook, Publisher, Office tools)
Rees CentronSQL System 2.0	Environmental monitoring and metrology system

ATTACHMENT A:
TFF HF SOP-Preconditioning pdf

WaterSep Technology Corporation
428 Maple Street, Suite 1 Marlborough, Mass. 01752
508- 970 0089, 508-970 0146 (FAX)

WaterSep PES Hollow Fiber

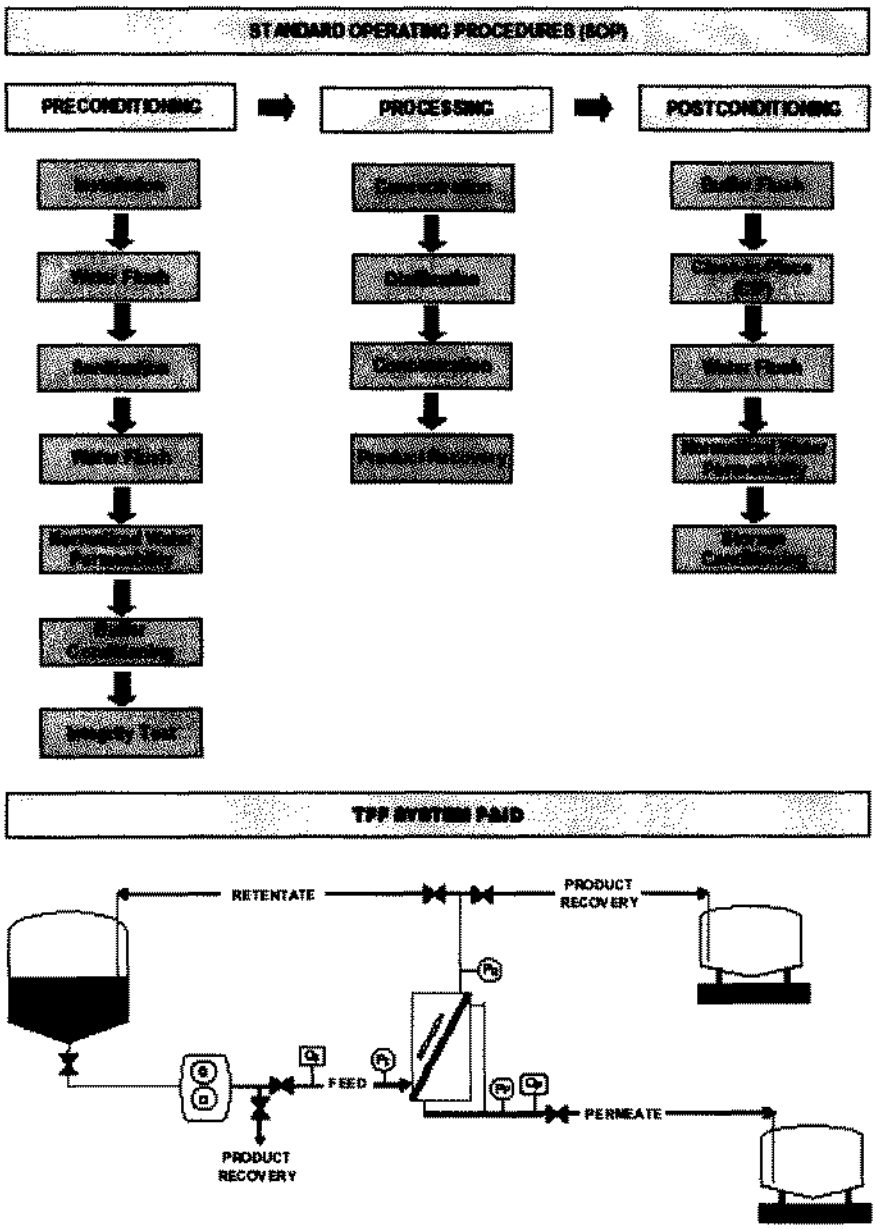
Standard Operating Procedure - SOP

Preconditioning for Use Procedure

Prepared by: John Rozembersky
Revision: January 2009

Tangential Flow Filtration (TFF)

Process Flowsheet for UF / DF



PRECONDITIONING

Installation

Hollow Fiber System

Attached the process stream lines to the feed, retentate and filtrate connections of the hollow fiber element.

Water Flush

1. **Flush storage agents from membrane element.**

1.1. Establish and set process conditions

1.1.1. Add water to feed vessel

Recommended Feed Volume:
50 – 80 liters per m^2 (5 – 8 liters per ft^2)

1.1.2. Open permeate and retentate valves completely.

1.1.3. Direct retentate and permeate streams to waste.

1.2. Flush feed - retentate process stream

1.2.1. Turn pump on and increase feed flow rate speed until a feed-to-retentate pressure differential of 2 - 6 psig is reached (fiber length – FL dependent). [10" FL = 2 psig, 20" FL = 3 psig, and 40" FL = 6 psig]

1.2.2. Continue to flush feed to retentate stream to waste for 1 - 3 minutes.

1.3. Flush permeate stream process stream

1.3.1. Decrease pump speed and retentate flow rate to ~5 - 10 %.

1.3.2. Close retentate valve completely. Then re-open valve to allow a minor flow rate in the retentate stream to prevent a dead-leg situation.

1.3.3. Increase pump speed and feed flow rate until a feed pressure of 10 – 15 psig reached for UF membranes (10kD – 500kD) or 3 – 5 psig for MF membranes (750kD – 0.45um).

1.3.4. Flush a minimum permeate volumetric throughput of ≥ 40 L/sqm (4 / sqft).

1.3.5. Turn off pump.

1.3.6. Remove water in feed vessel to bottom port. Keep lines flooded to prevent pump cavitation.

Sanitization

2. Sanitization of TFF System

2.1. Establish and set process conditions

2.1.1. Add 0.3 – 0.5N NaOH to feed vessel at 30 – 40°C.

Recommended Fee Volume:
8 – 12 liters per m^2 (0.8 – 1.2 liters per ft^2)

2.1.2. Open permeate and retentate valves completely.

2.1.3. Direct permeate to waste.

2.1.4. Direct retentate back to feed vessel.

2.2. Stabilized Feed-Retentate loop with NaOH

2.2.1. Turn pump on and increase pump speed until feed-to-retentate pressure differential of 2 - 4 psig is reached.

2.2.2. Allow ~ 2 - 3 minutes for retentate recirculation to stabilize.

2.3. Flood permeate with NaOH (Static sanitization of permeate)

2.3.1. Decrease pump speed to ~ 5- 10% of retentate flow rate

2.3.2. Close retentate valve completely. Then re-open valve 5 - 10%

2.3.3. Increase pump speed up until feed pressure is 10 – 15 psig

2.3.4. Continue to flush membrane and flood permeate with NaOH until permeate stream condition reaches equilibrium to feed concentration.

2.3.5. Proceed to next step with pump running.

2.4. Sanitization of Feed – Retentate Stream

2.4.1. Open retentate valve completely.

2.4.2. Close permeate valve completely

2.4.3. Increase pump speed until feed-to-retentate pressure differential of 3-5 psig is reached

2.4.4. Continue to recirculate

Recommended Sanitization Time : 30 – 45 min

2.4.5. Drain NaOH from feed tank and feed/retentate stream

2.4.6. Turn off pump

Water Flush

3. Flush sanitization agent from TFF System

3.1. Establish and set process conditions

3.1.1. Add water at ~ 25°C – 40°C to feed vessel

Recommended Volume: 50 – 80 liters per m^2 (5 – 8 liters per ft^2)

3.1.2. Open permeate and retentate valves completely.

3.1.3. Direct retentate and permeate streams to waste.

3.2. Flush feed - retentate process stream

3.2.1. Turn pump on and increase feed flow rate speed until a feed-to-retentate pressure differential of 5-10 psig is reached.

3.2.2. Continue to flush feed to retentate stream to waste for 1 - 3 minutes.

3.3. Flush permeate stream process stream

3.3.1. Decrease pump speed and retentate flow rate to ~5 - 10 %.

3.3.2. Close retentate valve completely first and then re-open ~10° o.

Note: feed pressure should not exceed 30 psig as retentate valve is closed. Decrease pump flow rate more should pressure rise beyond this point.

3.3.3. Increase pump speed and feed flow rate until a feed pressure of 10 – 20 psig reached.

3.3.4. Flush a minimum permeate volumetric throughput of 40 L/sqm (4 / sqft).

3.3.5. Continue to next step

Normalized Water Permeability

4. Normalized Water Permeability

4.1. Measure water flux rates

4.1.1. Reduce pump speed to feed pressure ~ 10 – 15 psig

4.1.2. Measure and Record

- Permeate Flow Rate (Q_P) – _____ Lpm
- Feed Pressure (P_f) – _____ psig
- Retentate Pressure (P_r) – _____ psig
- Permeate Pressure (P_p) – _____ psig
- Filtrate Temperature – _____ °C

4.1.3. Calculate

- Calculate TMP = $[(P_f+P_r)/2]-P_p$ – _____ psig
- Calculate filtrate flux rate (J) = Q_P / Area = liters/m^2/hr (LMH)

  J – _____ LMH
- Determine temperature correction factor ($TCF_{20°C}$) = _____

$$TCF = 0.0005T2 - 0.0449T + 1.7021$$

- Calculate filtrate flux rate at 20°C $(J)_{20^\circ C}$ = J x $TCF_{20^\circ C}$

J_{20° = _____LMH

- Calculate Normalized Water Flux Rate (NWP) = J_{20° / TMP

$NWP_{20^\circ C}$ = _____LMH/psig

4.2. Determine Percent Membrane Recovery (%MR)

4.2.1. Initial $NWP_{20^\circ C}$ constant (clean) = _____LMH/psig

If this is first time use for this membrane lot, the value calculated in 4.1.3. is the initial $NWP_{20^\circ C}$ constant (clean)

4.2.2. If element is in re-use mode, calculate %MR at this point.

%MR = NWP (step 4.1.3) / Initial NWP

%MR = _____ %

Buffer Conditioning

5. Buffer Condition TFF System

5.1. Establish and set process conditions

5.1.1. Add Buffer solution to feed vessel with same temperature as feed solution.

Volume required:
10 – 20 liters per m^2 (1 – 2 liters per ft^2)

5.1.2. Open permeate and retentate valves completely

5.1.3. Direct permeate to waste

5.1.4. Direct retentate back to feed vessel

5.2. Stabilized Feed-Retentate loop with buffer

5.2.1. Turn pump on and increase pump speed until feed-to-retentate pressure differential of 3-5 psig is reached.

5.2.2. Allow ~ 2 - 3 minutes for retentate recirculation to stabilize..

5.3. Flood permeate with buffer

5.3.1. Decrease pump speed to 5- 10% of retentate flow rate

5.3.2. Close retentate valve completely first and then re-open ~10°.

5.3.3. Increase pump speed and feed flow rate until a feed pressure of 10 – 20 psig reached.

5.3.4. Flush permeate stream to waste with 10–20 liters per m^2 (1 – 2 liters per ft^2)

5.4. Reduce the buffer in the feed vessel.

5.4.1. Drain buffer down to the bottom of the feed vessel near the exist port.

Feed – retentate lines should remain flooded with buffer. Do not permit air to be drawn in the feed – retentate. Lines should remain flooded and air free.

5.4.2. Turn off pump

Integrity Test

6. Integrity Testing of Membrane Element (optional)

Pressure hold test at 10 psig – HOLD for ~1-2 minutes
Air Diffusion at 10 psig < 10 cc/min