TOXIKON
ADVANCING YOUR INNOVATION

FINAL GLP REPORT: 15-03990-G1

HUMAN BLOOD HEMOLYSIS TEST (INDIRECT CONTACT) – AUTIAN

Test Article
THERMOLAST ® M TM9LFT

21 CFR Part 58 Compliance
Good Laboratory Practice for Nonclinical Laboratory Studies

Report Date
12/23/2015

Study Director
Franck Grall, Pharm.D., Ph.D.

Sponsor
KRAIBURG TPE GmbH & Co. KG
Friedrich-Schmidt-Str. 2
Waldkraiburg 84478
Germany

TABLE OF CONTENTS

STUDY SUMMARY

The potential hemolytic activity of human blood in response to exposure to the extract of the test article, THERMOLAST ® M TM9LFT, was determined. The test article was first extracted in Physiological Saline (NaCl) for 24 ± 2 hours at 70 ± 2°C, and then incubated with human blood for 60 ± 4 minutes at 37 ± 2 °C, in triplicate. Negative, positive, and untreated controls were analyzed in parallel. The absorbance of each sample was measured, and the average values were calculated. The percent (%) hemolysis was determined for each sample, and the average values were calculated.

The test article led to a hemolysis index of 0.00%. The response obtained from the blood exposed to the positive and negative control article extracts confirmed the suitability of the test system.

Based on the criteria of the protocol and the ISO 10993-4 guidelines, the test article meets the requirements of the test, and is considered non-hemolytic.

QUALITY ASSURANCE STATEMENT

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

The final report was reviewed to assure that the report accurately describes the methods and standard operating procedures. The reported results accurately reflect the raw data of the nonclinical study conducted per the protocol.

Phase	Inspection Date	Date Reported to Study Director	Date Reported to Management
ADDITION OF BLOOD	12/17/2015	12/17/2015	12/17/2015
DATA	12/22/2015	12/22/2015	12/22/2015
FINAL REPORT	12/23/2015	12/23/2015	12/23/2015

RK. Churchill

Ryan K. Churchill, B.S.
Quality Assurance

12/23/2015

Date

GLP COMPLIANCE STATEMENT

This study meets the technical requirements of the protocol.

This study was conducted in compliance with the current U.S. Food and Drug Administration 21 CFR, Part 58 Good Laboratory Practices for Nonclinical Laboratory Studies.

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, 21 CFR, Part 58.105, and its mixture with carriers, 21 CFR, Part 58.113.

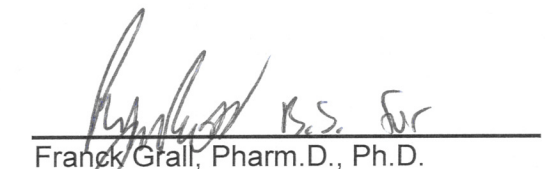
SIGNATURES

Signature Information	
Protocol Number	P15-1819-00A
Study Director	Franck Grall, Pharm.D., Ph.D.
Study Supervisor	Franck Grall, Pharm.D., Ph.D.
Company	Toxikon Corporation

VERIFICATION DATES

The study initiation day is the date the protocol is signed by the Study Director.

Verification Dates	
Test Article Receipt	11/11/2015
Project Log	11/11/2015
Study Initiation	12/11/2015
Study Completion	12/23/2015



Franck Grall, Pharm.D., Ph.D.
Study Director

12/23/15
Date

1.0 PURPOSE

The purpose of the study was to determine the potential hemolytic activity, by presence of increased levels of free plasma hemoglobin in rabbit blood, in response to the test article extract.

2.0 REFERENCES

The study was based upon the following references:

2.1 Autian Method, ATTP–I, Material Sciences Toxicology Laboratories, University of Tennessee Center for the Health Sciences, Memphis, TN, April 18, 1977.

2.2 Hemolysis – Rabbit Blood, Evaluation of Hemodialyzers and Dialysis Membranes, DHEW Publication # (NIH) 77–1294, pg. 213, 1977.

2.3 Feldman, Bernard F., Joseph G. Zinkl, and Nemi C. Jain, eds. Schalm's Veterinary Hematology. 5th edition. Baltimore: Lippincott Williams & Wilkins, 2000. 858–859.

2.4 ISO 10993–4, 2002, Biological Evaluation of Medical Devices – Part 4: Selection of Tests for Interactions with Blood, as amended 2006.

2.5 ISO 10993–12, 2012, Biological Evaluation of Medical Devices – Part 12: Sample Preparation and Reference Materials.

2.6 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 Nonclinical Laboratory Studies.

4.0 IDENTIFICATION OF TEST AND CONTROL ARTICLES

The Sponsor supplied the following information on a GLP 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:

Name	THERMOLAST ® M TM9LFT
CAS/Code Number	Not Supplied by Sponsor (N/S)
Lot/Batch Number	N/S
Physical State	Solid
Density	1,10

4.2 Negative Control Article (Toxikon Supplied):

Name	Negative Control High Density Polyethylene Equivalent to Negative Control USP High Density Polyethylene Reference Standard (Negative Control Plastic)
Toxikon QC Number	CSC-04-05-009-CC

4.3 Positive Control Article (Toxikon Supplied, unless specified by the Sponsor):

Name	USP Sterile Water for Injection (SWFI)
Toxikon QC Number	CSC-15-07-00216

4.4 Untreated Control (Extraction Medium; Toxikon Supplied):

Name	Physiological Saline (NaCl)
Toxikon QC Number	CSC-15-08-00085

5.0 IDENTIFICATION OF TEST SYSTEM

The test system was human blood with EDTA as an anticoagulant. The blood was obtained from an inside source (Toxikon donors).

6.0 JUSTIFICATION OF TEST SYSTEM AND ROUTE OF ADMINISTRATION

6.1 Justification of Test System:

The human blood is the system analyzed in this test.

6.2 Route of Administration:

The test article was extracted and administered *in vitro* through a medium compatible with the test system, as indicated on the GLP Test Requisition Form.

7.0 EXPERIMENTAL DESIGN AND DOSAGE

7.1 Preparation of the Test and Control Articles:

7.1.1 The test article was prepared and extracted following an ISO 10993-12/Autian ratio, as itemized in the table below.

Sample	Amount	Medium	Volume	Ratio	Time/Temperature
Test Article	172.2 cm^2	NaCl	57.4 mL	3 cm^2/mL	24 ± 2 hours at 70 ± 2 °C
Negative Control	120 cm^2	NaCl	40.0 mL	3 cm^2/mL	24 ± 2 hours at 70 ± 2 °C
Untreated Control	N/A	NaCl	40.0 mL	N/A	24 ± 2 hours at 70 ± 2 °C

N/A: Not Applicable

7.1.2 Properly prepared test article was placed in an extraction vessel and the appropriate medium was added. The medium completely covered the test article.

7.1.3 The negative control article (Negative Control Plastic, 0.06 cm thick) was prepared following an ISO 10993–12/Autian ratio and extracted with the same medium at the same temperature and for the same duration as the test article, as itemized in the table above.

7.1.4 An untreated control (blank) was prepared for parallel treatments and comparison. The untreated control was the extraction medium that is subject to the same temperature and for the same duration as the test article, as itemized in the table above.

7.1.5 After the completion of the extraction, the extracts were kept at room temperature and were used the same day the extraction was completed. The test article appeared unchanged by the extraction procedure and the extract was clear and free from particulates. No storage of the extracts occurred.

7.1.6 Each extract was agitated vigorously prior to administration.

7.1.7 The test articles and control articles were prepared and extracted in one replicate.

7.1.8 The pH and optical absorbance of the extracts were not measured.

7.1.9 Each extract was tested at 100% (neat) concentration.

7.2 Pre–Dose Procedure:

7.2.1 Blood Collection:

Whole human blood was collected into tubes containing Ethylene Diamine Tetra Acetate (EDTA) as the anticoagulant. The blood was from donors that have not taken aspirin, acetaminophen, ibuprofen, or other anti–inflammatory drugs, or contraceptive medications within 10 days of blood collection. The blood was used within 4 hours.

7.2.2 Blood Preparation:

The blood was diluted sufficiently in NaCl until 0.2 mL of blood mixed with 10.0 mL of SWFI gave a spectrophotometric reading of 1.0 ± 0.1 absorbance (A) at 545 nm.

7.3 Dose Administration:

7.3.1 Liquid Article Preparation:

The positive control was SWFI in a glass vessel.

7.3.2 Temperature Equilibration:

The vessels containing the positive control article were capped and incubated for at least 30 minutes at 37 ± 2 °C in a water bath, with gentle agitation, for temperature equilibration.

7.3.3 Addition of Blood:

After temperature equilibration incubation or extraction, human blood, prepared as specified in Section 7.2.2, was added to each vessel at a ratio of 0.2 mL of blood per 10.0 mL of medium, as itemized in the table below.

Sample	Volume of Extract	Volume of Blood
Test Article	10.0 mL	0.2 mL
Negative Control	10.0 mL	0.2 mL
Positive Control	10.0 mL	0.2 mL
Untreated Control	10.0 mL	0.2 mL

7.3.4 Replication:

All dosing was done in triplicate.

7.4 Post–Dose Procedure:

7.4.1 Incubation:

All vessels were capped or covered and incubated for 60 ± 4 minutes at 37 ± 2 °C in a waterbath with gentle agitation.

7.4.2 Hemolysis Measurement:

7.4.2.1 After incubation, the vessels were centrifuged at 750 *g* for approximately five minutes. The supernatant from each vessel was carefully collected into cuvettes and the presence of any coloration or precipitate was recorded, as itemized in the table below.

Sample	Color	Precipitate
Test Article	Colorless	Absent
Negative Control	Colorless	Absent
Positive Control	Reddish	Absent
Untreated Control	Colorless	Absent

7.4.2.2 The absorbance of each supernatant was determined against a NaCl blank at 545 nm on a calibrated spectrophotometer.

7.4.3 Calculations:

The average absorbance values (A^X) for the blood exposed to the test article extract, negative control article extract, positive control article, and untreated control were calculated. These averages were used to determine the percent (%) hemolysis of the test article extract and negative control article extract according to the following calculation:

$$\text{Article Hemolysis} = \frac{A^S - A^B}{A^P - A^B} \times 100\%$$

Where A^S is the average absorbance of the test or negative control article, A^B is the average absorbance of the untreated (blank) control and A^P is the average absorbance of the positive control article.

If a significant absorbance was noted in the extract, this pre-existing absorbance was subtracted from the absorbance of the test article.

8.0 EVALUATION CRITERIA

8.1 Test System Suitability:

The test system will be considered suitable if the following condition is met:

- The % hemolysis for blood exposed to the negative control article extract is $\leq$ 2%.

If the test system is not considered suitable, the test will be repeated.

8.2 Determination of the Hemolytic Effect of the Test Article:

The test article meets the requirements of the test, and is not considered to have a hemolytic activity potential, if the % hemolysis is $\leq$ 5%. The biological significance will also be considered in the evaluation of the results.

8.3 Control of Bias Statement:

The study and its design employed methodology to minimize uncertainty of measurement and control of bias for data collection and analysis, which included but was not limited to: concurrent control data, system suitability assessment, blanks, and replicates.

9.0 RESULTS

The optical absorbance readings and hemolysis index % are summarized in the following table:

Replicate	Test Article	Negative Control Article	Positive Control	Untreated Control
1	0.0224	0.0178	0.9735	0.0231
2	0.0099	0.0146	0.9484	0.0157
3	0.0129	0.0181	0.9809	0.0227
Mean Absorbance	0.0151	0.0168	0.0168	0.0205
Standard Deviation	0.0065	0.0019	0.0170	0.0042
Hemolysis Index %	0.00%	0.00%	100.00%	0.00%

10.0 CONCLUSION

Based on the criteria of the protocol and the ISO 10993-4 guidelines, the test article meets the requirements of the test, and is considered non-hemolytic.

11.0 RECORDS

11.1 Original raw data will be archived by Toxikon Corporation.

11.2 A copy of the final report and any report amendments will be archived by Toxikon Corporation.

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

11.4 The test article shall be disposed by Toxikon.

11.5 Test article retention upon study completion is the responsibility of the Sponsor.

12.0 CONFIDENTIALITY AGREEMENT

Per corporate policy, confidentiality shall be maintained in general, and in specific accordance with any relevant agreement specifically executed between Toxikon and the Sponsor.

13.0 UNFORESEEN CIRCUMSTANCES

Any unforeseen circumstances were documented in the raw data. However, no unforeseen circumstances that affected the integrity of the study were noted.

14.0 PROTOCOL AMENDMENTS/DEVIATIONS

There were no protocol amendments or deviations. No changes to the protocol were required.

APPENDIX I
Software Systems

Software	Use	Publisher/Vendor	Location
Adobe Acrobat 8, 9 and 10 Professional	Document preparation	Adobe Systems, Inc.	San José, CA
Lotus Domino Rel. 5	Client–server application for Sponsor, sample, test codes, and quotation management application databases	IBM Corporation	Armonk, NY
Matrix Gemini 5.3.5	Laboratory Information Management System	Autoscribe Limited	Reading, UK
MS Office 2010 Small Business Suite and MS Office 2013 Professional Suite	Business software (suite includes Word, Excel, PowerPoint, Outlook, Publisher, Office tools)	Microsoft Corporation	Redmond, WA
Rees CentronSQL System 2.0	Environmental monitoring and metrology system	Rees Scientific	Trenton, NJ
TMS Web 7	Document management for SOPs and training records management software system	Quality Systems Integrators	Eagle, PA
Toxikon Protocol Manager 1.0	Protocol requisition application	Custom developed	Toxikon Corporation, Bedford, MA
UV WinLab 6.0.4.0738	UV/Vis spectrophotometer software	Perkin Elmer	Waltham, MA