TEST REPORT

APPLICANT	:	**GUANGZHOU TOP-BOND ENVIRONMENTAL TECHNOLOGY CO.，LTD**
ADDRESS	:	E501, NO. 5 FACTORY BUILDING, NO. 9, LAN YU 4TH STREET, HUANGPU DISTRICT, GUANGZHOU CITY
TESTED SAMPLE DESCRIPTION	:	UV ADHESIVE
TESTED ITEM NO.	:	YS-33221
AGE REQUESTED ON APPLICATION FORM	:	NOT PRESENT
SAMPLE RECEIVED DATE	:	NOV. 21, 2022
TEST PERIOD	:	NOV. 23, 2022 TO MAR. 01, 2023
REMARK	:	Subcontracting test, see test report CSTBB2022110895, generated by CCIC Huatongwei International Inspection（Suzhou）Co., Ltd.

**********FOR FURTHER DETAILS, PLEASE REFER TO THE FOLLOWING PAGE(S)**********

SIGNED FOR AND ON BEHALF OF
EUROFINS PRODUCT TESTING HONG KONG LTD.

Alex Fung
General Manager

中国认可
国际互认
检测
TESTING
CNAS L13034

In Vitro Cytotoxicity Test

MTT Method

Final Report

Verification

Report Number: CSTBB2022110895

Article Name: YS-33221

Method Standard: ISO 10993-5: 2009

Sponsor

Guangzhou TOP-BOND Environmental Technology CO.，Ltd

E501, No. 5 factory building, No. 9, Lan Yu 4th Street, Huangpu district, Guangzhou City

Test Facility

CCIC Huatongwei International Inspection（Suzhou）Co., Ltd.

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Notices

1. Please apply for rechecking within 15 days of receiving the report if there is any objection.
2. Any erasure or without special testing seal renders the report null and void.
3. The report is only valid when signed by the persons who edited, checked and approved it.
4. The report is only responsible for the test results of the tested samples.
5. The report shall not be reproduced except in full without the written approval of the company.

Abstract

In this study, mammalian L-929 cells were cultured in vitro according to ISO 10993-5:2009 to test the potential cytotoxicity of the test article.

The test articles and the control material were separately placed in MEM medium containing 10% fetal bovine serum, and extracted in a 37 ℃ incubator for 24 hours. After the end of the extraction, the cell culture medium in the 96-well plate (10^4 cells/well) cultured for 24 hours was removed and replaced with the corresponding extract, cultured in 37 ℃, 5% CO_2, >90% humidity for 24 hours. After the culture, the morphology and cell lysis of the cells were observed under the microscope, and the cytotoxicity of the test samples was determined by MTT assay.

The results showed that the cells in the blank control group and the negative control group (high density polyethylene) were well-formed throughout the experiment and showed no cytotoxic reaction. A severe cytotoxic response was shown in the positive control group (ZDEC). While in test article group, after the cells were incubated for 24 hours, it could be found destroyed cell layer and lysed cells. The cell growth was inhibited and the cell viability was 7.7%. The data of each group met the acceptance criteria, and the results of this test are valid.

Based on the above results, it can be concluded that under the experimental conditions, the test article has potential toxicity to L-929 in the MTT method.

Study Verification and Signature

Protocol Number	SST2211037901BB
Protocol Effective Date	2022-11-21
Technical Initiation Date	2022-11-21
Technical Completion Date	2022-11-23
Final Report Completion Date	2023-03-01

Personnel	Betty Zhuang	2023-03-01 Date Completed
Approved	Study Director	2023-03-01 Date Completed
Supervisory	Test Facility Manager	2023-03-01 Date Completed

CCIC Huatongwei International Inspection（Suzhou）Co., Ltd.

Quality Assurance Statement and GLP Statement

Quality Assurance Statement

The Quality Assurance Unit conducted inspections on the following dates. The findings were reported to the Study Director and to the HTW'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 Inspected	Date	Study Director	Management
Experiment	2022-11-21	2022-11-21	2022-11-21
Raw Data	2022-11-23	2022-11-23	2022-11-23
Final Report	2023-03-01	2023-03-01	2023-03-01

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

Hongxia Li
Quality Assurance

2023-03-01
Date

GLP Statement

This study was conducted in compliance with current 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 HTW, exempt from this Good Laboratory Practice Statement, included characterization and stability of the test article and its mixture with carriers, 21 CFR, Part 58.105 and 58.113.

Study Director

2023-03-01
Date

1.0 Purpose

The purpose of the test is to determine the potential cytotoxicity of the test article towards a mammalian cell culture (mouse fibroblast L-929 cells).

2.0 Reference

Biological evaluation of medical devices-Part 5: Tests for In Vitro Cytotoxicity (ISO 10993-5: 2009)

Biological evaluation of medical devices-Part 12: Sample preparation and reference materials (ISO 10993-12: 2021)

Use of International Standard ISO 10993-1, "Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process"

3.0 Test and control articles

Groups	Test article	Negative Control Article	Positive Control Article	Blank Control
Name	YS-33221	High Density Polyethylene Film	ZDEC	MEM medium, with addition 10% FBS
Manufacture	Not Provided	Hatano Research Institute. FDSC	Sigma-Aldrich.	Hyclone
Size	Not Provided	3 cm×10 cm (5 sheets)	25 g	500 ml
Model	UVadhesive	/	/	/
Lot Batch#	See package	C-212	BCBQ6847V	AH30006415
Test Article Material	Not Provided	/	/	/
Physical State	Solid	Solid	Solid	Liquid
Color	Not Provided	White	White	Pink
Package material	Not Provided	/	/	/
Sterilized or Not	Not Sterilized	No	No	Yes
Concentration	/	/	0.1%	/
Surface (cm^2)	/	/	/	/
Weight (g)	/	/	/	/
Storage Condition	Room Temp.	Room Temp.	Room Temp.	4℃
Note：The information about the test article was supplied by the sponsor wherever applicable.				

4.0 Identification and justification of test system

L-929 mouse fibroblast cells obtained from American Type Culture Collection (ATCC). Cell cultures were free of mycoplasma and microbial contamination upon use.

L-929 cells have been used for cytotoxicity studies because they demonstrate sensitivity to extractable cytotoxic articles. Also, the test article is extracted and administered in vitro to mouse fibroblast L929 cells through a solvent compatible with the test system, which is the optimal route of administration available in this test system as recommended in ISO 10993-5.

5.0 Equipment and reagents

5.1 Instruments

Vertical pressure steam sterilizer (SHB026), Shaker incubator (SHB203), CO_2 Incubator (SHB002), Steel Straight Scale (SHB076), Electronic Balance (SHB016), Clean bench (SHB014), Multiskan Spectrum Microplate Spectrophotometer (SHB003), Bench type low speed centrifuge (SHB306), Inverted microscope (SHB005)

5.2 Reagents

MEM (Hyclone, AH30006415), FBS (Clark, JC65980), Penicillin-Streptomycin (Biosharp, 22019209), Trypsin (Gibco, 2445462), PBS (Hyclone, AH29787894), MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetr azolium bromide) (Solarbio, 530R054), Isopropyl alcohol (Rhawn, RH397824)

6.0 Sample preparation

According to the table below, aseptic extraction of the test article sealed and incubated in MEM medium (10% FBS) at 37 ℃, 5% CO_2 and 60 rpm for 24 hours.

Groups	Sampling		Aseptic Extraction In Inert Container				Final Extract
	Sampling Manner	Actual sampling	Ratio	Extracts	Condition	pH	Clear or Not
Test article	Random	5.1 g	0.2g:1ml	25.5 ml	37 ℃ 24 h	7.4	Clear
Negative Control	Random	60.0 cm^2	3 cm^2: 1 ml	20.0 ml	37 ℃ 24 h	7.4	Clear
Positive Control	Random	0.02 g	0.1 g: 100 ml	20.0 ml	37 ℃ 24 h	7.4	Clear
Blank Control	/	/	/	20.0 ml	37 ℃ 24 h	7.4	Clear

The changes of the leaching solution was observed after extraction. No particulates or color changes were observed in pre- and post-extraction, the color and pH of the extraction solution did not change before and after use, and the pH value was 7.4, the status of the extract was shown in the figure below. The extraction solution and the pH value did not been adjusted, filtered, centrifuged, diluted and other processes before used.The extraction of the test article can be stored at 4℃ for no more than 24 h, but in our test, the test article extract was immediately be used after leaching. Prepare blank control (MEM medium with 10% FBS) and negative/positive control under the same conditions.

Vehicle	Time Observed	Groups	Condition of Final Extracts		
			Color	Clear or Not	Particulates
MEM medium (10% FBS)	Before Extraction	Test article	Pink	Clear	None
		Negative Control	Pink	Clear	None
		Positive Control	Pink	Clear	None
		Blank Control	Pink	Clear	None
	After Extraction	Test article	Pink	Clear	None
		Negative Control	Pink	Clear	None

		Positive Control	Pink	Clear	None
		Blank Control	Pink	Clear	None

7.0 Test method

Aseptic procedures were used for handling cell cultures. L-929 cells were cultured in MEM medium (10% FBS, 1% Penicillin-Streptomycin solution) at 37 ℃ in a humidified atmosphere of 5% CO_2, then digested by 0.25% trypsin containing EDTA to get single cell suspension. 1×10^5 cells/ml suspension were obtained by centrifuging (1000 rpm, 5 min) and re-dispersing in MEM medium.

The suspended cells were dispensed at 100 µl per well in 96-well plates, and cultured in a cell incubator (5% CO_2, 37 ℃, >90% humidity). Cell morphology was evaluated to verify that the monolayer was satisfactory.

After 24 h incubation which made the cells grow to about 70% and form a monolayer, original culture medium was discarded. The 96-well plates were then treated with 100 µl of extract of test article (100%、75%、50%、25%), control article, negative article and positive article respectively. The 96-well plate was incubated at 37 ℃ in cell incubator of 5% CO_2 and >90% humidity for 24 h. Six replicates of each test were tested.

After incubation, observe the cell morphology first and then discard the culture medium. Add 50 µl MTT (1mg/ml) to each well and then incubated at 37 ℃ in a humidified atmosphere of 5% CO_2 for 2 hours. The liquid in each well was tipped out and 100 µl Isopropyl alcohol was added to each well to suspend the cell layer.

Evaluate the suspension above with a dual-wavelength spectrophotometer with the measurement wavelength at 570 nm.

8.0 Statistical method

Mean±standard deviation ($\bar{x}$ ±s)

The cell Viab. (%) = OD_{570} of test (or positive or negative) article group/ OD_{570} of blank control group×100%.

Table 1 Qualitative morphological grading of cytotoxicity of extracts

Grade	Reactivity	Conditions of all cultures
0	None	Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth
1	Slight	Not more than 20 % of the cells are round, loosely attached and without intracytoplasmatic granules, or show changes in morphology; occasional lysed cells are present; only slight growth inhibition observable.
2	Mild	Not more than 50 % of the cells are round, devoid of intracytoplasmatic granules, no extensive cell lysis; not more than 50 % growth inhibition observable.
3	Moderate	Not more than 70 % of the cell layers contain rounded cells or are lysed; cell layers not completly destroyed, but more than 50 % growth inhibition observable.
4	Severe	Nearly complete or complete destruction of the cell layers.

9.0 Evaluation criteria

9.1 The 50% extract of the test article should have at least the same or a higher viability than the 100% extract. Otherwise the test should be repeated.

9.2 The lower the Viab.% value, the higher the cytotoxic potential of the test article is.

9.3 If viability is reduced to < 70% of the blank, it has a cytotoxic potential.

9.4 The Viab.% of the 100% extract of the test article is the final result.

9.5 A test does not meet acceptance criteria if a cytotoxic effect is observed for the negative controls or no cytotoxic effect is elicited for the positive controls.

10.0 Results of the test

10.1 Results of the cell morphology

Table 2 Observation of the cell morphology

Group	Before inoculation	Before treated with extract	24 h after treatment
Blank control	0	0	0
Negative control	0	0	0
Positive control	0	0	4
100% Test article extract	0	0	4
75% Test article extract	0	0	4
50% Test article extract	0	0	4
25% Test article extract	0	0	4

10.2 Results of the MTT cytotoxicity test

Table3 Results of the MTT cytotoxicity test

Group	OD value								Viab. (%)
	1	2	3	4	5	6	$\bar{x}$	s	
Blank control	0.617	0.627	0.621	0.631	0.612	0.612	0.620	0.008	100.0
Negative control	0.631	0.634	0.623	0.629	0.633	0.617	0.628	0.007	101.3
Positive control	0.053	0.052	0.057	0.051	0.057	0.056	0.054	0.003	8.8
100% test article extract	0.048	0.046	0.045	0.051	0.046	0.050	0.048	0.002	7.7
75% test article extract	0.055	0.061	0.060	0.062	0.056	0.056	0.058	0.003	9.4
50% test article extract	0.066	0.059	0.059	0.058	0.059	0.068	0.062	0.004	10.0
25% test article extract	0.115	0.109	0.120	0.129	0.103	0.106	0.114	0.010	18.3

11.0 Conclusion

Under the conditions of this study, the test article has potential toxicity to L-929 cells.

12.0 Compliance

US FDA Good Laboratory Practice Regulations 21 CFR 58, effective June 20, 1979, as amended 52 FR 33780, Sept. 4, 1987, and subsequent amendments

Standard operating procedure of CCIC Huatongwei International Inspection（Suzhou）Co., Ltd.

13.0 Protocol amendment/deviations

There were no amendments or deviations that occurred during the course of this study.

14.0 Record

All raw data pertaining to this study and a copy of the final report are to be stored in the designated archive

files at Huatongwei.

15.0 Confidentiality Agreement

Statements of confidentiality are as agreed upon prior to study initiation.