中国认可
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检测
TESTING
CNAS L10463

FINAL REPORT

Study Name: Low Density Polyethylene Film/Bags- In Vitro Cytotoxicity Test

Study Number: MED202012320-05-EN

Sponsor

Name: Changzhou Dreure Packaging Material Co., LTD.

Address: No.502 Changting Road, Wujin Economic Development Zone, Changzhou, Jiangsu, China

Testing Facility

Name: EPIN Suzhou Ltd.

Address: No.558 Fenhu Avenue, LiLi Town, Wujiang District, Suzhou, China

SUPPLEMENTARY EXPLANATION

1. Please apply for rechecking within 15 days after receiving the report if there is any objection.
2. The report is only valid with the dated signatures by person responsible and cross-page seal.
3. The results in this report relate only to the article tested.
4. The test report shall not be reproduced except in full, without written approval of EPIN Suzhou Ltd.
5. ILAC-G8:09/2019 was employed as the decision rules of statement conformity, where applicable.

TEST ARTICLE CONFIRMATION AND SIGNATURE



Edited by: Arain Yan Date: 2021-01-08
Arain Yan (Report Drafter)

Checked by: aliciawang Date: 2021-01-08
Alice Wang (Study Director)

Approved by: Julie Wang Date: 2021 01 08
Julie Wang (Authorized Signatory)

GLP COMPLIANCE STATEMENT

This study was performed in compliance with a quality management system according to ISO/IEC 17025:2017 , *Accreditation criteria for the competence of testing and calibration laboratories*,which has been established at EPIN Suzhou Ltd.

The EPIN Suzhou Ltd. quality management system includes the quality requirements prescribed by the international guidelines for Good Laboratory Practice (GLP).

Study Director: Alice Wang

Date: 2021-01-08

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 EPIN, 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.

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

Inspections	Date of Inspections	Date of Reported
Test article preparation	2020-12-30	2020-12-30
Dosing process	2020-12-30	2020-12-30
Observing process	2020-12-31	2020-12-31
Raw data +Final report	2021-01-08	2021-01-08

Quality Assurance Unit: Bill Yu Date: 2021-01-08

Bill Yu

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SUMMARY

1. Purpose

The purpose of the test is to determine the biological reactivity of a mammalian cell culture (mouse fibroblast L-929 cells) in response to the test article Low Density Polyethylene Film/Bags.

2. Process Description

L-929 cells were cultured in MEM medium (10% FBS, Penicillin 100 U/mL, Streptomycin sulfate 100 μg/mL) at 37°C in a humidified atmosphere of 5% CO2 and then digested by 0.25% trypsin to obtain a cell suspension. The suspended cells were dispensed in 35 mm plat and cultured under the same condition.

After 24 h, discarded the culture medium. Added the fresh MEM medium into the plate. Then put the sample, negative control and positive control on the cell layer. Bipartite in each group and cultured the plate at 37°C, 5% CO2 condition.

After 24 h incubation, observed the cell morphology microscopically.

3. Result

The direct contect tese method results showed that the cytotoxicity grade of the test article was 1. The results of control groups showed the test was valid.

4. Conclusion

Under the conditions of this study, the test article did not show potential toxicity to L-929 cells.

1. STUDY SUMMARIES

1.1. Study Name (Study No.)

Low Density Polyethylene Film/Bags - In Vitro Cytotoxicity Test (MED202012320-05-EN)

1.2. Purpose

The purpose of the test is to determine the biological reactivity of a mammalian cell culture (mouse fibroblast L-929 cells) in response to the test article.

1.3. Referred Standard

- USP43-NF38<87>
  BIOLOGICAL REACTIVITY TESTS, IN VITRO

1.4. Compliance

- Good Laboratory Practice Regulations, 21 CFR, Part 58

1.5. Testing Facility

Name:	EPIN Suzhou Ltd.
Address:	No.558 Fenhu Avenue, Lili Town, Wujiang District, Suzhou, China

1.6. Sponsor

Name:	Changzhou Dreure Packaging Material Co., LTD..
Address:	No.502 Changting Road, Wujin Economic Development Zone, Changzhou, Jiangsu, China
ATTN:	Yu Zheng
Contact Information:	+86 135 8430 7736/519 8988 1356/qa@dreure.com

1.7. Quality Guarantee

Quality Assurance Unit: Bill Yu

The methods and raw data involved in this test were inspected and this report was reviewed by the Quality Assurance Unit (QAU). The key practices during the procedure were inspected regularly by QAU required by related SOP.

1.8. Study Alteration Treatment

Before the study start, the study protocol was approved by Study Director and Sponsor. Meanwhile, QAU finished the protocol review in accordance with the requirements for GLP. Any study alteration should be approved by Study Director.

1.9. Deviation(s) and Incident(s) Treatment

If any deviation or incident occurred during the test, the related information would be recorded timely and a deviation report should be submitted with the final report to interpretate the specific effect(s) on the final result caused by the deviation or incident.

1.10. Major Laboratory Personnel(s)

Study Director:	Alice Wang
Main Operation Personnels:	Alice Wang, Arain Yan

1.11. Schedule of the Study

Test Article Received Date:	2020-12-22
Protocol Effective Date:	2020-12-25
Technical Initiation Date:	2020-12-29
Technical Completion Date:	2020-12-31
Report Signature Date:	2021-01-08

2. TEST MATERIAL

2.1. Test Article

2.1.1. General information[1)]

Name:	Low Density Polyethylene Film/Bags
Initial State:	Non-sterile
Size:	N/S[2)]
Model:	N/S
Lot/ Batch#:	S2012008
Physical State:	Solid
Color:	N/S

Density:	N/S
Stability:	N/S
Solubility:	N/S
Storage Condition:	Room temperature
Test Article Material:	N/S
Packaging Material:	N/S
Expiry Date:	N/S
Manufacturer Name:	Changzhou Dreure Packaging Material Co., LTD..
Manufacturer Address:	No.502 Changting Road, Wujin Economic Development Zone, Changzhou, Jiangsu, China

1) The information about the test article was supplied by the sponsor wherever applicable. The Sponsor is responsible for all test article characterization data as specified in the GLP Regulations.
2) N/S means not supplied by the sponsor.

2.1.2. Retention of test article(s)

Retention Volume:	/
Retention Location:	/

2.1.3. Handling of residual test article(s)

Tested Article(s):	Distroy and waste
Untested Article(s):	Distroy and waste

2.2. Blank Control

Name:	MEM medium with 10% FBS
Manufacturer:	Hyclone
Size:	500 mL
Lot/ Batch#:	AF29549370
Physical State:	Liquid
Color:	Pink
Storage Condition:	(2-8) °C

2.3. Negative Control

Name:	High Density Polyethylene Film
Manufacturer:	Hatano Research Institute, FDSC
Size:	Ca. 3 cm×10 cm (5sheets)
Lot/Batch#.:	C-191
Physical State:	Solid
Color:	White
Storage Condition:	Room temperature

2.4. Positive Control

Name:	0.1% ZDEC Polyurethane Film
Manufacturer:	Hatano Research Institute, FDSC
Size:	Ca. 10 cm×15 cm
Lot/Batch#.:	A-202K
Physical State:	Solid
Color:	White
Storage Condition:	Room temperature

2.5. Main Instruments and Reagents

2.5.1 Main instruments

Name	No.	Calibration Due Date
CO_2 Incubator	EPB-122	2021-09-28
Shaking Water Bath	EPB-232	2021-09-28
Inverted Microscope	EPB-003	2021-09-28

2.5.2 Main reagents

Name	Lot/ Batch#	Source
Fetal bovine serum (FBS)	2064652	Gibco
Minimum essential medium (MEM)	AF29549370	Hyclone
Penicillin streptomycin sulfate	J190044	Hyclone
Trypsin	J200009	Hyclone

3. JUSTIFICATION OF THE TEST SYSTEM

Historically, mouse fibroblast L-929 cells have been used for cytotoxicity studies because they demonstrate sensitivity to test articles.

4. IDENTIFICATION OF TEST SYSTEM

L-929 mouse fibroblast cells obtained from Shanghai Cell Line Resources, CAS.

5. ROUTE OF ADMINISTRATION

The test article administrated in vitro to mouse fibroblast L-929 cells through direct touch with the test system. This was the optimal route of administration available in this test system as recommended in the guidelines.

6. TEST DESIGN

6.1. Test Article Preparation

Aseptically cut the test article into 1×1 cm squares, put the squares in the middle of culture dish and maked sure them contacted with cell directly.
The positive control and negative control were cut in the same way and sterilized with UV.

6.2. Experimental Process

Aseptic procedures were used for handling of cell cultures.
L-929 cells were cultured in MEM medium (10% FBS, Penicillin 100 U/mL, Streptomycin 100 μg/mL) at 37°C in a humidified atmosphere of 5% CO_2, then digested with 0.25% trypsin to get single cell suspension. And obtained a 1×10^5 cells/mL suspension by centrifuging (1000 rpm, 5 min) and re-suspended in MEM medium finally.
The suspended cells were dispensed at 2 mL per well in 35 mm-plates, and cultured them in cell incubator (5% CO_2, 37°C). Cell morphology was evaluated to verify that the monolayer was satisfactory.
After the cells grew to form a monolayer, original culture medium was discarded. Added the fresh MEM medium 0.8 mL into the plate. Then put the test article, negative control and positive control in the center of

cell layer. Bipartite in each group and cultured the plate at 37°C, 5% CO_2 condition.
After 24 h incubation, observed the cell morphology microscopically.

7. EVALUATION CRITERION

- Reactivity grades for elution test:

Grade	Reactivity	Description of reactivity zone
0	None	No detectable zone around or under specimen
1	Slight	Some malformed or degenerated cells under specimen
2	Mild	Zone limited to area under specimen or extending specimen size to 4.5 mm
3	Moderate	Zone extending specimen size up 4.5 mm to 10 mm
4	Severe	Zone extending farther than 1.0 cm beyond specimen

- For a valid test, an intact cell layer should be found under the negative control.
- The grade greater than 2 was considered cytotoxic.

8. ALTERATION AND DEVIATION

Alteration and deviation did not happen in this study.

9. RESULTS

Table 1 Reactivity grades

Group	Parallel 1	Parallel 2	Grade	Cytotoxicity
Test	1	1	1	Slight
Negtive Control	0	0	0	None
Positive Control	4	4	4	Severe

10. CONCLUSION

Under the conditions of this study, the test article Low Density Polyethylene Film/Bags did not show potential toxicity to L-929 cells.

11. ARCHIVING

All correspondence, including original copy of the protocol, original copy of the test report, and all raw data generated during the study (i.e., documentation forms as well as any other notes of raw data, printouts of instruments and computers) are stored in the archives room of the EPIN Suzhou Ltd.

End of the Report