INDUSTRIAL BIOTECHNOLOGY RESEARCH CENTRE
Building 19, SIRIM Complex
1, Persiaran Dato' Menteri, Section 2, P. O. Box 7035
40700 Shah Alam, Selangor Darul Ehsan, MALAYSIA
Tel: 603 - 5544 6953 / 6960 Fax: 603 - 5544 6988
Website: www.sirim.my

TEST REPORT

EVALUATION OF SMART COAT IN THE BALB/c 3T3 NRU CYTOTOXICITY STUDY

Job No. J463/20

Report No. R463/20/B19/03

Sponsor:
Titanium World Technology Sdn Bhd.,
No. 16-3, Jalan Jalil 6,
Jalil Link, Bukit Jalil,
5700 Kuala Lumpur.

Sponsor Representative:
Jason Kuan/Wong Weng Heng

Test Facility:
Industrial Biotechnology Research Centre (IBRC),
Building 19, SIRIM Berhad.

Study Initiation Date:
18 April 2020

Experimental Start Date:
20 April 2020

Experimental End Date:
23 April 2020

Study Completion Date:
04 May 2020

5.7 Interpretation of Result

The OD_{570} data was transferred to a Microsoft Excel spreadsheet and the relative cell viability was presented as a percentage of each optical density value against mean optical density of vehicle control. The calculated values with its equivalent test item concentrations was supposed to be applied to a Hill function analysis using GraphPad Prism® version 6.04 for Windows software. The concentration corresponding to the IC_{50} was to be calculated as follows:

$$\log IC_{50} = \log EC_{50} - [\log ((Top-Bottom)/(Y-Bottom) - 1) / HillSlope]$$

where
IC_{50} is the concentration producing 50% toxicity;
EC_{50} is the concentration producing a response midway between the Top and Bottom responses;
Top is the maximum response (100% viability, maximum survival);
Bottom is the minimum response (0% viability, maximum toxicity);
Y=50 (i.e. 50% viability); and
HillSlope describes the slope of the response.

A mean IC_{50} from two main tests was to be calculated as the final result and applied to the following regression formula for estimation of a median lethal dose or LD_{50} in mg/kg:

$$\log LD_{50} (mg/kg) = 0.872 \log IC_{50} (\mu g/mL) + 2.024$$

Starting dose for the Up-and-Down Procedure (UDP) method is the next dose lower than the estimated LD_{50} in the default dose progression. The default dose progression for UDP is 1.75, 5.5, 17.5, 55, 175, 550 and 2,000 mg/kg for the 2,000 mg/kg limit test.

6.0 RESULT AND DISCUSSION

6.1 Solubility Determination

The test item was soluble in Chemical Dilution Medium (CDM) at the highest stock concentration of 200,000 µg/mL. Chemical Dilution Medium (CDM) was used as the vehicle control.

6.2 Range Finder Test

Range Finder Test was carried out at the highest treatment concentration of 100,000 µg/mL. The concentration-response curves are presented in Figure 1. Individual NRU data and the calculated relative viability percentages are presented in the Appendix (A-1.1 and A-1.2).

6.3 Main Test

Main test was not carried out because a concentration-response curve could be not established from the range finder test.

6.4 Controls

Positive control was carried out at the highest treatment concentration of 250 µg/mL. The concentration-response curves are presented in Figure 2. Individual NRU data and the calculated relative viability percentages are presented in the Appendix (A-2.1 and A-2.2).

7.0 CONCLUSION

Under the condition of this study, the median inhibition concentration (IC_{50}) level of Smart Coat predicted an LD50 value of more than 2,000 mg/kg body weight. Therefore, the proposed starting dose for acute oral toxicity test according to the Up-and-Down Procedure is 2,000 mg/kg.

8.0 RETENTION OF RECORDS

One report will be forwarded to the Sponsor. The other report, together with all generated raw data, is maintained at the Industrial Biotechnology Research Centre Archives.

9.0 REFERENCE

9.1 Federal Register, Vol. 73, No. 58, 25 March 2008, pp 15757-15758. Availability of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Test Method Evaluation Report: In Vitro Cytotoxicity Test Methods for Estimating Starting Doses for Acute Oral Systemic Toxicity Tests and the Final Background Review Document for In Vitro Cytotoxicity Test Methods for Estimating Acute Oral Systemic Toxicity.

9.2 ICCVAM Test Method Evaluation Report Appendix C: Recommended Test Method Protocols (November 2006)

9.3 OECD (2010). Guidance Document on Using Cytotoxicity Tests to Estimate Starting Doses for Acute Oral Systemic Toxicity Tests. Environmental Health and Safety Monograph Series on Testing and Assessment No 129.

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APPENDIX
Range Finder Test

A-1.1 Range Finder Test: NRU data of Smart Coat
Corrected optical density values (OD_{570} values minus mean OD_{570} of blank) and vehicle controls

Vehicle Control	Smart Coat (µg/mL)								Vehicle Control
	100000	10000	1000	100	10	1	0.1	0.01	
*0.028	0.039	0.034	0.041	*0.039	*0.036	*0.034	*0.045	0.042	0.042
0.034	0.035	0.037	0.042	0.040	*0.041	*0.048	0.047	*0.036	0.038
0.034	*0.049	0.040	*0.046	*0.050	0.053	0.051	0.051	*0.050	*0.052
0.030	*0.047	*0.052	*0.047	*0.045	*0.057	0.053	*0.059	0.041	*0.052
0.037	*0.042	*0.047	*0.049	0.041	0.051	0.052	0.051	*0.033	0.041
0.032	0.038	0.038	0.040	0.044	0.049	0.051	*0.040	0.040	0.039

Note
Cell passage number = 5
Vehicle control: Difference between Column 2 and the mean = 9.6 %
Data marked * omitted from relative cell viability calculation

A-1.2 Range Finder Test 1: Relative viability percentage of Smart Coat
Percentage of OD_{570} against mean vehicle control, together with the calculated mean and standard deviation (s.d)

	Smart Coat (µg/mL)							
	100000	10000	1000	100	10	1	0.1	0.01
	107.0%	94.4%	112.8%	-	-	-	-	115.5%
	96.6%	101.0%	114.4%	109.5%	-	-	130.4%	-
	-	109.8%	-	-	146.0%	140.3%	140.6%	-
	-	-	-	-	-	146.6%	-	111.7%
	-	-	-	111.7%	139.7%	144.1%	138.9%	-
	105.6%	105.6%	109.2%	119.9%	135.3%	140.3%	--	109.5%
Mean	103.1%	102.7%	112.2%	113.7%	140.4%	142.8%	136.6%	112.2%
s.d.	5.7%	6.6%	2.7%	5.5%	5.4%	3.1%	5.5%	3.1%

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APPENDIX
Positive Control

A-2.1 Positive Control: NRU data of Sodium Lauryl Sulphate
Corrected optical density values (OD_{570} values minus mean OD_{570} of blank) and vehicle controls

Vehicle Control	Sodium lauryl sulphate (µg/mL)								Vehicle Control
	250.0	142.9	81.6	46.6	26.7	15.2	8.7	5.0	
*0.039	-0.002	-0.004	0.000	0.017	*0.035	*0.053	*0.063	*0.063	0.063
0.046	*0.002	-0.004	0.002	0.019	*0.044	0.059	0.069	0.072	0.070
0.062	-0.004	*-0.004	*-0.002	*0.028	0.057	*0.083	0.067	*0.091	0.073
0.050	-0.002	0.001	0.002	0.017	0.059	*0.076	*0.080	*0.083	*0.083
0.053	*0.001	0.001	0.003	0.018	0.057	0.062	0.066	0.073	0.073
0.058	*0.004	*0.003	*-0.004	*0.023	0.050	0.067	*0.071	0.073	0.068

Note
Cell passage number = 5
Positive control: IC_{50} = 37.5 µg/mL and R^2 = 0.9913
Vehicle control: Difference between Column 2 and the mean = 12.9%
Data marked * omitted from relative cell viability calculation

A-2.2 Positive Control: Relative viability percentage of Positive Control
Percentage of OD_{570} against mean vehicle control, together with the calculated mean and standard deviation (s.d)

	Sodium lauryl sulphate (µg/mL)							
	250.0	142.9	81.6	46.6	26.7	15.2	8.7	5.0
	-3.9%	-6.8%	0.8%	27.2%	-	-	-	-
	-	-6.2%	4.0%	31.6%	-	95.7%	111.3%	116.2%
	-6.0%	-	-	-	92.2%	-	108.7%	-
	-2.8%	1.4%	2.7%	27.7%	95.1%	-	-	-
	-	1.4%	4.5%	29.0%	92.2%	100.8%	106.8%	119.1%
	-	-	-	-	81.5%	108.4%	-	117.9%
Mean	-4.2%	-2.5%	3.0%	28.9%	90.2%	101.6%	108.9%	117.7%
s.d.	1.6%	4.6%	1.7%	2.0%	6.0%	6.4%	2.3%	1.5%