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Toxicology

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Summary Report

To: JLic
Copy: JNP, QA
From: FKBi

Re: Asparaginase, batch PPV33595
***In Vitro* Cytotoxicity Test:**
Neutral Red Uptake in BALB/c 3T3 cell culture
Study No.: 20128030

PURPOSE

The purpose of this study was to screen for the cytotoxic potential of Asparaginase, batch PPV33595.

TEST MATERIALS

Name:	Asparaginase, batch PPV33595
Received from:	RPP, 19. March 2012.
Appearance:	brown liquid
pH:	8.0 at the highest concentration of dosing solution (30 mg/mL)
Osmolarity:	332 mOsm at the highest concentration of dosing solution (30 mg/mL)

SCHEDULE

Study Initiation Date:	22 March 2012
Experimental Starting Date:	27 March 2012
Experimental Completion Date:	30 March 2012
Study Completion Date:	12 April 2012

METHOD

The test is performed according to standard method TOX-SM-1013.01, ver. 2.0. BALB/c 3T3 cells were grown in growth medium. A 96-well micro-plate was added 100µl (5×10^5) cell suspension per well. The plate was incubated for 24 hours at 37°C, 90% ± 10% humidity and 5% CO_2/air, establishing an approximately 50% confluent cell culture. 50 µL growth medium and 50 µl double concentrated test material dilutions in growth medium were added (8 replicate testing) to each well and the plate incubated for 48 hours at 37°C, 90% ± 10% humidity and 5% CO_2/air.

After 48 hours incubation the test material was removed, wells washed with 250 µl DPBS (+$CaCl_2$, +$MgCl_2$) and 250 µL Neutral Red working solution added to each well. The plate was incubated in the CO_2 incubator for 3 hours at 37°C, 90% ± 10% humidity and 5% CO_2/air , thereafter washed twice with 250 µl DPBS (+$CaCl_2$, +$MgCl_2$)/well, before 100 µL Neutral Red desorb solution was added to each well to leach the stain from the cells.

The plate was agitated well to evenly distribute the released Neutral Red, and the absorbance at 540 nm (OD_{540}) of each well was measured to indicate the number of cells surviving exposure to the test materials. The concentration of the test substance required to reduce the viability of the treated test system to 50% of that of the untreated control test system was determined as the endpoint (NRU_{50}).

Dosing:

The following concentrations were selected for the test materials for determination of cytotoxicity in this test model: 30, 10, 3, 1, and 0.3 mg per mL growth medium.

Positive control: 0.16, 0.13 and 0.10 mg SDS per mL growth medium.

Appropriate growth medium control wells (negative control cells) and blank controls (growth medium alone without cells) were included on the micro-plate.

DEVIATIONS

The positive control, SDS, was by mistake, not included in this assay. The assay is however considered to be valid, because of the values obtained for the negative control and thus the deviation is judged not to have influenced the result of the assay.

RESULTS AND DISCUSSION

The results obtained in this investigation appear from table 1 and are illustrated in figure 1.

The concentration of the test substance required to reduce the viability of the treated test system to 50% of that of the untreated control test system was determined as the endpoint (NRU_{50}).

The NRU_{50} for Asparaginase, batch PPV33595 was calculated from the linear part of the curve (10 to 1 mg amylase/mL) and determined to be 5.72 mg/mL (see table 1).

The variability of the negative control was within the acceptance criteria i.e. control mean values from negative controls on each site of the test plate, respectively, did not differ more than 15% from the mean of all negative controls.

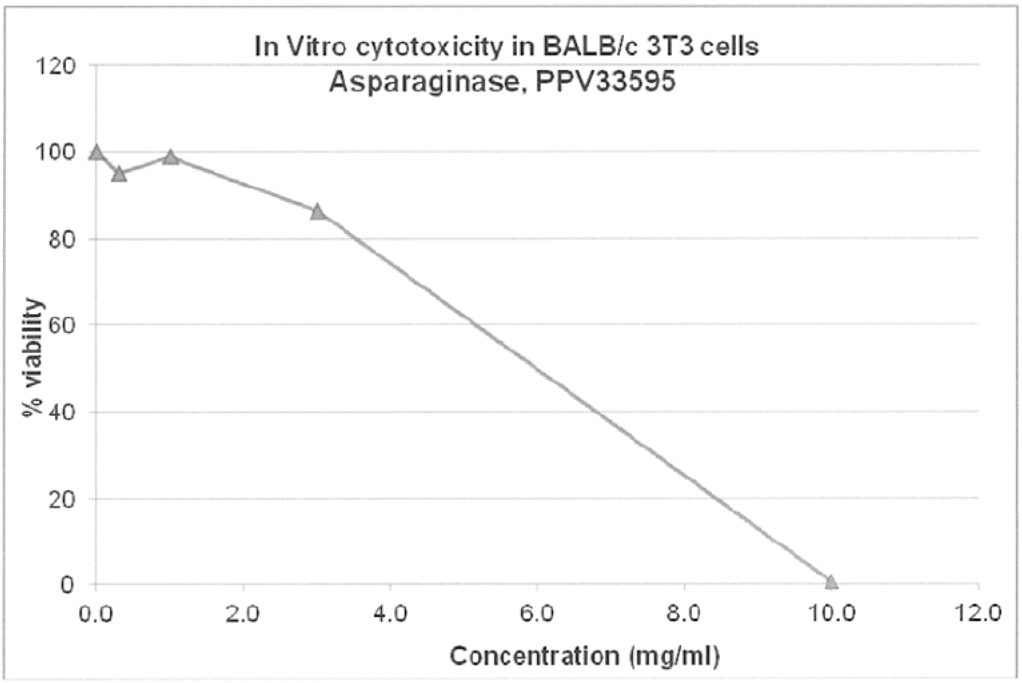
The OD values for the negative control were >0.3 thereby meeting the acceptance criteria for a valid test.

Thus the test was considered to be valid.

Table 1:

Test substance	Concentration mg/mL	Viability %	NRU_{50} mg/mL
Asparaginase, batch PPV33595	0	100	5.72
	0.3	95	
	1	99	
	3	86	
	10	0	
	30	0	

Figure 1:



CONCLUSION

The NRU_{50} value for Asparaginase, batch PPV33595 is 5.72 mg/ml in the present *in vitro* Neutral Red Uptake assay applying the mouse fibroblast cell line BALB/c 3T3 as test system.

Date: 12 April 2012

[redacted]

Study Director