



Study Reportr S167321

Comments:

Number

Study report Page 7 (Table 1), regarding the list of HCS-based endpoints

[Comment 1](#)

Study report Page 9 (Table 2 and Table 3), regarding concentration ranges used in RTCA and HCS experiments.

[Comment 2](#)

Study report Page 9 (section 8.2), regarding seeding density for RTCA and HCS experiments.

[Comment 3](#)

Study report Page 30 (Figure 32A), regarding plot title.

[Comment 4](#)

Study report Page 9 (section 8.1.4) regarding analytical determination of nicotine and carbonyls

[Comment 5](#)

Cell cycle endpoint not included in the study report but included in the related publication

[Comment 6](#)



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1 Comment description

Comment 1 (Page 7, table 1). The report indicates that 13 different HCS-based endpoints were measured in the study. The number of endpoints listed in Table 1 is 14, because the cell membrane permeability endpoint was measured in two different assays (cytotoxicity screening panel and apoptosis / necrosis). Due to interference of 3R4F TPM with the dye used to measure cell membrane permeability in the cytotoxicity screening panel, only the cell membrane permeability data from the apoptosis / necrosis assay was used for the purpose of the study report and the related publication by Gonzalez-Suarez et al (PMID 26651182).

Comment 2 (Page 9, Table 2 and Table 3). The report includes two tables with a summary of the concentrations tested for the different items and fractions. Some typographical errors were detected only after the report was completed. The corrected tables are shown below:

		DOSES (puff / L)																					
3R4F TPM	Exp.1	X	X	X	X	X	X	X	X	X	64	52	X	42	X	32	26	X	X	13	X	X	Vehicle
	Exp.2	X	X	X	X	X	X	X	X	X	64	52	X	42	X	32	X	X	16	X	8	X	Vehicle
	Exp. 3-5	X	X	X	X	X	X	X	X	X	64	52	X	42	X	32	26	X	16	13	X	X	Vehicle
	Exp. 6-8	X	X	X	X	X	X	X	X	X	64	52	X	42	X	32	X	X	16	X	8	X	Vehicle
P1 TPM	Exp. 1-6	450	375	X	300	225	X	150	X	75	X	X	X	X	38	X	X	X	X	X	X	X	Vehicle
	Exp. 7-8	450	375	X	300	225	X	150	X	75	X	X	X	X	X	X	X	X	X	X	X	X	Vehicle
3R4F GVP	Exp. 1-5	X	X	X	X	X	200	X	100	X	X	X	50	X	X	X	X	25	X	13	X	6	Vehicle
P1 GVP	Exp. 1-3	X	X	350	300	X	200	X	100	X	X	X	50	X	X	X	X	25	X	X	X	X	Vehicle
3R4F sbPBS	Exp. 1-6	X	X	X	X	X	200	X	100	X	X	X	50	X	X	X	X	25	X	13	X	6	Vehicle
P1 sbPBS	Exp. 1-3	X	X	350	300	X	200	X	100	X	X	X	50	X	X	X	X	25	X	X	X	X	Vehicle

Table 2. Dose ranges tested in NHBE cells by RTCA analysis

		DOSES (puff / L)																									
3R4F TPM	Exp 1.	X	X	X	X	X	X	X	X	X	X	X	X	X	65	52	X	42	X	X	33	26	X	X	13	X	6
	Exp. 2-6	X	X	X	X	X	X	X	X	X	X	X	X	X	65	52	X	42	X	X	33	X	X	16	X	8	X
	Exp.1	450	380	X	300	X	226	X	X	150	X	X	75	X	X	X	X	X	38	X	X	X	X	X	X	X	X
P1 TPM	Exp. 2-3	450	380	X	300	X	226	X	X	150	X	X	75	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3R4F GVP	Exp. 1-6	X	X	X	X	X	X	X	200	X	X	100	X	X	X	X	50	X	X	X	X	X	25	X	13	X	6
P1 GVP	Exp. 1-3	X	X	350	300	X	X	X	200	X	X	100	X	X	X	X	50	X	X	X	X	X	25	X	X	X	X
3R4F sbPBS	Exp. 1-6	X	X	X	X	X	X	X	200	X	X	100	X	X	X	X	50	X	X	X	X	X	25	X	13	X	6
P1 sbPBS	Exp. 1-3	X	X	350	X	280	X	210	X	X	140	X	X	70	X	X	X	X	X	35	X	X	X	X	X	X	X

Table 3. Dose ranges tested in NHBE cells by HCS analysis



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1. Comment description

Comment 3 (Page 9, section 8.2). The study report indicates that 4.8×10^3 cells per well were seeded for RTCA experiments and 8×10^3 cells per well were seeded for HCS experiments (3×10^3 cells for the proliferation endpoint). These numbers are not correct. The correct seeding densities were 7.2×10^3 cells per well for the RTCA experiments and 12×10^3 cells for the HCS experiments (3×10^3 cells for the proliferation endpoint).

Comment 4. (Page 30, Figure 32A), The study report indicates that the title of the plot is Cell Count 4h. The title is not correct. The correct title was Nuclear size 4 h.

Comment 5. (Page 9, section 8.1.4) The study report indicates that nicotine concentrations were determined in TPM samples and carbonyl levels were determined in GVP and sbPBS samples. As detailed in the SP deviation document, nicotine concentrations could not be determined in this study. With regards to carbonyl concentrations in GVP and sbPBS fractions, the data was unintentionally omitted in the final version of the report, although the data was included in the related publication by Gonzalez-Suarez et al (PMID 26651182). The results are summarized in the table below:

	GVP FRACTION				GVP FRACTION			
	3R4F (µg/stick)		P1 (µg/stick)		3R4F (µg/stick)		P1 (µg/stick)	
	Average	STDEV	Average	STDEV	Average	STDEV	Average	STDEV
FORMALDEHYDE	19.8	2.3	2.1	1.1	38.9	6.7	2.6	1.6
ACETALDEHYDE	1336.0	86.8	198.9	26.7	1319.3	180.0	203.6	36.3
ACETONE	555.2	28.1	34.7	3.3	574.2	52.1	35.1	6.4
ACROLEIN	139.4	8.4	8.3	0.7	128.5	9.7	8.6	1.4
PROPIONALDEHYDE	76.8	10.8	12.3	1.4	72.6	22.5	12.0	3.9
CROTONALDEHYDE	59.5	5.7	3.5	0.5	61.7	6.4	3.4	0.6
METHYL ETHYL KETONE	124.0	9.6	6.9	0.7	132.6	13.4	6.8	1.3
BUTYRALDEHYDE	22.4	3.2	10.6	2.2	23.8	5.9	11.6	2.6

Comment 6 (cell cycle endpoint). The report does not include the results from the cell cycle analysis using a thymidine analogue (5 ethynyl 2 deoxyuridine, EDU). This HCS-based endpoint was not part of the original study plan. The cell cycle analysis endpoint was established at PMI laboratories only after the study report was finalized. The results of the cell cycle analysis were only included in the publication by Gonzalez-Suarez et al (PMID 26651182).



Effective Date: see EDMS

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3 Assessment on Report Quality and Integrity

The comments in this addendum related to bona fide errors that occurred during the preparation of the report and have little to no impact on the results of the study. Comment 6 is included for clarification, as some of the results included on the publication by Gonzalez-Suarez et al (PMID 26651182) were not part of the study plan and report for study S167321 .

Approval

Name

Date and Signature

Study Director

Ignacio Gonzalez Suarez

April -21-2017