



PMI_RD_FOR_000339

Version N°: 3.0

Effective

Global Quality Management

Quality Implementation

Effective Date: see EDMS

Study Number S167320

Deviation from:	Number
<input checked="" type="checkbox"/> Study Plan Throughout document, regarding SP number.	Deviation 1
<input checked="" type="checkbox"/> Study Plan Throughout document, regarding naming conventions.	Deviation 2
<input checked="" type="checkbox"/> Study Plan Page 6 (section4.1.2), regarding test item batch number used in the study.	Deviation 3
<input checked="" type="checkbox"/> Study Plan Page 14 (table 10) regarding maximum concentrations of 3R4F used in the study.	Deviation 4
<input checked="" type="checkbox"/> Study Plan Page 14 (figure 2), page 17 (figure 4), page 20 (figure 6) and page 21 (figure 7) regarding plate layouts for RTCA, HCS and gene expression experiments.	Deviation 5
<input checked="" type="checkbox"/> Study Plan Page15 (section 4.2.2.2) regarding the positive control used for RTCA experiments.	Deviation 6
<input checked="" type="checkbox"/> Study Plan Page18 (section 4.2.3) regarding the positive control used for HCS experiments.	Deviation 7
<input checked="" type="checkbox"/> Study Plan Page 1 (Work Package name) regarding the name of the Work Package	Deviation 8
<input checked="" type="checkbox"/> Study Plan Page 10 (Section 4.1.2.3) regarding the analytical determination of nicotine in TPM samples	Deviation 9



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1 Description of Deviations

Deviation 1 (throughout the document). The study plan (SP167320) comprises the assessment of smoke constituents (harmful / potentially harmful smoke constituents or HPHCs), ZRH 4.2 (also known as P1 or THS 2.2) aerosol fractions and 3R4F smoke fractions. Due to the broad scope of the study, and in order to better organize the results, separate reports with different study numbers were prepared.

Deviation 2 (throughout the document). Different naming conventions were used for test item, smoking regimen and smoke/aerosol fractions in study plan, study report and the related publication by Gonzalez-Suarez et al (PMID 26651182).

Deviation 3 (Page 6, section 4.1.2) The study plan indicates that batch number B-05879 will be used for the test item (ZRH). Due to the duration of the study, more than one batch of test items were used (B-05879 and B-08164).

Deviation 4 (Page 14, table 10) The study plan indicates the maximum concentrations of TPM, GVP and sbPBS generated from 3R4F smoke and THS 2.2 aerosol that NHBE cells can be exposed to. For 3R4F smoke fractions some of these concentrations could not be tested due to the fact that high levels of cytotoxicity were observed at a lower concentration.

Deviation 5. (Page 14, figure 2; page 17, figure 4; page 20, figure 6 and page 21, figure 7) The study plan indicates the layout of the plates used in RTCA, HCS and gene expression experiments. During the course of the study, the position of the different samples within a plate was modified in order to optimize the number of items and concentrations to be tested.

Deviation 6 (Page 15, section 4.2.2.2). The study plan indicates that staurosporine 300µM will be used as a positive control in RTCA experiments. The correct positive control used in the study was carbonyl cyanide m-chlorophenyl hydrazine (CCCP) 50µM.

Deviation 7 (Page 18, section 4.2.3). The study plan indicates that staurosporine 30, 300µM will be used as a positive control for apoptosis/necroptosis HCS endpoint. The correct positive control used in the study was CCCP. In addition, the study plan indicates that two different concentrations will be tested for each positive controls. During the course of the study, multiple concentrations were tested for each positive control.

Deviation 8. (Page 1, Work Package name). The study plan indicates that the study belongs to the Work Package "Biological Impact Assessment of MRTPs". During the course of the study, the scope of the work package increased and title name was modified accordingly to "Biological Impact Assessment of MRTPs, Aerosol Constituents, Mixtures and E-liquids"



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1 Description of Deviations

Deviation 9. (Page 10, Section 4.1.2.3). The study plan indicates that nicotine concentration will be measured in TPM samples. However, for technical reasons, nicotine determinations could not be performed in this study.



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2 Corrective Action

Deviation 1. The report containing the results of the assessment of HPHCs in normal human bronchial epithelial (NHBE) cells was given the following study number (S167320). The report for containing the results of the assessment of ZRH (also known as P1 and THS 2.2) aerosol fractions and 3R4F smoke fractions in NHBE cells was given the following study number (S167321).

Deviation 2. The following table summarizes the naming equivalencies between study plan, study report and related publication by Gonzalez-Suarez et al (PMID 26651182)..

	Study plan	Study report	Manuscript
1 Test item	ZRH (4.2)	P1	THS 2.2
2 Smoking regimen	HC (Health Canada)	HCI (Health Canada Intense)	Health Canada
3 MRTTP / RRP	pMRTTP (prototypic Modified Risk Tobacco Product)	RRP (Reduced risk product)	candidate MRTTP
4 smoke / aerosol fraction	sbPBS (smoke-bubbled PBS)	sbPBS (smoke-bubbled PBS)	AE (Aqueous extract)

Deviation 3. Batches B-05879 and B-08164 were used for real-time cellular analysis (RTCA) and high-content screening (HCS) experiments. Batch B-08164 was used for gene expression analysis.

Deviation 4. Final concentrations used in the study were included in the study report.

Deviation 5. No impact on study results was to be expected by the changes in plate layout. No corrective action was deemed necessary.

Deviation 6. The appropriate positive control used in the study was included in the study report.

Deviation 7. The following table summarizes positive controls and concentrations used in the study.\

	Assay	Endpoint	Positive control
1	Cytotoxicity	Cytochrome c release mitochondrial membrane potential Mitochondrial mass	CCCP
2	apoptosis / necrosis	Caspase 3/7 activity Membrane permeability	CCCP
3	Oxidative stress	ROS formation	Tacrine
4	GSH content	GSH content	Ethacrynic acid
5	DNA damage	DNA damage	Mytomicyn C
6	Proliferation	Proliferation	Nocodazole
7	Stress kinase	Stress kinase	Colchicine

Deviation 8. The updated name of the Work Package is included in the study report.



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2 Corrective Action

Deviation 9. Analytical determination of nicotine is used as a QC for TPM fractions. However, nicotine could not be measured in the study. Carbonyl concentrations are used as QC for GVP and sbPBS fractions. GVP and TPM fractions are generated simultaneously from the same smoke / aerosol generation runs. Therefore, for the purpose of this study, for any GVP sample not passing the QC for carbonyls, we considered that the corresponding TPM fraction did not pass the QC either.



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3 Assessment on Study Quality and Integrity

Most deviations described on this document are to be considered as minor and thus, little to no effect is to be expected on the study results. .

Approval	Name	Date and Signature
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Study Director	Ignacio Gonzalez Suarez	
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April - 21 - 2017

If decided by Study Director

Sponsor	Not Applicable (NA)	NA
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Program Management	NA	NA
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In Case of a Multi Site Study and if applicable

Principal Investigator	NA	NA
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Management of Site Facility (optional)	NA	NA
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