Annex 1:
Status report of ongoing and summary of completed studies

In this Annex the significant findings in publications is provided for the following products:

Product Name	FDA STN number
IQOS System Holder and Charger	PM0000479/MR0000133
IQOS 3 System Holder and Charger	PM0000634
Marlboro Amber HeatSticks	PM0000424/MR0000059
Marlboro Green Menthol HeatSticks	PM0000425/MR0000060
Marlboro Blue Menthol HeatSticks	PM0000426/MR0000061

Completed Studies

Summary of completed studies

Reporting Period	March 1, 2021 – February 28, 2022

Category	Study Number	Product Tested	Study Title	Completion Date	Study Objectives	Study Summary
Aerosol Chemistry and Physics	(b) (4)	Marlboro Amber *Heatsticks*	Leaching of chemicals of environmental concern from used heated tobacco sticks to natural water and comparison with leaching from smoked conventional cigarettes	July 31, 2021	To identify and quantify the chemical components of environmental importance that are leached from HeatSticks and cigarette butts and to assess and compare their leaching behavior. This research was funded by an Investigator-Initiated Study grant by Philip Morris Products S.A., Switzerland (IIS.PMI.2017.30). The study protocol was written by the investigator, who also conducted the study. Philip Morris Products S.A. had no involvement in the study conduct, data analysis, and writing of the manuscript. Publication: - Koutela, N., Fernández, E., Saru, M.-L. et al. (2020). A comprehensive study on the leaching of metals from heated tobacco sticks and cigarettes in water and natural waters. Sci Total Environ. 714:136700 - Stefano Alberti, Maria Sotiropoulou, Elena Fernández, Nicoleta Solomou, Maurizio Ferretti, Elefteria Psillakis, UV-254 degradation of nicotine in natural waters and leachates produced from cigarette butts and heat-not-burn tobacco products, Environmental Research,Volume 194, 2021.	The leaching behavior of Al, Cr, Ni, Cu, Zn, As, Se, Cd, Ba, Hg and Pb in water from two types of heat-not-burn tobacco sticks is presented here, and compared to that from conventional cigarettes. The total concentration of each metal in solid tobacco products was initially determined. Concentrations in used and unused tobacco sticks were similar and generally, lower than those in unused conventional cigarettes. Studies on the contribution of paper, filter and tobacco revealed that tobacco was the major source of metal contamination. Smoking conventional cigarettes reduced the total metal concentrations since a substantial amount of metals was retained in the ash; a post-consumption waste that is difficult to collect. Batch leaching tests were performed to determine dissolved concentrations as a function of time. With the exceptions of As and (in most cases) Hg that were not detected, metals were released at varying rates. At 24 h of soaking the percentage of metals leached ranged from 0.2–43%. The contribution of paper, filter and tobacco to the dissolved concentrations at 24 h of leaching was investigated and in almost all cases tobacco was the major source of metal contamination. The dissolved concentrations from ash were low as metals were strongly bound. Varying the pH, ionic strength and humic acids content at environmentally relevant values did not affect leaching of metals at 24 h of soaking. The use of river water, rainwater and seawater as leachants was also not found to alter dissolved concentrations at 24 h compared to ultrapure water. The results presented here suggest that the consequences of improper disposal of tobacco products in the environment are two-sided and that next to the generation of plastic litter, discarded tobacco products can also act as point sources of metal contamination. Public education campaigns focusing on the environmental impact and best disposal practices are urgently needed. Nicotine is an important emerging contaminant widely detected in water resources. The main nicotine sources are human excretions from users and leaching from discarded tobacco product waste, which represents the most commonly littered item in urban areas and coasts. In this study, the UV254 photolytical fate of nicotine in natural water and leachates produced from conventional cigarettes (CCs) and the new generation heat-not-burn (HnBs) tobacco products is examined for the first time. The effect of UV254 irradiation on nicotine depletion in ultrapure water was initially studied. The reaction was pseudo first-order with respect to nicotine concentration at low concentrations and shifted to lower order at higher concentrations, an effect associated to absorption saturation. Although nicotine removal was fast, only 9.5% of the total organic carbon was removed after irradiation due to the formation of by-products. The chemical structures of six photo-products were derived by means of liquid and gas chromatography coupled to mass spectrometry. The photodegradation kinetics was found to depend on pH and faster kinetics were recorded when the monoprotonated form of nicotine was dominant (pH = 5–8). The presence of humic acids was found to slightly delay kinetics as they competed with nicotine for lamp irradiance, whereas the presence of salt had no effect on the direct photolysis of nicotine. [redacted]
Aerosol Chemistry and Physics	(b) (4)	Marlboro Blue *Heatsticks* Marlboro Green *Heatsticks*	Non-targeted differential screening activities in support of a supplementary pre-market tobacco application for two Heatstick variants with a modified Heatstick design for the IQOS Tobacco Heating System	October 22, 2021	Comparative assessment of differences in aerosol chemistry between two new heatstick variants (one regular and one high-menthol) with modified heatstick design and the respective currently authorized heatsticks. The study will apply non-targeted differential screening methods (liquid chromatography with high-resolution accurate mass spectrometry (LC-HRAMS) and comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (GCxGC-TOFMS)). For aerosol constituents identified as being more abundant in the aerosol of the new heatstick variants in comparison to aerosol of the authorized heatstick comparators also the semiquantitative yields in 1R6F cigarette smoke will be provided.	The findings presented in this report indicate that the overall differences in aerosol composition observed between the modified regular and the authorized regular product as well as between the modified mentholated and the authorized mentholated product were minimal. The number of differences observed were very low, with only 15 and 35 aerosol constituents determined as being higher in concentration in the modified regular and mentholated products, respectively. Overall, 74% of the chemical constituents determined as being higher in abundance in the modified products had observed concentration increases of less than 30%, and only 3 (out of 50) chemical constituents (6%) were increased in concentration by more than 100% (2-fold).
Behavioral	(b) (4)	N/A	Quantitative assessment of intent to use IQOS among adults populations in Brazil	May 01, 2021	The overall aim of this study is to assess responses to instances of *IQOS* label and labelling within adult smokers, adult former smokers, adult never smokers in general and adult never smokers LA-24. Note: 'Label' refers to the display of printed text or graphical material, including branding on the pack containing *IQOS* sticks, or the packaging box of the *IQOS* device. 'Labeling' refers to printed or graphical material, which accompanies *IQOS*. For example, labelling could refer to (i) leaflets inserted into the packaging of the *IQOS* device or the pack of *IQOS* Tobacco Sticks, and (ii) other similar text, which is not printed on the pack but is on the materials accompanying the pack.	The results of this four-arm study, representing the instances of IQOS label, labelling, and marketing material (IQOS Brochure, HEETS Mock-up Packaging, and IQOS Diagram Card) among four participant groups show the following: 1. Adult Smokers showed substantial levels of Intent to Use IQOS. Their positive Intention to Use varied from 14.2% to 20.0% across study ARMs. 2. The level of Intention to Use IQOS within Adult Former Smokers was ≤5.0% across the ARMs. Intent to Use IQOS was similar to Intent to Use CC, Electronic Cigarettes and Any Nicotine-Containing Products. 3. LA-24 Never Smokers and LA-64 Never Smokers Intention to Use IQOS was ≤2.5%. Intent to Use IQOS was similar to Intent to Use CC, E-cigarettes and Any Nicotine-Containing Products. 4. The results of Intent to Use IQOS were similar when comparing the instances of IQOS label, labelling, and marketing material with the Brazilian Official Health Warnings and the material with the PMI Important Warning, developed by PMI. The study findings show that IQOS is likely to be a potential alternative to CC for Adult Smokers, while it is unlikely to attract the Adult Former Smokers and the Adult Never Smokers population.
Human study	(b) (4)	All tobacco sticks variants available for sale in the US market during the study.	Qualitative Study to Develop P1 Product Messages	December 20, 2021	The objective of this qualitative study was to explore IQOS potential product messages to develop IQOS Label, Labeling and Marketing Materials (LLM) by: 1. Understanding study participants' response in terms of use intention. 2. Understanding the believability and the relevance of the product messages. 3. Exploring comprehension of the product messages (overall and for specific section). 4. Exploring risk perception for IQOS in comparison with combustible cigarettes (CC) and cessation 5. Understanding how wording, amount of information and tone of voice contribute to participants' perception, comprehension, believability, relevance and use intention. 6. Improving messages/concepts based on study outcomes.	(b) (4) [redacted]

	Post-Market Studies	(b) (4)	All tobacco sticks variants available for sale in the Japanese market during the study	Repeated Cross-Sectional Survey on the Use of Tobacco and Nicotine Containing Products in the General Adult Population and among Adult Users of IQOS in Japan (Year 4, 2020-2021)	October 2021	The overall aim of the repeated cross-sectional studies in Japan is to investigate the current and past TNP use in the general adult Japanese population and among Japanese adult IQOSTM users registered in the IQOS user database of PMI's affiliate in Japan to understand and characterize how PMI's smoke-free product IQOS is used by the adult Japanese population and among adult IQOS users compared to other TNPs. More specifically, the study objectives in the target populations are: 1. Estimate the prevalence/frequency of TNP use broken down into (i) current, former, and never use; (ii) daily and non-daily use; and (iii) exclusive, dual, and poly use. 2. Describe the past TNP use status to estimate (i) TNP initiation (based on first product regularly used); (ii) relapse and reinitiation; and (iii) intention to quit, quit attempts, and quitting of TNPs in the target populations. 3. Estimate self-reported risk perceptions related to smoking cigarettes and using IQOS among current IQOS users. 4. Estimate self-reported perceived changes in health outcomes as well as in hygiene, beauty, and fitness related benefits among current IQOS users.	(b) (4)
	Standard & System Toxicology	(b) (4)	The patients were given a choice to use any tobacco sticks variants available for sale in the Japanese market during the study.	Omics study on samples from a clinical study in Japan (P1-OHS-01-JP) to evaluate the effect of switching from cigarette smoking to the use of IQOS in smokers with generalized chronic periodontitis on the response to mechanical periodontal treatment and oral health status	November 16, 2021	• Analysis of subgingival plaques samples, collected before periodontal therapy and after 6 months, to analyze the changes in the oral bacterial composition by next generation sequencing. • Analysis of gingival crevicular fluid sample, collected before periodontal therapy and after 3 months, to determine quantitative changes in the inflammatory response by measuring pro-inflammatory and immuno-regulatory mediators. • Analysis of Buccal swab samples, collected before periodontal therapy and after 3 and 6 months to evaluate the gene expression changes in patients switching to *IQOS* use compared to those continuing to smoke cigarettes.	Initially 514 samples (BUSW-R) were received and 178 passed the RNA acceptance criteria. Backup samples (BUSW-L) were then requested and from the 335 extracted, 42 passed the RNA acceptance criteria. BUSW-R and BUSW-L were consolidated into a final dataset of 220 RNA samples to be processed for the downstream gene expression microarray workflow. Two hundred-twenty .CEL files were delivered for analysis. After the microarray QC analysis, 11 samples were requested for re-hybridization and 11 .CELmfiles were delivered for data analysis. Due to poor yields during the RNA isolation process, the miRNA Genechips were not processed.
	Standard & System Toxicology	(b) (4)	*IQOS* 4.0 tobacco stick; Monitor (P1M2) tobacco stick; *IQOS* 2.4	Determination of the in vitro cytotoxic potency of two mainstream aerosol fractions generated from the test item, IQOS 4 tobacco sticks, and the reference items, IQOS 4 comparator and P1M2, in the neutral red uptake assay	March 2021	To determine the cytotoxic potency (via the EC50 metric) of TPM and GVP fractions derived from the mainstream aerosol of IQOS 4 tobacco sticks, IQOS 4 comparator and P1M2 in the Balb/c 3T3 cell NRU assay.	The aim of this study was to determine the cytotoxic potency (via the EC50 metric) of the mainstream aerosol fractions (TPM and GVP) of the test item (IQOS4 tobacco sticks) and reference item (head-to-head comparator: IQOS4 comparator) as well as that of counterpart fractions generated from the reference item (monitor). In vitro cytotoxic potency was determined using the NRU assay conducted in BALB/c 3T3 cells across 3 independent tests. All data were judged to be acceptable and, thus, justified the derivation of EC50-related cytotoxic potency values for each item batch on each test occasion. Importantly, the positive control (SDS) as well as aerosol fractions derived from the reference item (monitor) performed in line with the PSTL's historical data. Similarly, chemical characterization data on the reference item (monitor)-derived aerosol fractions across the study were also consistent with the PSTL's historical data. While not the objective of the present study, these findings allow comparisons to be made on the cytotoxic potency of the GVP and TPM aerosol fractions derived from IQOS4 tobacco sticks and the IQOS4 comparator via a comparability model developed and administered by PMP SA.
	Standard & System Toxicology	(b) (4)	*IQOS* 4.0 tobacco stick; *IQOS* 4.0 comparator stick *IQOS* 4.0; *IQOS* 2.4	Determination of the in vitro mutagenicity of two mainstream aerosol fractions generated from the test item, IQOS 4 tobacco sticks, and the reference item, IQOS 4 comparator, in the AMES test	June 2021	To determine the mutagenicity of TPM and GVP fractions derived from the mainstream aerosol of IQOS 4 tobacco sticks and the IQOS 4 comparator in the Ames test.	In final conclusion, while GVP (up to 4 mg TPM equivalent/plate) and TPM (up to 10 mg/plate) aerosol fractions from the two items were generally found to be non-mutagenic via both evaluation approaches, there was some evidence of bacterial mutagenicity, e.g. reference item-derived TPM in strain TA1535 (+S9). However, application of the stringent acceptance criteria led to the rejection of 12 experiments, an outcome that severely hinders the ability to interpret the data meaningfully. Moreover, given that this study was conducted on exploratory batches of test and reference items and not confirmatory batches, it is recommended that the study is repeated on more appropriate items via the refined Ames test.
	Standard Toxicology (Pre clinical)	(b) (4)	Blind, Method Development	Determination of the cytotoxic potency of the aerosol fractions derived from 9 blinded test items, A-I, and the reference item P1M3 in the BALB/C 3T3 cell line-based neutral red uptake assay	April 22, 2021	The aim of this study was to determine the cytotoxic potencies of the mainstream aerosol fractions (total particulate matter (TPM) and aqueous-soluble portion of the gas-vapour phase (GVP)) of 9 blinded P1 variants as well as those from counterpart fractions generated from the P1M3 reference item. *In vitro* cytotoxic potency was determined using the neutral red uptake (NRU) assay conducted in BALB/c 3T3 cells across 2 independent tests. All presented data were judged to be acceptable and, thus, justified the derivation of half maximal effective concentration (EC50) cytotoxic potency values for each item batch on each test occasion. Importantly, the positive control as well as aerosol fractions derived from the reference item performed in line with the laboratory's historical data. Similarly, chemical characterisation data on the reference item-derived aerosol fractions across the study were also consistent with the laboratory's historical data. These findings allow insights to be drawn on the cytotoxicity data derived from the 9 blinded P1 variants.	All presented data were judged to be acceptable and, thus, justified the derivation of half maximal effective concentration (EC50) cytotoxic potency values for each item batch on each test occasion. Importantly, the positive control as well as aerosol fractions derived from the reference item performed in line with the laboratory's historical data. Similarly, chemical characterization data on the reference item-derived aerosol fractions across the study were also consistent with the laboratory's historical data. These findings allow insights to be drawn on the cytotoxicity data derived from the 9 blinded P1 variants.
	Standard Toxicology (Pre clinical)	(b) (4)	Marlboro Amber *Heatsticks* Marlboro Blue *Heatsticks* Marlboro Green *Heatsticks*	Determination of the cytotoxic potency of the aerosol or smoke fractions derived from the test items Amber, Green and Blue (via the THD 2.4 device), the monitor item P1M3 (via the THD 2.4 device) and the reference item 1R6F in the BALB/c 3T3 cell linebased neutral red uptake assay	October 28, 2021	The aim of this study is to determine the cytotoxic potency of the aerosol or smoke fractions derived from the test items Amber, Green and Blue (via the THD 2.4 device), the monitor item P1M3 (via the THD 2.4 device) and the reference item 1R6F in the BALB/c 3T3 cell linebased neutral red uptake assay	For each test item-related experiment, 9 replicates of test item-derived TPM and GVP, 1 replicate of P1M3-derived TPM and GVP and the positive control sodium dodecyl sulfate (SDS) were assessed. In addition, 1 replicate of 1R6F-derived smoke fractions was assessed in each of 3 experiments alongside SDS. All reported data were judged to be acceptable and, thus, justified the derivation of half maximal effective concentration (EC50) cytotoxic potency values for each item. Importantly, the positive control SDS as well as the aerosol/smoke fractions derived from the monitor and reference items performed in line with the laboratory's historical data on these substances. Similarly, characterization data on the monitor and reference item-derived aerosol/smoke fractions across the study were also consistent with the laboratory's historical data. Taken together, these findings allow insights to be drawn on the cytotoxic potency data generated from the Amber, Green and Blue test items. For example, the test item-derived aerosol fractions were markedly less potently cytotoxic (92.3-96.3%) than the counterpart smoke fractions derived from the 1R6F reference item.

	System Toxicology	(b) (4)	Marlboro Amber *Heatsticks* - Japan	THS 2.2 exposure and CYP activity in liver cells	March 31, 2021	The aim of this study is to determine the impact of THS 2.2 exposure on CYP activity in liver cells	Although the clozapine/CS interaction experiment did not work, this study was the first to use a multi organs-on-a-chip system to study the effects on xenobiotic metabolism of liver spheroids connected to bronchial epithelial tissue cultures exposed to CS or THS aerosol. The combination of a smoke/aerosol exposure system, 3D tissue cultures physiologically copying the functionality of human organs, and a system to recreate the interaction of these organs has made it possible to recreate in vitro the effects observed on humans in clinical studies. Although requiring some optimizations, this platform will allow studying the effects of repeated exposure to any aerosol on 3D respiratory tissue (nasal, bronchial, small airway) as well as on liver surrogates.
	Standard Toxicology (Pre clinical)	(b) (4)	4 different *Heatstick* variants	Determination of Cytotoxicity of Selected Heated Tobacco Products Using Neutral Red Uptake Assay	November 01, 2021	The aim of this study was to assess the cytotoxic activity of both the total particulate matter (TPM) and the aqueous solution -soluble portion of the gas-vapour phase (GVP) aerosol fractions of one P1 tobacco stick variants. The *in vitro* cytotoxic potency was determined using the Neutral Red Uptake (NRU) assay using BALB/c 3T3 cells. Aftercut (AC) Argos, AC Mint, Fauvery, AC Wilge, AC Matamec.	The four product variants were tested in the *in vitro* cytotoxicity NRU assay with either the parent aftercut or a mentholated version and assessed against current applicable comparability model. All products complied against comparability model.

On-Going Studies

Status report of the ongoing studies

Reporting Period	March 1, 2021 – February 28, 2022

Category	Study Number	Product Tested	Study Title	Study Objectives	Estimated Completion
(b) (4)					

(b) (4)

(b) (4)