Annex 2:
MRTP Use and Health Risk - Toxicology

Product	Marlboro Amber *HeatSticks* Marlboro Green Menthol *HeatSticks* Marlboro Blue Menthol *HeatSticks* IQOS System Holder and Charger
FDA STN	MR0000059, MR0000060, MR0000061, MR0000133
Reporting Period	March 1, 2021 to February 28, 2022

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1. OVERVIEW

As part of the initial product characterization of the IQOS system, non-targeted differential screening (NTDS) analyses of the aerosol generated from Marlboro Amber *HeatSticks* (MR0000059), Marlboro Green Menthol *HeatSticks* (MR000060), and Marlboro Blue Menthol *HeatSticks* (MR000061) with the IQOS System Holder and Charger (MR0000133) were performed to identify compounds which were potentially new, or significantly increased in IQOS aerosol relative to 3R4F smoke. A hazard identification protocol was developed to determine the genotoxic and carcinogenic potential of both these inhaled tobacco product constituents and their potentially reactive and toxic metabolites.

The protocol was accepted by FDA on February 24, 2021. PMP SA appointed two Contract Research Organizations (CROs) for the performance of the study, ForthTox Limited (PO Box 13550, Linlithgow, West Lothian, EH49 7YU, U.K.) and Bibra toxicology advice & consulting Ltd (Cantium House, Wallington, SM6 0DZ, UK). The study was divided in 3 phases:

- The project Phase 1 is intended to determine the genotoxicity and/or carcinogenicity potential of the 80 chemicals (parent compounds) identified as potentially new, or significantly increased in IQOS aerosol relative to 3R4F smoke.
- The project Phase 2 is intended to determine the potential metabolites of the 80 chemicals relevant to humans
- The project Phase 3 is intended to determine the genotoxicity and/or carcinogenicity potential of the relevant metabolites.

The phase 1 has been completed and the outcome is presented on the next section. Phase 2 completion is expected by Q3 2022 and Phase 3 completion by end of Q2 2023.

2. PHASE 1 RESULTS

The genetic *in silico* toxicity (GIST) protocol described by Hasselgen et al. (2019)[1] was applied. For each compound, the final assessment of the genotoxic/carcinogenic potential is mentioned (positive/negative), and the level of confidence linked to the prediction (low, medium, or high). Indications given as to the confidence are based on recent guidance from Johnson et al. (2022),[2] as described in Table 1. The results are summarized in Table 2.

Table 1: Assessment confidence level

Confidence	Description
High	The assessment is likely to be true and further research is unlikely to change its confidence
Medium	The assessment is likely to be true but further research might change its confidence
Low	Further research is needed to improve the confidence in the assessment

Table 2: Genotoxic and carcinogenic potential prediction of the 80 identified chemicals

CAS-Number	Chemical	Genotoxic potential	Genotoxicity confidence	Carcinogenic potential	Carcinogenicity confidence
100-51-6	Benzyl alcohol	Positive	Medium	Negative	High
10267-21-7	Labdane-8,15-diol, (13S)	Negative	Medium	Positive	Low
10458-14-7	Menthone	Negative	High	Negative	Medium
105-43-1	3-Methylvaleric acid	Negative	Low	Positive	Low
105453-16-5	Germacrene D	Negative	Medium	Negative	Medium
106-33-2	Ethyl dodecanoate (Ethyl laurate)	Negative	Medium	Positive	Low
111-61-5	Stearate, ethyl-	Negative	Medium	Negative	Medium
1125-80-0	Isoquinoline, 3-methyl	Positive	Low	Negative	Low
116-09-6	1-Hydroxy-2-propanone	Positive	High	Negative	Medium
1191-41-9	Ethyl linolenate	Positive	Medium	Negative	Medium
1192-58-1	2-Formyl-1-methylpyrrole	Negative	Low	Negative	Low

[1] Hasselgren C, Ahlberg E, Akahori Y et al. Genetic toxicology in silico protocol. Regulatory Toxicology and Pharmacology, 2019, 107: 104403. https://doi.org/10.1016/j.yrtph.2019.104403

[2] Johnson C, Anger LT, Benigni R et al. Evaluating confidence in toxicity assessments based on experimental data and in silico predictions. Computational Toxicology, 2022, 21: 100204. https://doi.org/10.1016/j.comtox.2021.100204

CAS-Number	Chemical	Genotoxic potential	Genotoxicity confidence	Carcinogenic potential	Carcinogenicity confidence
119903-95-6	Bourbonene, beta	Negative	Medium	Negative	Medium
121-32-4	Ethyl Vanillin	Positive	High	Negative	Medium
122-78-1	Phenylacetaldehyde	Negative	High	Negative	High
13246-52-1	Ethyl 2,4-dioxohexanoate	Negative	Medium	Negative	Medium
139559-06-1	Isolinderanolide	Negative	Medium	Negative	Medium
1490-04-6	Menthol	Negative	High	Negative	High
15506-54-4	2-Methylcyclobutane-1,3-dione	Positive	Low	Negative	Low
1560-82-3	Heneicosane, 2-methyl-	Negative	Medium	Negative	Medium
1560-84-5	Eicosane, 2-methyl-	Negative	Medium	Negative	Medium
179694-78-1	Isopulegone caffeate	Positive	Low	Negative	Medium
18881-04-4	(S)-cis-Verbenol	Negative	Medium	Negative	Low
20296-29-1	3-Octanol	Negative	Medium	Negative	Medium
2230-87-7	Neomenthyl acetate	Negative	Low	Negative	Medium
23283-97-8	Isomenthol	Negative	Medium	Negative	Medium
2601-90-3	Oleoylglycine	Negative	Medium	Negative	Medium
3008-43-3	Cyclohexane-1,2-dione, 3-methyl	Negative	Medium	Negative	Medium
3188-00-9	3(2H)-Furanone, dihydro-2-methyl	Negative	Medium	Negative	Medium
33691-73-5	2H-Pyran-2-one, tetrahydro-5-hydroxy	Positive	Low	Positive	Low
3674-21-3	trans-4-Hydroxymethyl-2-methyl-1,3dioxolane	Negative	Medium	Negative	Medium
3856-25-5	Copaene	Negative	High	Negative	Medium
3857-25-8	2-Furanmethanol, 5-methyl	Positive	Low	Positive	High
38877-21-3	2,3-Dihydro-5-hydroxy-6-methyl-4Hpyran-4-one (Dihydromaltol)	Positive	Low	Negative	Medium
4230-97-1	Octanoic acid, 2-propenyl ester	Negative	Medium	Positive	Low

CAS-Number	Chemical	Genotoxic potential	Genotoxicity confidence	Carcinogenic potential	Carcinogenicity confidence
491-07-6	Cyclohexanone, 5-methyl-2-(1methylethyl)-, cis- (D,L-Isomenthone)	Negative	Medium	Negative	Medium
494-90-6	Menthofuran	Negative	Medium	Positive	High
497-23-4	2(5H)-Furanone	Positive	Medium	Negative	Medium
5077-67-8	1-Hydroxy-2-butanone	Positive	Medium	Negative	Medium
53907-91-8	1,4-Dioxane, 2-ethyl-5-methyl	Negative	Low	Negative	Medium
544-35-4	Ethyl linoleate	Negative	Low	Negative	Low
54750-70-8	Anhydro linalool oxide	Negative	Medium	Negative	Medium
57-55-6	Propylene glycol	Negative	High	Negative	High
57-87-4	Ergosterol	Positive	Low	Positive	Medium
581-49-7	Anatabine	Positive	Low	Negative	Low
58319-05-4	cis-sesquisabinene hydrate	Negative	Medium	Negative	Medium
587-45-1	Tyrosine, 3-hydroxy	Positive	High	Positive	Low
592-20-1	2-Propanone, 1-(acetyloxy)	Positive	Low	Negative	Medium
611-13-2	Methyl furoate	Negative	Medium	Positive	Low
61142-76-5	Benzene, 1,2,3,4-tetramethyl-4-(1methylethenyl)	Negative	Medium	Positive	Low
620-02-0	2-Furancarboxaldehyde, 5-methyl- (5-Methylfurfural)	Equivocal	Low	Positive	Medium
623-05-2	Benzenemethanol, 4-hydroxy-	Negative	Medium	Negative	Medium
628-97-7	Hexadecanoic acid, ethyl ester	Negative	Medium	Negative	Medium
6387-89-9	Glycidyl acetate	Positive	High	Positive	Medium
6418-46-8	Eicosane, 3-methyl	Negative	Medium	Negative	Medium
65-23-6	Pyridoxin	Positive	Low	Negative	Medium
67402-83-9	4(H)-Pyridine, N-acetyl	Negative	Low	Negative	Low

CAS-Number	Chemical	Genotoxic potential	Genotoxicity confidence	Carcinogenic potential	Carcinogenicity confidence
6890-88-6	Lanost-8-en-3-ol, 24-methylene-, (3beta)	Negative	Medium	Positive	Medium
7786-67-6	Cyclohexanol, 5-methyl-2-(1methylethenyl)- (Menth-8-en-3-ol; Isopulegol)	Negative	Medium	Negative	Medium
80933-73-9	Maltoxazine	Negative	Low	Negative	Low
89-48-5	Menthyl acetate	Negative	High	Negative	Medium
89-81-6	2-Cyclohexen-1-one, 3-methyl-6-(1-methylethyl)- (Piperitone)	Negative	Medium	Positive	Low
942-43-8	1H-Indene, 2,3-dihydro-1,1,5,6-tetramethyl	Negative	Low	Negative	Low
96-48-0	Butyrolactone	Negative	Low	Negative	High
96937-49-4	Benzoic acid, 2,5-dihydroxy-methyl	Negative	Low	Negative	Low
97-99-4	2-Furanmethanol, tetrahydro	Negative	Medium	Negative	Medium
98-55-5	3-Cyclohexene-1-methanol, α,α,4trimethyl- (Terpineol)	Negative	High	Negative	Medium
99-49-0	2-Cyclohexen-1-one, 2-methyl-5-(1methylethenyl)- (6,8-P-Menthadien-2-one)	Negative	High	Negative	Medium
57605-80-8	alpha-cembratriene-diol	Negative	Medium	Negative	Medium
102-62-5	1,2,3-Propanetriol, diacetate (1,2-diacetin)	Negative	Medium	Negative	Low
106-61-6	1,2,3-Propanetriol, 1-acetate (1-monoacetin)	Positive	Medium	Negative	Medium
128-37-0	Butylated hydroxytoluene	Negative	High	Positive	Low

CAS-Number	Chemical	Genotoxic potential	Genotoxicity confidence	Carcinogenic potential	Carcinogenicity confidence
25395-31-7	Diacetin (1,3-diacetin)	Negative	Medium	Negative	High
28564-83-2	Pyranone	Positive	Medium	Negative	Medium
487-06-9	5,7-Dimethoxycoumarin	Negative	Medium	Negative	Medium
765-87-7	Cyclohexane, 1,2-dioxo-	Negative	Low	Negative	Medium
930-60-9	2-Cyclopentene-1,4-dione	Positive	Medium	Negative	Medium
556-52-5	Glycidol	Positive	High	Positive	High
96-24-2	1,2-Propanediol, 3-chloro	Negative	Medium	Positive	High
98-00-0	Furanmethanol	Negative	Low	Positive	High
98-01-1	Furfural	Equivocal	Low	Positive	High

After completion of Phase 2 and Phase 3 of the project, identified hazards will be reported for each group of compounds (parents and metabolites) and segmented according to the quality and the reliability of data. Any collected data will be integrated into a narrative by human experts to evaluate and discuss all relevant factors associated with the data to help understand the formation of metabolites from parent compounds as well as the potential genotoxicity/carcinogenicity risk of the parent and the metabolite compounds.