Appendix 2

The methodology of Excess Lifetime Cancer Risk (ELCR) cumulated (ELCRc) calculation consisted in different steps and follows the approach outlined in the FDA memorandum on Calculating Excess Lifetime Cancer Risk in ENDS Premarket Tobacco Product Application[1]:

1) Hazard identification of constituents to be included in the ELCRc calculation.

   The list of considered aerosol/smoke constituents are derived from the Non-Targeted Differential Screening (NTDS) study results and targeted aerosol chemistry characterization studies (i.e., PMI-58 list of HPHCs) submitted in our original Modified Risk Tobacco Product Application (MRTPA), specifically:

   a. From the PMSS – THS 2.2 computational toxicology study, after hazard identification refinement (Appendix 1), 40 compounds determined as increased or new in IQOS aerosols compared to 3R4F cigarette smoke in the NTDS study were considered as potential carcinogens and the list is detailed in Table 1.

   b. From the PMI-58 list of HPHCs, 39 compounds were included in the ELCRc calculation because they are recognized as carcinogens by the FDA (established list of HPHCs)[2] (Table 2).

2) Reference values identification:

   the Inhalation Unit Risk (IUR) values were gathered from toxicological databases following the FDA Memorandum Use of Reference Values in the Toxicological Evaluation of Inhaled Tobacco Products[3].

   a. When available, IUR values was adjusted and expressed in µg/day associated with a 1 in 100000 ELCR, 52 years exposure duration, assuming an inhalation volume of 20 m^3, as per formula described in FDA memorandum on Calculating Excess Lifetime Cancer Risk in ENDS Premarket Tobacco Product Application.

   b. If no IUR value is available, a default Threshold of Toxicological Concern (TTC) value of 1.5 µg/day was used instead.

3) Cumulative ELCR calculation:

   a. Emission values:
      i. PMI-58 list of HPHCs:
         - To obtain a representative dataset of constituent's levels from IQOS (THS 2.2) aerosols and 3R4F cigarette smoke, the PMI-58 study results from the three *HeatSticks* variants or 3R4F

[1] U.S. Food and Drug Administration (FDA) Memorandum: Calculating the Excess Lifetime Cancer Risk in ENDS Premarket Tobacco Product Applications. (2024) https://www.fda.gov/media/180610/download?attachment

[2] Harmful and Potentially Harmful Constituents in Tobacco Products and Tobacco Smoke: Established List | FDA. https://www.fda.gov/tobacco-products/rules-regulations-and-guidance-related-tobacco-products/harmful-and-potentially-harmful-constituents-tobacco-products-and-tobacco-smoke-established-list

[3] U.S. Food and Drug Administration (FDA) Memorandum: Use of Reference Values in the Toxicological Evaluation of Inhaled Tobacco Products (2019). Use of Reference Values in the Toxicological Evaluation of Inhaled Tobacco Products

reference cigarette, were combined and the mean value was calculated (Table 2).

- For aerosol constituents measured below the level of quantification (LOQ) or detection (LOD), the respective LOQ or LOD values were used as worst-case assumption.

ii. NTDS / PMSS – THS 2.2 computational toxicology list:

- Because not all 40 compounds with genotoxic/carcinogenic potential were increased or new in all three *HeatSticks* variants aerosols compared to cigarette smoke, the mean semi-quantified level was considered when the constituent was reported in more than one *HeatStick*s variant (Table 1).

- If the increased constituent was reported in only one *HeatStick* variant, the level was used as a representative value.

b. A daily consumption of 20 *HeatSticks* or 20 cigarettes was considered to determine the daily exposure to HPHCs emitted from the IQOS aerosol or cigarette smoke.

c. The constituent-specific ELCR was calculated as per described formula:

$$ELCR = \frac{\text{Daily exposure in µg/day (emission} * \text{20 sticks or cigarettes)}}{\text{Potency value in µg/day (adjusted IUR or TTC 1.5 µg per day)}}$$

d. The cumulative ELCR per product is calculated by summing the constituents-specific ELCRs from all constituents with cancer hazards (Table 3).

4) Relative cancer risk calculation:

a. After the ELCRc of IQOS aerosol and 3R4F reference cigarette smoke are determined, the ELCRc of IQOS aerosol is compared to the ELCRc from 3R4F cigarette smoke, considered as 100%, and expressed into a percentage value (Table 3).

Table 1. Summary table of potential genotoxic/carcinogenic chemical constituents yields increased or new in IQOS aerosols compared to 3R4F cigarette smoke from NTDS study and PMSS – THS 2.2 computational toxicology study.

Constituent	*HeatStick* Amber (µg/stick)	*HeatStick* Green (µg/stick)	*HeatStick* Blue (µg/stick)	*HeatStick* Mean (µg/stick)	3R4F (µg/cig.)			3R4F mean (µg/cig.)
Labdane-8,15-diol, (13S)	0.143	0.071	0.175	0.13	0.015	0.09		0.0525
Germacrene D	< 3R4F	0.019	0.164	0.092	0.001	0.11		0.056
Isoquinoline, 3-methyl	6.29	6.3	< 3R4F	6.295	4.99			4.99
Ethyl linolenate	0.614	0.274	2.05	0.979	0.153	0.429		0.291
2-Formyl-1-methylpyrrole	0.128	0.095	< 3R4F	0.111	0.064			0.064
2-Methylcyclobutane-1,3-dione	2.78	2.06	< 3R4F	2.42	0.71			0.71
Heneicosane, 2-methyl-	0.063	0.051	< 3R4F	0.057	0.021			0.021
Eicosane, 2-methyl	0.05	0.033	0.158	0.080	0.014	0.081		0.048
Isopulegone caffeate	< 3R4F	0.107	< 3R4F	0.107	0.001			0.001
3-Octanol	< 3R4F	0.103	< 3R4F	0.103	0			0
2H-Pyran-2-one, tetrahydro-5-hydroxy	4.45	4.09	5.41	4.65	3.11	2.94		3.025
2-Furanmethanol, 5-methyl	0.123	0.082	< 3R4F	0.103	0.029			0.029
2,3-Dihydro-5-hydroxy-6-methyl-4Hpyran-4-one (Dihydromaltol)	< 3R4F	< 3R4F	0.421	0.421	0.304			0.304
Menthofuran	< 3R4F	0.524	0.158	0.341	0.012	0.007		0.0095
2(5H)-Furanone	5.32	1.38	2.28	2.993	1.99	0.808	1.06	1.286
1-Hydroxy-2-butanone	0.947	0.842	< 3R4F	0.895	0.465			0.465
Anhydro linalool oxide	0.457	< 3R4F	< 3R4F	0.457	0.291			0.291
Ergosterol	3.18	2.68	3.54	3.133	1.58	1.33		1.455
Anatabine	< 3R4F	< 3R4F	15.6	15.6	12			12

(Table continues)

Tyrosine, 3-hydroxy	< 3R4F	< 3R4F	2.82	2.82	2.42			2.42
2-Propanone, 1-(acetyloxy)	16.9	< 3R4F	9.68	13.29	8.01	6.62		7.315
Benzene, 1,2,3,4-tetramethyl-4-(1methylethenyl)	0.006	< 3R4F	< 3R4F	0.006	0.005			0.005
Benzenemethanol, 4-hydroxy-	0.011	< 3R4F	< 3R4F	0.011	0.0			0
Glycidyl acetate	< 3R4F	< 3R4F	0.178	0.178	0.14			0.14
Pyridoxin	0.699	0.618	0.898	0.738	0.526	0.498		0.512
Lanost-8-en-3-ol, 24-methylene-, (3beta)	6.3	5.22	8.06	6.527	1.61	1.65		1.63
Cyclohexanol, 5-methyl-2-(1methylethenyl)-(Menth-8en-3-ol)	< 3R4F	< 3R4F	0.325	0.325	0.028			0.028
Maltoxazine	0.077	0.07	< 3R4F	0.0735	0.038			0.038
Benzoic acid, 2,5-dihydroxy-methyl	4.55	4.04	5.23	4.607	2.18	1.87		2.025
1,2,3-Propanetriol, 1-acetate	< 3R4F	1.98	< 3R4F	1.98	0.696			0.696
Butylated hydroxytoluene	0.132	0.154	0.149	0.145	0.007	0.009		0.008
1,2-Diacetin	1.23	2.23	< 3R4F	1.73	0.381			0.381
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	0.231	< 3R4F	< 3R4F	0.231	0.135			0.135
5,7-Dimethoxycoumarin	< 3R4F	< 3R4F	1.81	1.81	1.59			1.59
Cyclohexane, 1,2-dioxo-	0.083	0.061	< 3R4F	0.072	0.046			0.046
2-Cyclopentene-1,4-dione	3.8	2.49	15.5	7.263	0.764	3.92		2.342
Glycidol	5.71	1.1	1.08	2.63	1.76	0.528	0.456	0.915
1,2-Propanediol, 3-chloro	9.94	5.39	8.25	7.86	5.93	7.08	1.44	4.817
2-Furanmethanol	39.2	47.1	41.6	42.633	7	9.13	6.16	7.43
alpha-cembratriene-diol	1.43	0.731	8.68	3.614	0.064	0.322		0.193
Mean cumulated yield (µg/stick)				**137.5**				**57.8**

Table 2. Emission level of IQOS aerosol and 3R4F reference cigarette smoke

Constituents	Unit emission level	3R4F Reference cigarette				THS 2.2 *HeatSticks*			
		Blue	Green	Amber	Mean	Blue	Green	Amber	Mean
1,3-Butadiene	µg/item	92.99	92.81	89.23	**91.68**	0.19	0.22	0.21	**0.21**
Isoprene	µg/item	926.76	955.87	891.20	**924.61**	1.27	1.51	1.51	**1.43**
Acrylonitrile	µg/item	23.54	22.06	21.20	**22.26**	<0.107 (LOQ)	0.13	0.14	**0.14**
Benzene	µg/item	73.53	79.67	77.26	**76.82**	0.47	0.45	0.45	**0.46**
Vinyl chloride	ng/item	100.77	92.53	93.42	**95.58**	<0.657 (LOD)	<2.19	<0.657 (LOD)	**2.19**
Ethylene oxide	µg/item	15.69	19.01	16.05	**16.92**	0.12	<0.119	<0.119	**0.12**
Propylene oxide	ng/item	920.51	1065.67	895.78	**960.65**	118.94	111.40	142.34	**124.23**
NNN	ng/item	273.78	273.78	271.33	**272.96**	7.75	7.01	10.11	**8.29**
NNK	ng/item	282.44	256.00	244.67	**261.0**	5.52	6.63	7.80	**6.65**
Pyridine	µg/item	27.03	26.84	30.93	**28.27**	6.05	6.22	6.58	**6.28**
Quinoline	µg/item	0.41	0.45	0.43	**0.43**	<0.011 (LOQ)	<0.011	<0.011	**0.011**
Styrene (oxide)	µg/item	11.95	12.32	13.87	**12.71**	0.46	0.46	0.58	**0.5**
Nitro benzene	µg/item	<0.038 (LOD)	ND	ND	**0.038**	<0.011 (LOD)	ND	ND	**0.011**
Acetamide	µg/item	14.15	14.03	13.01	**13.73**	2.94	2.98	2.96	**2.96**
Acrylamide	µg/item	5.00	4.67	4.50	**4.72**	1.68	1.64	1.58	**1.63**

(Table continues)

Hydroquinone	µg/item	92.91	97.23	94.51	**94.88**	6.48	4.99	6.55	**6.01**
Catechol	µg/item	100.83	92.79	84.06	**92.56**	14.12	11.54	14.00	**13.22**
p-Cresol	µg/item	10.06	9.83	9.60	**9.83**	<0.034 (LOQ)	0.05	0.07	**0.06**
m-Cresol	µg/item	4.14	4.23	4.24	**4.2**	<0.019 (LOQ)	0.03	0.04	**0.03**
o-Cresol	µg/item	4.98	4.58	4.81	**4.79**	0.04	0.05	0.08	**0.06**
Benz[a]anthracene	ng/item	25.64	26.48	28.43	**26.85**	1.51	1.80	2.65	**1.99**
Benzo[a]pyrene	ng/item	14.44	12.51	13.29	**13.41**	0.45	0.54	0.74	**0.57**
Dibenz[a,h]anthracene	ng/item	1.02	<0.689	<0.689	**1.02**	<0.124 (LOD)	ND	ND	**0.124**
Formaldehyde	µg/item	92.93	67.94	79.42	**80.09**	9.97	15.15	14.12	**13.08**
Acetaldehyde	µg/item	1656.02	1612.44	1602.20	**1624**	191.87	206.44	192.09	**196.8**
Acrolein	µg/item	161.03	143.95	157.91	**154.3**	9.32	9.79	8.32	**9.14**
Crotonaldehyde	µg/item	49.86	48.23	49.26	**49.12**	<3.29 (LOQ)	<3.29	<3.29	**3.29**
Butyraldehyde	µg/item	79.58	83.45	81.32	**81.45**	18.85	21.12	20.70	**20.23**
1-Aminonaphthalene	ng/item	21.67	20.92	20.88	**21.2**	0.06	0.04	0.04	**0.050**
2-Aminonaphthalene	ng/item	16.96	17.32	17.54	**17.3**	0.03	0.02	0.02	**0.020**
3-Aminobiphenyl	ng/item	4.51	4.61	4.60	**4.57**	0.01	0.01	0.01	**0.010**
4-Aminobiphenyl	ng/item	3.02	3.21	3.21	**3.14**	0.01	0.01	0.01	**0.0100**
o-Toluidine	ng/item	106.09	101.92	96.17	**101.39**	0.98	0.94	1.10	**1**
Cadmium	ng/item	103.91	89.15	89.15	**94.07**	0.30	<0.28	<0.28	**0.3**
Lead	ng/item	30.17	31.23	31.23	**30.88**	<1.62 (LOQ)	1.87	2.23	**2.05**
Chromium (VI)	ng/item	<11.9 (LOD)	ND	ND	**11.9**	<11.0 (LOQ)	ND	<11.0	**11**

(Table continues)

Nickel	ng/item	<12.9 (LOD)	<43.1	ND	**12.9**	<15.9 (LOD)	ND	<15.9	**15.9**
Arsenic	ng/item	<7.49 (LOQ)	<7.49	<7.49	**7.49**	<1.20 (LOQ)	<1.2	ND	**1.2**
Mercury	ng/item	4.13	3.70	3.68	**3.84**	1.89	1.32	1.38	**1.53**

<LOD: below level of detection; <LOQ: below level of quantification; ND: Not detected

Table 3. Excess Lifetime Cancer Risk cumulated from 3R4F reference cigarette and IQOS aerosol calculation and relative cancer risk.

Constituents	U.S. FDA Classification	Inhalation Unit Risk $(\mu g/m^3)^{-1}$	Sources[4,5,6]	Adjusted IUR (µg/day)	Unit emission level	3R4F Reference cigarette			THS 2.2 *HeatSticks (IQOS aerosol)*		
						Mean emission level	Daily exposure (µg/day)	ELCR (10^{-5})	Mean emission level	Daily exposure (µg/day)	ELCR (10^{-5})
1,3-Butadiene	CA, RT, RDT	$3.0x10^{-5}$	EPA IRIS	8.97	µg/item	91.68	1833.6	204.32	0.21	4.2	0.468
Isoprene	CA	$5.4x10^{-6}$	EPA IRIS	49.86	µg/item	924.61	18492.2	370.90	1.43	28.6	0.574
Acrylonitrile	CA, RT	$6.8x10^{-5}$	EPA IRIS	3.96	µg/item	22.26	445.2	112.44	0.14	2.8	0.707
Benzene	CA, CT, RDT	$7.8x10^{-6}$	EPA IRIS	34.52	µg/item	76.82	1536.4	44.51	0.46	9.2	0.267
Vinyl chloride	CA	$4.4x10^{-6}$	EPA IRIS	61.19	ng/item	95.58	1.9116	0.03	2.19	0.0438	0.001
Ethylene oxide	CA, RT, RDT	$3.0x10^{-3}$	EPA IRIS	0.09	µg/item	16.92	338.4	3770.74	0.12	2.4	26.743
Propylene oxide	CA, RT	$3.7x10^{-6}$	EPA IRIS	72.77	ng/item	960.65	19.213	0.26	124.23	2.4846	0.034
NNN	CA	$4.0x10^{-4}$	Cal/EPA	0.67	ng/item	272.96	5.4592	8.11	8.29	0.1658	0.246
NNK	CA	$5.2x10^{-3}$	Marano et al., (2018)	0.05	ng/item	261.0	5.22	100.84	6.65	0.133	2.569
Pyridine	CA	N/A	N/A	1.50	µg/item	28.27	565.4	376.93	6.28	125.6	83.733
Quinoline	CA	N/A	N/A	1.50	µg/item	0.43	8.6	5.73	0.011	0.22	0.147

(Table continues)

[4] U.S. Environmental Protection Agency (EPA) Integrated Risk Information System (IRIS) Assessments. IRIS Assessments | IRIS | US EPA

[5] California Environmental Protection Agency (Cal/EPA) Office of Environmental Health Hazard Assessment (OEHHA). Chemicals - OEHHA

[6] Marano KM, Liu C, Fuller W, Gentry PR. Quantitative risk assessment of tobacco products: A potentially useful component of substantial equivalence evaluations. Regul Toxicol Pharmacol. 2018 Jun;95:371-384. doi: 10.1016/j.yrtph.2018.03.026. Epub 2018 Mar 31. PMID: 29614342.

Styrene (oxide)	CA	$4.6x10^{-5}$	Cal/EPA	5.85	µg/item	12.71	254.2	43.43	0.5	10	1.709
Nitro benzene	CA, RT, RDT	$4.0x10^{-5}$	EPA IRIS	6.73	µg/item	0.038	0.76	0.11	0.011	0.22	0.033
Acetamide	CA	$2.0x10^{-5}$	Cal/EPA	13.46	µg/item	13.73	274.6	20.40	2.96	59.2	4.398
Acrylamide	CA	$1.0x10^{-4}$	EPA IRIS	2.69	µg/item	4.72	94.4	35.06	1.63	32.6	12.109
Hydroquinone	CA	N/A	N/A	1.50	µg/item	94.88	1897.6	1265.07	6.01	120.2	80.133
Catechol	CA	N/A	N/A	1.50	µg/item	92.56	1851.2	1234.13	13.22	264.4	176.267
p-Cresol	CA, RT	N/A	N/A	1.50	µg/item	9.83	196.6	131.07	0.06	1.2	0.800
m-Cresol	CA, RT	N/A	N/A	1.50	µg/item	4.2	84	56.00	0.03	0.6	0.400
o-Cresol	CA, RT	N/A	N/A	1.50	µg/item	4.79	95.8	63.87	0.06	1.2	0.800
Benz[a]anthracene	CA, CT	$1.1x10^{-4}$	Cal/EPA	2.45	ng/item	26.85	0.537	0.22	1.99	0.0398	0.016
Benzo[a]pyrene	CA	$6.0x10^{-4}$	EPA IRIS	0.449	ng/item	13.41	0.2682	0.60	0.57	0.0114	0.025
Dibenz[a,h]anthracene	CA	$1.2x10^{-3}$	Cal/EPA	0.224	ng/item	1.02	0.0204	0.09	0.124	0.00248	0.011
Formaldehyde	CA, RT	$1.1x10^{-5}$	EPA IRIS	24.5	µg/item	80.09	1601.8	65.44	13.08	261.6	10.688
Acetaldehyde	CA, RT, AD	$2.2x10^{-6}$	EPA IRIS	122	µg/item	1624	32471	265.33	196.8	3936	32.163
Acrolein	RT, CT, CA (IARC 2A)	N/A	N/A	1.50	µg/item	154.3	3086	2057.33	9.14	182.8	121.867
Crotonaldehyde	CA	$3.27x10^{-5}$	N/A	8.23	µg/item	49.12	982.4	119.32	3.29	65.8	7.992
Butyraldehyde	CA	N/A	N/A	1.50	µg/item	81.45	1629	1086.00	20.23	404.6	269.733
1-Aminonaphthalene	CA	$5.1x10^{-4}$	Marano et al., (2018)	0.528	ng/item	21.2	0.423	0.80	0.050	0.0010	0.002
2-Aminonaphthalene	CA	$5.1x10^{-4}$	Marano et al., (2018)	0.528	ng/item	17.3	0.345	0.65	0.020	0.0004	0.001
3-Aminobiphenyl	CA	N/A	N/A	1.50	ng/item	4.57	0.091	0.06	0.010	0.0002	0.000
4-Aminobiphenyl	CA	$6.0x10^{-3}$	Cal/EPA	0.0449	ng/item	3.14	0.063	1.40	0.0100	0.0002	0.004

(Table continues)

o-Toluidine	CA	$5.1x10^{-5}$	Cal/EPA	5.28	ng/item	101.39	2.0278	0.38	1	0.02	0.004
Cadmium	CA, RT, RDT	$1.8x10^{-3}$	EPA IRIS	0.15	ng/item	94.07	1.8814	12.58	0.3	0.006	0.040
Lead	CA, CT, RDT	$1.2x10^{-5}$	Cal/EPA	22.4	ng/item	30.88	0.6176	0.03	2.05	0.041	0.002
Chromium (VI)	CA, RT, RDT	$1.8x10^{-2}$	EPA IRIS	0.015	ng/item	11.9	0.238	15.91	11	0.22	14.709
Nickel	CA, RT	$2.6x10^{-4}$	Cal/EPA	1.04	ng/item	12.9	0.258	0.25	15.9	0.318	0.307
Arsenic	CA, CT, RDT	$4.3x10^{-3}$	EPA IRIS	0.0626	ng/item	7.49	0.1498	2.39	1.2	0.024	0.383
Mercury	CA, RDT	N/A	N/A	1.50	ng/item	3.84	0.0768	0.05	1.53	0.0306	0.020
NTDS potential genotoxic compounds mean cumulated yield	CA	N/A	N/A	1.50	µg/item	57.8	1156	770.67	137.5	2750	1833.3
						ELCR cumulated		**12243.5**			**2683.4**
						Relative ELCRc (%)		**100**			**21.9**

CA: Carcinogen; CT: Cardiovascular Toxicant; N/A: Not available; RDT: Reproductive/Development Toxicant; RT: Respiratory Toxicant