Article

1,2-Propylene Glycol: A Biomarker of Exposure Specific to e-Cigarette Consumption

Therese Burkhardt [1], Nikola Pluym [1], Gerhard Scherer [1] and Max Scherer [1,*]

[1] ABF Analytisch-Biologisches Forschungslabor GmbH, Semmelweisstr. 5, 82152 Planegg, Germany; therese.burkhardt@abf-lab.com; nikola.pluym@abf-lab.com; gerhard.scherer@abf-lab.com

* Correspondence: max.scherer@abf-lab.com

Table S1. Method validation parameters for the determination of propylene glycol and glycerol in urine and plasma

Analyte	Parameter	Plasma	Urine
Propylene glycol	Accuracy LLOQ (N = 18)	0.1 µg/mL 92.2 %	0.1 µg/mL 100.1 %
	Calibration range	0.1 – 150.0 µg/mL	0.1 – 150.0 µg/mL
	Accuracy (N= 5)*	0.5 µg/mL: 97.0 % 5.0 µg/mL: 101.2 % 100 µg/mL: 99.9 %	0.5 µg/mL: 97.0 % 5.0 µg/mL: 101.2 % 100 µg/mL: 99.9 %
	Intraday precision (N= 5)	0.9 µg/mL: 8.5 % 5.2 mg/mL: 3.9 % 84.0 µg/mL: 2.0 %	0.3 µg/mL: 18.0 % 7.8 µg/mL: 9.4 % 117.2 µg/mL: 7.8 %
	Interday precision (N= 6)	0.9 µg/mL: 11.0 % 5.2 mg/mL: 6.0 % 84.0 µg/mL: 7.1 %	0.3 µg/mL: 13.2 % 7.8 mg/mL: 3.1 % 117.2 µg/mL: 8.8 %
	Re-injection (N= 3)	0.9 µg/mL: 4.5 % 82.3 µg/mL: 2.0 %	0.3 µg/mL: 8.0 % 115.0 µg/mL: 4.6 %
	Carry-over	150 µg/mL: 0 %	150 µg/mL: 0 %
	Short-term stability (24 h, 21 °C)	0.9 µg/mL: 103.5 % 85.0µg/mL: 109.0 %	0.3 µg/mL: 108.6 % 117.2 µg/mL: 89.6 %
	Long-term stability (-20 °C)	1.0 µg/mL: 21 months 6.2 µg/mL: 21 months 89.0 µg/mL: 21 months	0.3 µg/mL: 26 months 7.3 µg/mL: 34 months 112.0 µg/mL: 34 months
	Freeze-thaw stability (6 cycles)	0.9 µg/mL: 101.0 % 85.0µg/mL: 107.4 %	0.3 µg/mL: 89.5 % 117.2 µg/mL: 86.3 %
	Post preparative stability (7 d, 10 °C)	0.9 µg/mL: 94.8 % 85.0µg/mL: 96.0 %	0.3 µg/mL: 83.9% 117.2 µg/mL: 96.6 %
Glycerol	Accuracy LLOQ (N = 6)	0.1 µg/mL 97.6 %	0.1 µg/mL 97.6 %
	Calibration range	0.1 – 150.0 µg/mL	0.1 – 150.0 µg/mL
	Accuracy (N= 5)*	0.5 µg/mL: 95.5 % 5.0 µg/mL: 92.0 % 100 µg/mL: 4.8 %	0.5 µg/mL: 95.5 % 5.0 µg/mL: 92.0 % 100 µg/mL: 106.4 %
	Intraday precision (N= 5)	0.3 mg/mL: 9.6 % 3.2 µg/mL: 4.0 % 72.0 µg/mL: 4.8 %	0.4 µg/mL: 10.5 % 5.8 µg/mL: 11.3 % 102.2 µg/mL: 11.8 %
	Interday precision (N= 6)	0.3 mg/mL: 15.2 % 3.2 µg/mL: 14.5 % 72.0 µg/mL: 3.0 %	0.4 µg/mL: 5.9 % 5.8 µg/mL: 10.2 % 102.2 µg/mL: 9.3 %
	Re-injection (N= 3)	1.0 µg/mL: 7.8 % 80.0 µg/mL: 3.4 %	0.5 µg/mL: 4.0 % 100 µg/mL: 6.6 %
	Carry-over	150 µg/mL: 0 %	150 µg/mL: 0 %
	Short-term stability (24 h, 21 °C)	0.3 mg/mL: 93.7 % 72.0 µg/mL: 101.5 %	0.4 µg/mL: 94.8 % 102.2 µg/mL: 99.9 %

Analyte	Parameter	Plasma	Urine
	Long-term stability (-20 °C)	Under investigation	0.4 µg/mL: 26 months 5.1 µg/mL: 34 months 91.0 µg/mL: 26 months
	Freeze-thaw stability (6 cycles)	0.3 mg/mL: 89.4 % 72.0 µg/mL: 93.7 %	0.4 mg/mL: 82.2 % 102.2 µg/mL: 99.9 %
	Post preparative stability (7 d, 10 °C)	0.3 mg/mL: 90.7 % 72.0 µg/mL: 109.7 %	0.4 mg/mL: 112.0% 102.2 µg/mL: 100.4 %

Acceptance criteria for accuracy: ± 15% of nominal concentrations; except ± 20% at 3x LLOQ. Acceptance criteria for precision: ± 15% coefficient of variation (CV); except ± 20% CV at 3 x LLOQ.

* Accuracy tests were conducted in water since no analyte-free matrix was available.

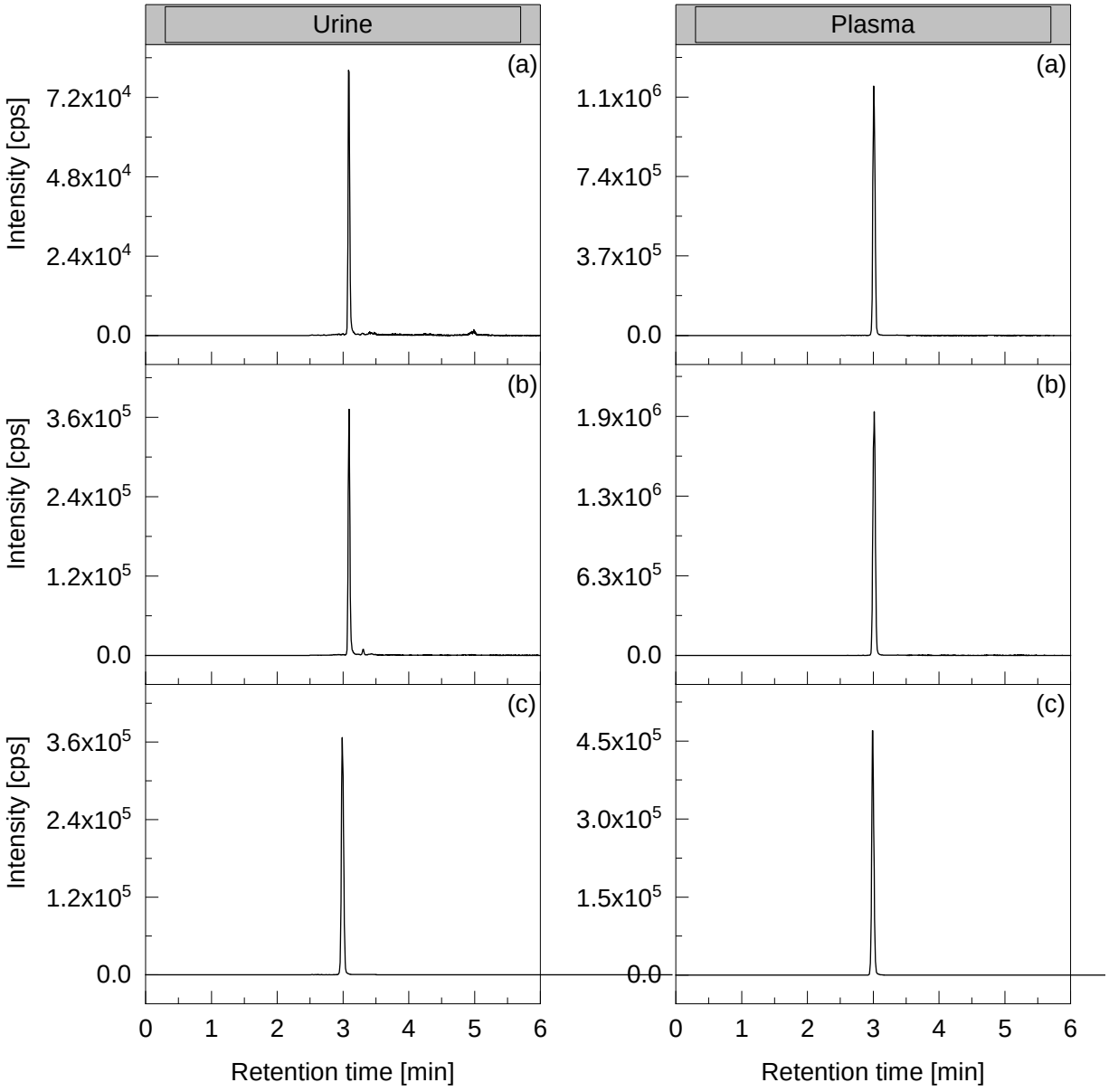

Figure S1. Representative chromatogram of glycerol (G) (MRM 405→283) of (a) low concentrated urine (0.21 μg/mL G, user of oral tobacco) and plasma (9.9 μg/mL G, non-user) samples, (b) high concentrated urine (9.7 μg/mL G, user of e-cigarettes) and plasma (24.4 μg/mL PG, user of e-cigarettes) samples, and (c) glycerol-d_5 (MRM 410→288, 2 μg/mL).

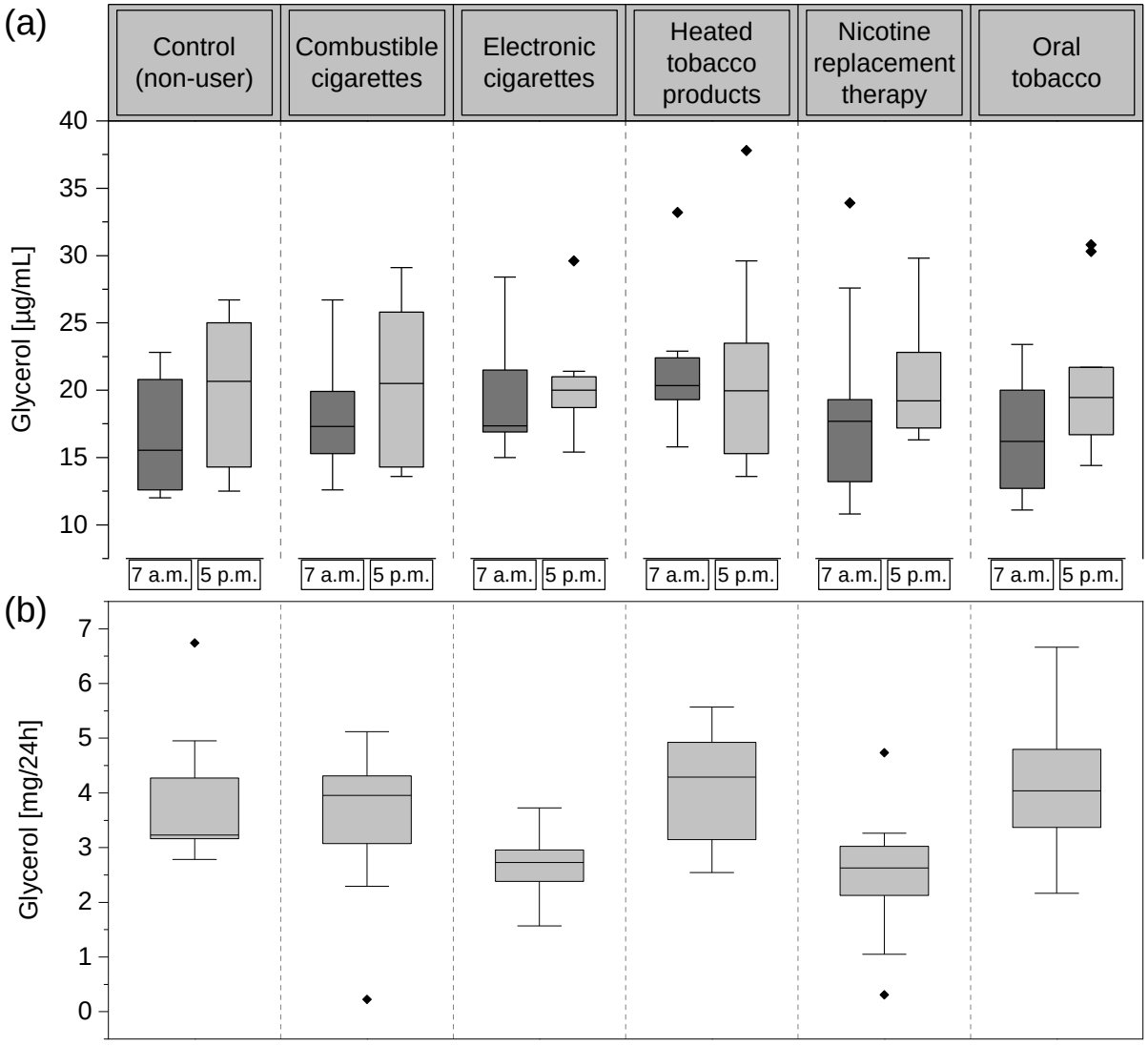

Figure S2. Box-and-whisker plots of glycerol (G) on day 3 in (a) plasma [µg/mL] at 7 a.m. and 5 p.m. and (b) urine [mg/24h] between different nicotine product user groups and the control. Box-and-whisker plots represent medians (horizontal lines) with 25 % and 75 % percentiles (boxes), 1.5xIQR (whiskers), and outliers (diamonds).

Table S2. Descriptive statistics of glycerol [µg/mL] in plasma of study samples for different nicotine user groups

User group	Day	Time	N total	Mean	SD	Median	Min	Max
Control (non-user, NU)	Day -1	5 p.m.	10	20.4	7.7	17.45	9.9	30
	Day 1	7 a.m.	10	19.2	3.5	17.9	14.5	26
		5 p.m.	10	15.5	4.1	15.1	8.78	23.5
	Day 2	7 a.m.	10	16.6	5.4	14.5	10.4	26
		5 p.m.	10	23.2	5.2	21.4	17.4	32.7
	Day 3	7 a.m.	10	16.7	4.2	15.55	12	22.8
		5 p.m.	10	20.0	5.6	20.65	12.5	26.7
Combustible cigarettes (CC)	Day -1	5 p.m.	10	20.2	5.5	18.8	12.9	30.3
	Day 1	7 a.m.	10	20.1	4.3	20.2	14.1	27.9
		5 p.m.	10	17.3	2.6	17.5	12.6	20.9
	Day 2	7 a.m.	10	17.2	2.9	17.4	13	22.3
		5 p.m.	10	20.0	5.5	17.4	14.5	31.3
	Day 3	7 a.m.	10	17.7	4.1	17.3	12.6	26.7
		5 p.m.	10	20.2	5.7	20.5	13.6	29.1
Electronic cigarettes (EC)	Day -1	5 p.m.	10	22.7	4.1	22.6	18	30.9
	Day 1	7 a.m.	10	20.6	6.2	18.75	13.7	36.2
		5 p.m.	10	19.1	4.0	18.55	15.1	28
	Day 2	7 a.m.	10	17.9	3.7	16.9	14	24.5
		5 p.m.	10	22.6	4.7	22.6	14.8	29.2
	Day 3	7 a.m.	10	19.5	4.7	17.35	15	28.4
		5 p.m.	10	20.5	3.7	20	15.4	29.6
Heated tobacco products (HTP)	Day -1	5 p.m.	10	24.4	10.4	21.1	16.3	51.6
	Day 1	7 a.m.	10	20.4	5.2	20	14	31.1
		5 p.m.	10	19.6	6.3	18.3	14	35.9
	Day 2	7 a.m.	10	18.1	4.6	17.05	10.9	26.4
		5 p.m.	10	23.7	7.4	21.05	17.1	39.9
	Day 3	7 a.m.	10	21.5	4.6	20.35	15.8	33.2
		5 p.m.	10	21.4	7.5	19.95	13.6	37.8
Nicotine replacement therapy (NRT)	Day -1	5 p.m.	10	19.1	5.5	19.95	10.4	27.9
	Day 1	7 a.m.	10	17.4	4.9	17.85	8.85	23.9
		5 p.m.	10	20.0	5.7	18.05	14.3	29.6
	Day 2	7 a.m.	10	19.0	5.6	16.7	12.6	28.7
		5 p.m.	10	18.0	5.9	16.7	11.9	32.7
	Day 3	7 a.m.	10	18.8	7.1	17.7	10.8	33.9
		5 p.m.	10	20.7	4.9	19.2	16.3	29.8
Oral tobacco (OT)	Day -1	5 p.m.	10	20.8	7.5	19.1	11.4	32.8
	Day 1	7 a.m.	10	20.3	4.5	18.55	15.4	29.8
		5 p.m.	10	18.5	5.2	17.35	10.6	28.6
	Day 2	7 a.m.	10	17.3	2.9	16.9	12.1	22.2
		5 p.m.	10	21.1	6.3	19.75	15.5	37.4
	Day 3	7 a.m.	10	16.5	4.0	16.2	11.1	23.4
		5 p.m.	10	20.4	5.8	19.45	14.4	30.8

Table S3. Descriptive statistics of glycerol [mg/24h] in urine of study samples for different nicotine user groups

User group	Day	N total	Mean	SD	Median	Min	Max
Control (non-user, NU)	Day 1	10	4.1	1.1	3.9	2.7	6.3
	Day 2	10	3.5	1.1	3.3	2.0	5.3
	Day 3	10	3.8	1.2	3.2	2.8	6.7
Combustible cigarettes (CC)	Day 1	10	4.3	1.1	4.4	2.0	5.9
	Day 2	10	4.3	0.9	4.4	2.4	5.6
	Day 3	10	3.5	1.4	4.0	0.2	5.1
Electronic cigarettes (EC)	Day 1	10	3.5	1.7	3.6	1.3	6.9
	Day 2	10	3.2	1.0	3.4	1.2	4.2
	Day 3	10	2.8	0.6	2.7	1.6	3.7
Heated tobacco products (HTP)	Day 1	10	4.8	2.0	4.5	2.0	8.4
	Day 2	10	3.7	1.5	3.0	2.0	5.7
	Day 3	10	4.1	1.1	4.3	2.5	5.6
Nicotine replacement therapy (NRT)	Day 1	10	3.3	0.9	3.4	2.1	4.7
	Day 2	10	2.6	1.6	2.3	0.6	5.3
	Day 3	10	2.5	1.2	2.6	0.3	4.7
Oral tobacco (OT)	Day 1	10	4.2	1.4	4.3	2.1	7.0
	Day 2	10	4.4	2.4	4.2	1.3	9.8
	Day 3	10	4.1	1.3	4.0	2.2	6.7

SD: Standard deviation, Min: Minimum, Max: Maximum

Table S4. Descriptive statistics of 1,2-propylene glycol [µg/mL] in plasma of study samples for different nicotine user groups

User group	Day	Time	N total	Mean	SD	Median	Min	Max
Control (non-user, NU)	Day -1	5 p.m.	10	0.27	0.36	0.10	0.04	1.18
	Day 1	7 a.m.	10	0.08	0.04	0.08	0.03	0.13
		5 p.m.	10	0.10	0.03	0.10	0.05	0.14
	Day 2	7 a.m.	10	0.05	0.02	0.05	0.02	0.07
		5 p.m.	10	0.09	0.02	0.10	0.06	0.13
	Day 3	7 a.m.	10	0.05	0.02	0.04	0.03	0.10
		5 p.m.	10	0.09	0.02	0.09	0.05	0.12
Combustible cigarettes (CC)	Day -1	5 p.m.	10	0.42	0.91	0.12	0.07	3.00
	Day 1	7 a.m.	10	0.09	0.05	0.07	0.03	0.17
		5 p.m.	10	0.11	0.04	0.10	0.06	0.17
	Day 2	7 a.m.	10	0.08	0.04	0.07	0.04	0.17
		5 p.m.	10	0.13	0.05	0.12	0.06	0.25
	Day 3	7 a.m.	10	0.08	0.04	0.06	0.04	0.17
		5 p.m.	10	0.12	0.03	0.12	0.08	0.19
Electronic cigarette (EC)	Day -1	5 p.m.	10	7.20	4.73	5.67	0.71	16.60
	Day 1	7 a.m.	10	1.75	2.32	0.78	0.29	7.59
		5 p.m.	10	9.76	7.83	7.29	1.27	26.90
	Day 2	7 a.m.	10	1.45	1.68	0.65	0.19	5.61
		5 p.m.	10	8.83	7.68	5.89	2.48	26.20
	Day 3	7 a.m.	10	1.25	1.01	0.78	0.25	2.95
		5 p.m.	10	8.37	8.88	5.47	2.38	31.90
Heated tobacco products (HTP)	Day -1	5 p.m.	10	0.83	1.95	0.06	0.03	6.34
	Day 1	7 a.m.	10	0.09	0.05	0.08	0.03	0.19
		5 p.m.	10	0.11	0.03	0.11	0.07	0.15
	Day 2	7 a.m.	10	0.05	0.02	0.04	0.03	0.09
		5 p.m.	10	0.11	0.04	0.11	0.05	0.19
	Day 3	7 a.m.	10	0.07	0.04	0.05	0.03	0.15
		5 p.m.	10	0.11	0.04	0.11	0.05	0.17
Nicotine replacement therapy (NRT)	Day -1	5 p.m.	10	0.57	0.92	0.17	0.04	3.00
	Day 1	7 a.m.	10	0.08	0.05	0.07	0.02	0.16
		5 p.m.	10	0.14	0.11	0.09	0.06	0.36
	Day 2	7 a.m.	10	0.09	0.06	0.07	0.03	0.20
		5 p.m.	10	0.14	0.10	0.12	0.04	0.40
	Day 3	7 a.m.	10	0.08	0.07	0.06	0.02	0.22
		5 p.m.	10	0.15	0.17	0.08	0.04	0.59
Oral tobacco (OT)	Day -1	5 p.m.	10	0.41	0.47	0.14	0.03	1.36
	Day 1	7 a.m.	10	0.11	0.06	0.09	0.04	0.23
		5 p.m.	10	0.20	0.13	0.18	0.07	0.51
	Day 2	7 a.m.	10	0.10	0.05	0.09	0.04	0.22
		5 p.m.	10	0.21	0.16	0.16	0.08	0.60
	Day 3	7 a.m.	10	0.11	0.04	0.10	0.06	0.20
		5 p.m.	10	0.19	0.12	0.19	0.07	0.43

Table S5. Descriptive statistics of PG [mg/24h] in urine of study samples for different nicotine user groups

User group	Day	N total	Mean	SD	Median	Min	Max
Control (non-user, NU)	Day 1	10	1.8	1.2	1.5	0.6	4.7
	Day 2	10	1.4	0.4	1.5	0.8	2.0
	Day 3	10	1.5	0.4	1.5	1.0	2.1
Combustible cigarettes (CC)	Day 1	10	2.8	4.2	1.4	1.1	14.7
	Day 2	10	1.7	0.4	1.6	1.3	2.4
	Day 3	10	1.8	0.5	1.7	1.0	2.7
Electronic cigarettes (EC)	Day 1	10	86.1	73.2	59.5	13.4	226.9
	Day 2	10	96.1	88.8	67.5	19.3	303.5
	Day 3	10	95.4	107.1	77.6	12.7	380.7
Heated tobacco products (HTP)	Day 1	10	3.9	5.6	2.2	1.2	19.7
	Day 2	10	2.0	1.1	1.6	1.1	4.6
	Day 3	10	2.1	1.5	1.6	0.9	5.9
Nicotine replacement therapy (NRT)	Day 1	10	2.2	1.6	2.1	0.2	5.1
	Day 2	10	1.6	1.7	1.0	0.2	5.9
	Day 3	10	1.7	2.3	1.1	0.4	8.1
Oral tobacco (OT)	Day 1	10	3.9	4.2	2.8	0.9	15.2
	Day 2	10	3.6	3.4	2.8	0.9	12.7
	Day 3	10	3.3	2.3	3.0	1.1	8.7

SD: Standard deviation, Min: Minimum, Max: Maximum

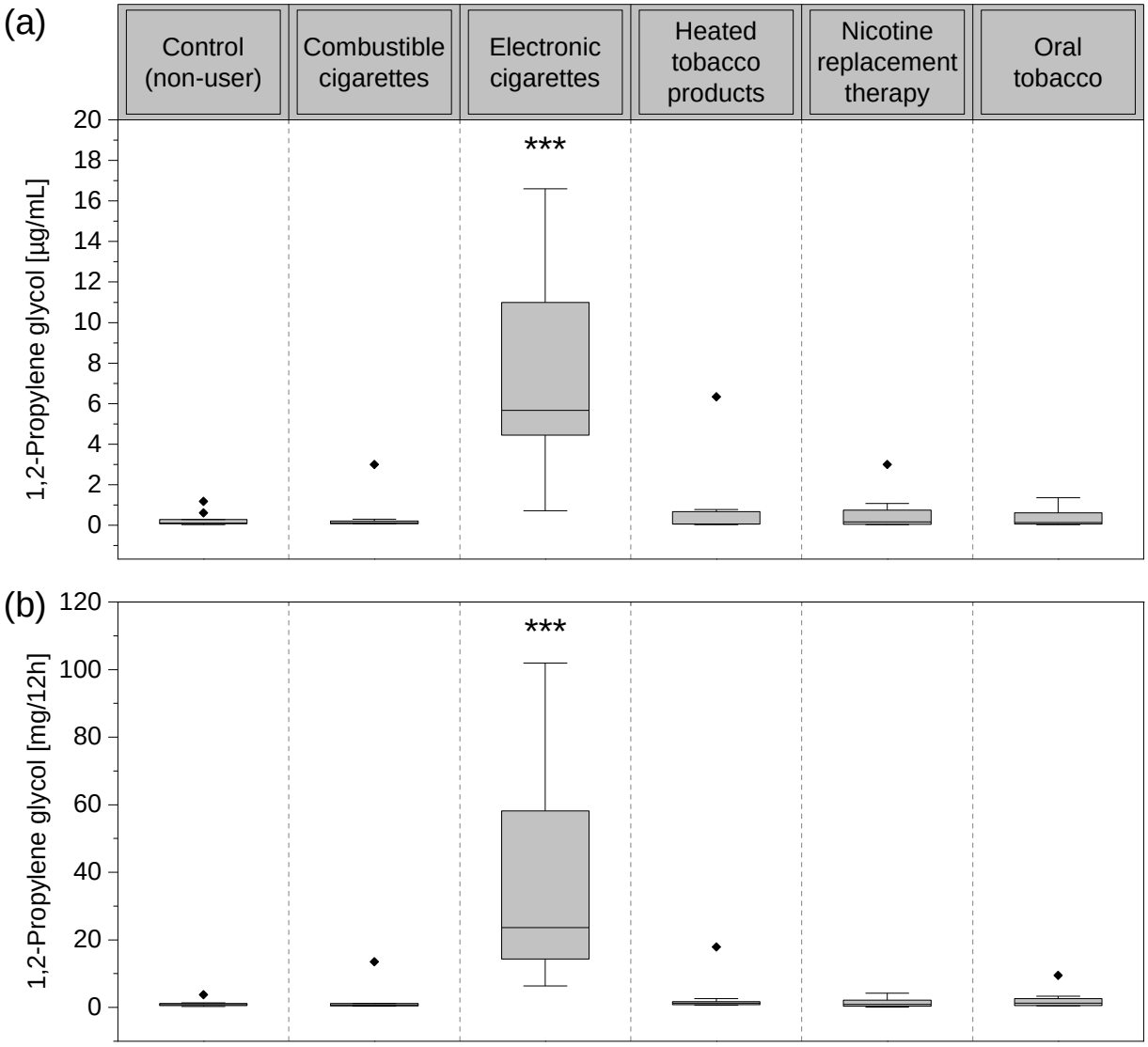

Figure S3. Box-and-whisker plot of 1,2-propylene glycol (PG) on day -1 in (a) plasma [µg/mL] at 5 pm (B0) and (b) urine [mg/12h] (U0) between different nicotine product user groups and the control. Box-and-whisker plots represent medians (horizontal lines) with 25 % and 75 % percentiles (boxes), 1.5xIQR (whiskers), and outliers (diamonds). *** $p < 0.01$ (comparison of EC vs all other groups).

Table S6. Descriptive statistics of PG in urine [mg/12h] and plasma [µg/mL] of study samples for different nicotine user groups at day -1

Matrix	User group	N total	Mean	SD	Median	Min	Max
Urine (U0)	Control (non-user, NU)	10	1.1	1.0	0.3	0.8	3.8
	Combustible cigarettes (CC)	10	2.0	4.1	0.4	0.7	13.6
	Electronic cigarettes (EC)	10	35.5	30.3	6.4	23.6	101.9
	Heated tobacco products (HTP)	10	2.9	5.3	0.6	1.3	17.9
	Nicotine replacement therapy (NRT)	10	1.4	1.3	0.1	0.9	4.2
	Oral tobacco (OT)	10	2.2	2.7	0.5	1.2	9.5
Plasma (B0)	Control (non-user, NU)	10	0.27	0.36	0.10	0.04	1.18
	Combustible cigarettes (CC)	10	0.42	0.91	0.12	0.07	3.00
	Electronic cigarettes (EC)	10	7.20	4.73	5.67	0.71	16.60
	Heated tobacco products (HTP)	10	0.83	1.95	0.06	0.03	6.34
	Nicotine replacement therapy (NRT)	10	0.57	0.92	0.17	0.04	3.00
	Oral tobacco (OT)	10	0.41	0.47	0.14	0.03	1.36

SD: Standard deviation, Min: Minimum, Max: Maximum