Electronic Supplementary Material (ESI) for Analytical Methods.

A Review of the Analysis of Biomarkers of Exposure to Tobacco and Vaping Products

Habibagahi et al, 2020

Table 1. Literature values and analytical methods for nicotine and total nicotine equivalents (TNEQ)

LOD Free (Total)	LOQ (LLOQ)	N	Median (Mean) [Range]	Sample Preparation	Instrumental Analysis	Ref.
1.1. Nicotine						
1.63 ng/L (1.55 ng/L)	-	94	-	**Method A** A 100 µL sample of urine was incubated with internal standards, HPLC water and 1600 units of enzyme at 37°C overnight (for free forms no enzyme was added). Cold acetone (4 °C) was added followed by cold centrifugation. After separation, the resultant liquid was acidified with MeOH/HCl. The acetone was removed by drying of the sample in an evaporator and the residual urine supernatant directly injected.	LC-MS/MS	[1]
(3.1 ng/L)	10.5 ng/L	240 (40 Smokers & 200 non-smokers)	1485 ng/L	**Method B** Sample preparation was performed using a robotic system. Urine samples (100 µL) were added to an internal standard solution. Enzymatic hydrolysis was then performed at 45 °C for 12 hours. Cold acetone (-20 °C) was added followed by centrifugation at -20 °C for 30 minutes. The supernatant was transferred, evaporated and reconstituted in HPLC water (250 µL).	LC-MS/MS	[2]
-	(10.0 ng/mL)	20 smokers	(Tobacco cigarette: 1126 ± 821 [634-1578]* µg/g*)	**Method C** Internal standards in HCl (0.01 M) were added to 1 mL of sample. After vortexing, perchloric acid (100 µL of 30% w/v) was added to precipitate proteins. The resulting solution was centrifuged and the supernatant decanted. Tripotassium phosphate (2 mL of 50% w/v) and methylene chloride (6 mL) were added followed by vortexing. This was centrifuged and placed in a dry ice-acetone mixture to freeze the aqueous layer. The organic layer was poured into 100 µL of 10% w/v HCl in MeOH, evaporated to dryness and reconstituted in 150 µL of 100 mM aqueous ammonium formate.	LC-MS/MS	[3]

1 ng/mL	-	-	(Electronic cigarette: 962 ± 1139 [202–1290]* µg/g**)	**Method D** Aliquots (1 mL) of urine samples were taken and internal standards in HCl (0.01 M) added. After vortexing, 0.5 mL of 2 M NaOH containing 0.2 M ammonia was added. A mixture of toluene and 1-butanol (70:30, 3 mL) was added and vortex mixed. The resulting solution was centrifuged and the aqueous layer frozen in a dry ice-acetone bath. The organic layer was discarded. Aqueous potassium carbonate was added (0.5 mL of 50% w/v containing 0.2 M ammonia) followed by 90:10 toluene: butanol (150 µL) and vortexing/centrifuging. The aqueous layer was frozen and the organic layer decanted and concentrated to 25 µL by heating at 85 °C.	GC/MS	[4]
-	0.2 ng/mL	13	-		GC/MS	[5]
-	13[a] ng/mL	364 African Americans	5.44 nmol/mL [2.9-11.0* nmol/mL]	**Method E** Urine samples were diluted 1:10 with water and 400 µL of 100 mM ammonium acetate added. Enzymatic hydrolysis of urine samples by β-glucuronidase was performed overnight at 37°C, followed by solid phase extraction using Oasis MCX 2mg cartridges. Columns were washed with 200 µL 0.5 % (v/v) formic acid and 400 µL methanol. Nicotine, cotinine and 3-HCOT were eluted with 50 µL 2% ammonium hydroxide in methanol.	LC-MS/MS	[6]
		311 Native Hawaiians	6.19 nmol/mL [3.32-11.4* nmol/mL]			
		437 Caucasians	5.42 nmol/mL [3.04-8.80* nmol/mL]			
		453 Latinos	4.41 nmol/mL [1.92-7.72* nmol/mL]			
		674 Japanese Americans	6.38 nmol/mL [3.54-11.8* nmol/mL]			

-	(10 ng/mL)	100	-	**Method F** Urine samples (1.0 mL) were basified and loaded onto a preconditioned Oasis HLB solid-phase extraction (SPE) cartridge. The sample was washed with NH_4OAc (pH 6.6) and then eluted with methanol. The eluent was evaporated to dryness and reconstituted in 200 µL of methanol. For the analysis of total cotinine and total trans-3´-hydroxycotinine, a 0.2 mL aliquot of urine was incubated at 37 °C for 20 to 24 hours with β-glucuronidase.	LC-MS/MS	[7]
-	-	12	[7.24 ± 3.41 µmol/24h]	**Method G** The volume of urine from active smokers was adjusted to 1 mL. After mixing with 1 mL of 50% aqueous K_2CO_3 the sample was extracted once with 2 mL of CH_2Cl_2. The CH_2Cl_2 layer was separated and mixed with 200 µL of CH_3OH. This solution was concentrated under a gentle stream of N_2 to a total volume of 100-200 µL of CH_3OH and then analyzed. Total cotinine and nicotine were assayed by treating the samples with 0.1 N NaOH for 30 min at 70 °C to release the aglycones prior to analysis for cotinine and nicotine.Trans-3'-Hydroxycotinine-Gluc present in urine was hydrolyzed by treating the samples with β-glucuronidase.	GC-MS	[8]
0.15 ng/mL	-	12 Smokers who were orally administered 2mg d(2)-nicotine 30 minutes prior	(d(2) nicotine in plasma: 2.47 ± 1.02 ng/mL Nicotine in plasma: 28.8 ± 8.1 ng/mL)	**Method H** Samples (100-200 µL) were with diluted in 900 µL of water and 2 mL of Dulbecco's 10 mM phosphate buffered saline (pH 7.4). Internal standards (10 µL) were added and the sample vortexed. SPE was performed using an Oasis MCX column that was activated with 3 mL methanol and equilibrated with 3 mL of water followed by 3 mL phosphate buffered saline (PBS). The columns were then washed with 3 mL each of water, 0.1 N HCl, and methanol, and the samples eluted with 3 mL of methylene chloride/isopropanol/ammonium hydroxide (78:20:2). Water (1–2 mL) was added to the eluted samples, which were then extracted and the aqueous layer discarded. The organic layer was then extracted with an equal volume of 1 N HCl. The aqueous layer was removed, an equal volume of 50% potassium carbonate added to it and cotinine and nicotine extracted into methylene chloride. To this, 200 µL of methanol was added. The samples were then dried under nitrogen gas to a volume of 50–100 µL of methanol.	LC-MS/MS	[9]
		10 Past smokers who were orally administered 2mg d(2)-nicotine 30 minutes prior	(d(2) nicotine in plasma: 2.7 ± 2.12 ng/mL Nicotine in plasma: Not detected)			
1.2. Total nicotine equivalents (TNEQ)						

-	-	52 exclusive adult cigarette smokers	(10.1) [0-29] ng/g creatinine	Method E	LC-MS/MS	[10]
		122 exclusive adult e-vapor users	(6.3) [0-29] ng/g creatinine			
-	-	364 African Americans	44.4 nmol/mL [27.1-74.0* nmol/mL]	**Method I** Urine samples were diluted 1:10 with water and 400 µL of 100 mM ammonium acetate added. Enzymatic hydrolysis of urine samples by β-glucuronidase was performed overnight at 37 °C, followed by solid phase extraction using Oasis MCX 2mg cartridges. Columns were then washed with 200 µL 0.5% (v/v) formic acid and 400 µL methanol. Nicotine, cotinine and 3-HCOT were eluted with 50 µL of 2% ammonium hydroxide in methanol.	LC-MS/MS	[6]
		311 Native Hawaiians	30.3 nmol/mL [19.4-46.8* nmol/mL]			
		437 Caucasians	36.3 nmol/mL [21.9-61.5* nmol/mL]			
		453 Latinos	32.2 nmol/mL [20.8-53.6* nmol/mL]			
		674 Japanese Americans	27.3 nmol/mL [15.8-43.14* nmol/mL]			
-	-	305 Electronic cigarette users	(9.9◊ mg/24h) [9.2-10.6◊ mg/24h]	**Method J** Internal standards (100 µL in 0.01 M HCl) were added to 1 mL of sample. After votexing, 100 µL of 30% (w/v) perchloric acid was added. The mixture was vortexed and centrifuged, with the supernate decanted. Tripotassium phosphate (2 mL of 50% w/v) was added followed by 6 mL of methylene chloride. After vortexing for 5 minutes, this was centrifuged and the aqueous layer frozen in an acetone/dry ice bath. The organic layer was taken and 100 µL of 10% HCl (w/v) added. After evapourating to dryness in a centrifugal vacuum evaporator, the extract was reconstituted in 150 µL of 100 mM aqueous ammonium formate.	LC-MS/MS	[11]
		102 Regular cigarette users	(10.1◊ mg/24h) [8.9-11.4◊ mg/24h]			

-	(0.500 ng/mL)	47 (Heated cigarette users)	(3.66◊ mg/day) [3.10-4.31◊ mg/day]	**Method K** Urine samples were diluted with citrate phosphate buffer solution (0.1 M, pH 5.0, 945 µL), and β-glucuronidase/arylsulfatase was added (30 µL, 3kU). Four-hundred microliter and 1.1 mL of the resulting solution were used for determination of the unconjugated form and total form, respectively. For determination of the total form, the sample was incubated at 37 ºC for 17 h and 0.5 mL of 0.28% aqueous ammonia were added followed by solid phase extraction (SPE) on an Empore universal resin plate (Sumitomo-3 M). The sample was eluted with 0.2 mL of methanol and analysed.	LC-MS/MS	[12]
		23 (Conventional cigarette users)	(13.9◊ mg/day) [11.1-17.6 ◊ mg/day]			
-	-	37	(12.27 ± 6.07 mg/g**)	**Method F**	LC-MS/MS	[13]
-	-	160	(Conventional cigarettes (6.0 mg tar/0.5 mg nicotine) 11.79 ± 6.98 mg/g**)		LC-MS/MS	[14]
			(Conventional cigarettes (10.6 mg tar/0.84 mg nicotine) 11.61 ± 6.37 mg/g**)			
			(Test cigarettes (6.0 mg tar) 17.20 ± 7.90 mg/g**)			
			(Test cigarettes (11 mg tar) 11.36 ± 6.07 mg/g**)			
-	-	47 Smokers – Pack tar 10mg/cig	(18.3 mg/day) [16.4-20.2◊ mg/day]		LC-MS/MS	[15]

		46 Smokers – Pack tar 10mg/cig	(15.8 mg/day) [13.7-17.9◊ mg/day]	**Method L** For total cotinine and total trans-3-hydroxycotinine analysis, the samples were hydrolysed overnight with β-glucuronidase (type 1α-A from Escherichia coli) at 37 °C. The deconjugated samples were subsequently analysed simultaneously for total nicotine, total cotinine and total trans-3'-hydroxycotinine using an SPE (Oasis HLB 3 cc/60 mg). The total cotinine and total trans-3'-hydroxycotinine were converted to molar nicotine equivalents and summed with the total nicotine to provide a concentration of total nicotine equivalents (TNeq). The analysis of total NNAL involved overnight hydrolysis with β-glucuronidase (type 1α-A from E. coli) at 37 °C followed by SPE (Oasis MAX, 3 cc/60 mg).		
		45 Smokers – Pack tar 6mg/cig	(14.4 mg/day) [12.2-16.5◊ mg/day]			
		44 Smokers – Pack tar 6mg/cig	(9.5 mg/day) [8.1-10.8◊ mg/day]			
		48 Smokers – Pack tar 1mg/cig	(8.3 mg/day) [7.0-9.6◊ mg/day]			
-	10 ng/mL	50 Non-smokers	(0.02 mg/day) [0.02-0.02◊ mg/day]		LC-MS/MS	[16]
		50 Smokers of 1mg tar cigarettes	(7.7 mg/day) [6.5-8.9◊ mg/day]			
		50 Smokers of 4mg tar cigarettes	(13.4 mg/day) [11.5-15.2◊ mg/day]			
		50 Smokers of 10mg tar cigarettes	(18.1 mg/day) [16.2-20.1◊ mg/day]			
-	-	49 Non-smokers	(0.03 mg/day) [0.02-0.03◊ mg/day]		LC-MS/MS	[17]

		42 Smokers of 4mg tar cigarettes	(14.5 mg/day) [12.7-16.2◊ mg/day]			
		48 Smokers of 9mg tar cigarettes	(15.1 mg/day) [13.4-16.8◊ mg/day]			
		49 Smokers of 14mg tar cigarettes	(18.9 mg/day) [17.4-20.5◊ mg/day]			

* Interquartile range ** Normalized per gram creatinine [a]1 µL of urine ◊ 95% confidence interval

Table 2. Literature values and analytical methods for nicotine metabolites

LOD Free (Total) (ng/L)	LOQ (LLOQ) (ng/L)	N	Median (Mean) [Range]	Sample Preparation	Instrumental Analysis	Ref.
2.1 Nicotine-N-oxide (NNO)						
0.75	2.5	240 (40 Smokers & 200 non-smokers)	<1000 ng/L	Method B, Table 1.1	LC-MS/MS	[2]
-	(5.0)	20 smokers	(Tobacco cigarette: 335 ± 231 [235-415]* µg/g**)	Method C, Table 1.1	LC-MS/MS	[3]

			(Electronic cigarette: 326 ± 399 [49-442]* µg/g**)			
0.29	0.71	94	-	Method A, Table 1.1	LC-MS/MS	[1]
-	-	363 African Americans	2.65 (1.37-4.89*) nmol/mL	Method I, Table 1.2	LC-MS/MS	[6]
		311 Native Hawaiians	1.88 (0.96-3.56*) nmol/mL			
		437 Caucasians	1.76 (0.90-3.04*) nmol/mL			
		453 Latinos	1.49 (0.64-3.07*) nmol/mL			
		673 Japanese Americans	2.16 (1.14-3.60*) nmol/mL			
2.2. Nornicotine (NorNIC)						
0.33 (0.41)	-	94	-	Method A, Table 1.1	LC-MS/MS	[1]
(0.75)	2.5	240 (40 Smokers & 200 non-smokers)	<150 ng/L	Method B, Table 1.1	LC-MS/MS	[2]

-	(5)	20 smokers	(Tobacco cigarette: 73 ± 39 [47-105]* µg/g**) (Electronic cigarette: 46 ± 45 [10-58]* µg/g**)	Method C, Table 1.1	LC-MS/MS	[3]
2.3. 4-Hydroxy-4-(3-pyridyl) butanoic acid (HPBA)						
0.4	1.4	240 (40 Smokers & 200 non-smokers)	<1000	Method B, Table 1.1	LC-MS/MS	[2]
-	-	12	(6.84 ± 3.86 µmol/24h)	Method G, Table 1.1	GC-MS	[8]
2.4. 4-oxo-4-(3-pyridyl)butanoic acid (Keto acid)						
-	-	12	1.49 ± 1.00 µmol/24h	Method G, Table 1.1	GC-MS	[8]
2.5. Nicotine glucuronide						
-	-	363 African Americans	0.26 [0.12-0.42*] nmol/mL	Method I, Table 1.2	LC-MS/MS	[6]
		311 Native Hawaiians	0.30 [0.18-0.42*] nmol/mL			
		437 Caucasians	0.34 [0.21-0.48*] nmol/mL			

		453 Latinos	0.37 [0.25-0.54*] nmol/mL			
		673 Japanese Americans	0.32 [0.21-0.44*] nmol/mL			
-	(5 ng/mL)	100	-	Method F, Table 1.1	LC-MS/MS .	[7]
-	-	12	(1.72 ± 1.03 µmol/24h)	**Method M** Urine sample (1 mL) was mixed with 1 mL of 50% aqueous K_2CO_3 and then extracted once with 2 mL of CH_2Cl_2. The CH_2Cl_2 layer was separated and mixed with 200 µL of CH_3OH. This solution was concentrated under a gentle stream of nitrogen to a total volume of 100–200 µL of CH_3OH and then analyzed. Total cotinine and nicotine were assayed by treating the samples with 0.1 N NaOH for 30 min at 70 °C to release the aglycones prior to analysis for cotinine and nicotine.Trans-3'-Hydroxycotinine-Gluc present in urine was hydrolyzed by treating the samples with β-glucuronidase	GC-MS	[8]
-	-	27 people who stopped smoking	(13.6 ± 8.22 nmol/mL urine)		GC-MS	[18]
2.6. Cotinine						
1.4 (3.53) ng/L	-	94	-	Method A, Table 1.1	LC-MS/MS	[1]
(1.4 ng/L)	4.6 ng/L	240 (40 Smokers & 200 non-smokers)	3555 ng/L	Method B, Table 1.1	LC-MS/MS	[2]

-	(10.0 ng/mL)	20 smokers	(Tobacco cigarette: 2287 ± 1381 [1344-2941]* µg/g**) (Electronic cigarette: 2048 ± 2102 [745-2211]* µg/g**)	**Method N** Internal standards in HCl (0.01 M) were added to 1 mL of sample. After vortexing, perchloric acid (100 µL of 30% w/v) was added to precipitate proteins. The resulting solution was centrifuged and the supernatant decanted. Tripotassium phosphate (2 mL of 50% w/v) and methylene chloride (6 mL) were added followed by vortexing. This was centrifuged and placed in a dry ice-acetone mixture to freeze the aqueous layer. The organic layer was poured into 100 µL of 10% (w/v) HCl in MeOH, evaporated to dryness and reconstituted in 150 µL of 100 mM aqueous ammonium formate.	LC-MS/MS	[3]
-	0.2 ng/mL	62	[5-100 ng/mL]	**Method O** Samples were taken and either diluted 1:20 with water or used as-is depending upon the concentration. Internal standards were added, followed by 1 mL of 100 g/L trichloroacetic acid. These were vortexed, centrifuged and the supernatant decanted. To this, 0.5 mL of 5 mol/L KOH was added followed by 6 mL of methylene chloride. After vortexing and centrifugation, the aqueous layer was removed by a water aspirator. Sodium sulphate columns were washed with 4 mL of methylene chloride, followed by passing of the sample through the column. The sample was then taken to dryness and dissolved in a further 200 µL of methylene chloride. This was allowed to evaporate overnight. After the sample was dry, 20 µL of toluene was added and the sample analysed.	LC-MS/MS	[19]
50 ng/L	-	59 high 28 low (nominal cutoff concentration of ~25 µg/L)	[0-500 ng/mL]		LC-MS/MS	[20]
-	0.02 to 0.1ng/mL	101	690 (1990) ng/L	Method C, Table 1.1	LC-MS/MS	[21]
		310	160 (830) ng/L			
10 ng/mL	-	-	-	Method D, Table 1.1	GC/MS	[4]

-	20[a] mg/mL	364 African Americans	10.7 [6.19-15.4*] nmol/mL	Method E, Table 1.1	LC-MS/MS	[6]
		311 Native Hawaiians	9.85 [5.62-14.1*] nmol/mL			
		437 Caucasians	10.7 [5.54-17.2*] nmol/mL			
		453 Latinos	9.34 [4.49-14.8*] nmol/mL			
		674 Japanese Americans	7.78 [4.34-12.8*] nmol/mL			
-	-	33	(1655 ± 1469*** ng/mg)	**Method P** Urine samples were stored at -20 °C before being couriered to ABS laboratories. The sample was assayed using an LC-MS/MS array method developed and validated by ABS laboratories. All the analytical methods were validated, and the analysis of the samples from this study was performed in accordance with the FDA Guidance for Industry and the EMA Guideline on bioanalytical method validation.	LC-MS/MS	[22]
		16 (Abstinent at 4 weeks)	(889 ± 959*** ng/mg)			
		17 (Smoking at 4 weeks)	(1227 ± 679*** ng/mg)			
(0.05 ng/mL)	-	5 Non-smokers	(0.16◊ ng/mL) [0.06-0.26]	**Method Q** Urine samples were absorbed onto CE1001 ChemElute columns after prewashing with 2 mL of 0.5 M KOH, followed by two successive elutions with 4 mL of methylene chloride. The combined eluant was passed through a sodium sulfate column, and taken to dryness in a vacuum evaporator at ambient temperature using cryopumping. The residue was dissolved and transferred in a small volume of methylene chloride to a prewashed autosampler	LC-MS/MS	[23]
			(0.44◊*** ng/mg) [0.16-0.79]			

		19 Hookah smokers	(0.65$^{\lozenge}$ ng/mL) [0.30-1.08] (0.44$^{\lozenge***}$ ng/mg) [0.16-0.79]	Total NNAL was performed by adding internal standards to 5 mL of urine sample followed by vortexing. Sodium potassium phosphate buffer (0.5 mL of 2 M, pH 7) was added followed by the addition of 50 µL of 50 mg/mL glucuronidase dissolved in 0.1 M phosphate buffer. The samples were incubated for 20-24 h at 37 °C. Potassium carbonate (0.5 mL of 50% w/v) was added and vortexed with 8 mL of toluene/1-butanol (70:30). This was centrifuged, frozen in a dry ice-acetone bath and the organic layer discarded. The aqueous layer was washed with 5 mL of ethyl acetate/toluene (2:1) and centrifuged. The acidic layer was frozen in dry ice-acetone and made basic with 0.8 mL of 50% (w/v) K_2CO_3 and extracted by vortex mixing 5 min with 4 mL of ethyl acetate/toluene (1:2). This was centrifuged, frozen, and the organic layer collected. The organic layer was evaporated to dryness before adding hexanoic anhydride (50 µL) and 10 µL of 50 mg/mL DMAP in toluene. The resulting solution was added to 0.5 mL of 1 M H_2SO_4, and the organic layers were discarded after vortexing, centrifuging, and freezing. The acid layers were made basic with 0.5 mL of 50% (w/v) K_2CO_3 and then extracted with 4 mL of 10% ethyl acetate in pentane by vortex mixing, centrifugation, and freezing the aqueous layers. The organic layer was evaporated to dryness and reconstituted in 125 µL of 10% methanol containing 12 mM HCl.		
-	(10 ng/mL free cotinine) (100 ng/mL Total cotinine) (10 ng/mL Cotinine in plasma)	100	-	Method F, Table 1.1 **Method R** Samples were aliquoted into a 96-well sample plate. A protein precipitation extraction and filter procedure was used for sample purification.	LC-MS/MS	[7]
-	-	343 Smokers who developed lung cancer	(13.0$^{***\lozenge}$) [11.2-15.1*] nmol/mg	**Method S** An Oasis MAX cartridge was conditioned with 6 mL of MeOH and 6 mL of 2% aq. NH_4OH. The sample was applied, and washed with 6 mL MeOH and 6 mL 2% aq. NH_4OH. The cartridge was dried and washed with 6 mL of 2% formic acid. For collection of the fraction containing MHBMA, DHBMA, HPMA, HBMA, and HEMA, 5 mL of 30% MeOH in 2% aq. formic acid was added. The cartridge was then washed with 5 mL of 50% MeOH in 2%	LC-MS/MS	[24]

		392 Smokers who remained cancer-free	(7.10$^{***◊}$) [6.14-8.20*] nmol/mg	aq. formic acid. The fraction containing SPMA was collected by addition of 5 mL of 90% MeOH in 2% aq. formic acid. The two fractions containing the mercapturic acids were concentrated. The residues were transferred to 200 µL autosampler vials with two aliquots of 80/20:CH_3CN/MeOH and concentrated to dryness. This was re-dissolved in 50 µL of 93% 15 mM NH_4OAc/7% MeOH and analysed.		
0.04 pmol/mL	-	155 Shanghai cohort cases	(3033*** ± 2244 pmol/mg)	**Method T** Urine samples (4.5 mL) were adjusted to pH 6-8 and β-glucuronidase added. This was incubated overnight at 37°C. After adjusting the pH to 2 with 1N HCl, the sample was partitioned twice with equal volumes of CH_2Cl_2. The aqueous phase was taken, adjusted to pH 7 using 1N NaOH and applied to a Chem-Elute cartridge. The cartridge was eluted with 3 x 8 mL aliquots of CH_2Cl_2 and dried. The residue was dissolved in 50 µL of methanol, 200 µL of 0.1 M potassium phosphate buffer (pH 7), and 250 µL of H_2O into a filter unit autosampler vial. Five microliters of an aqueous solution of the collection markers consisting of 50 µg of 2-pyridylmethyl acetate and 50 µg of 3-acetylpyridine were added to each vial. The sample was injected on the HPLC system, monitored at 254 nm, and the fraction between the two marker compounds (11–16 min) was collected. This fraction was concentrated to dryness and transferred with two 75 µL portions of methanol, which was subsequently removed by vacuum. Five microliters of N,O-Bis(trimethylsilyl) trifluoroacetamide (BSTFA) and 2 ng of natriuretic peptide type A (NPPA) were added. The vial was capped, heated at 50 °C for 60 min, and mixed intermittently before injection for analysis.	Gas chromatography with nitrosamine selective detection	[25]
		152 Shanghai cohort controls	(1972*** ± 1573 pmol/mg)			
		91 Singapore cohort cases	(2873*** ± 1758 pmol/mg)			
		93 Singapore cohort controls	(2517*** ± 1825 pmol/mg)			
-	-	12	(10.3 ± 4.67 µmol/24h)	Method G, Table 1.1	GC-MS	[8]
-	-	27 people who stopped smoking	(13.1 ± 6.71 nmol/mL urine)		GC-MS	[18]

-	-	9 Non-smokers exposed to environmental tobacco smoke	30 ng/mL urine (170 ± 160 pmol/mL) (120*** ± 100 pmol/mg) [Not detected-550 pmol/mL urine]	**Method U** The pH of the urine was adjusted to 7, and the urine was extracted four times with ethyl acetate. The extracts were combined and internal standard added. The solution was dried and concentrated to produce fraction 1, which contained unconjugated NNAL. The aqueous layer was treated with 25,000 units of β-glucuronidase type IXA. Internal standard was added, the pH was adjusted to 2, and the mixture was extracted with ethyl acetate. The ethyl acetate extracts were discarded. The pH was adjusted to 7, and the resulting mixture was extracted four times with methylene chloride. The extracts were dried and concentrated to produce fraction 2, which contained NNAL released from NNAL glucuronide. Samples were run on both GC-MS/MS and GC-TEA (gas chromatography-thermal energy analyser).	GC-MS	[26]
0.25 ng/mL	-	12 Smokers who were orally administered 2mg d(2)-nicotine 30 minutes prior	(d(2) cotinine in plasma: 17.8 ± 6.6 ng/mL) (Cotinine in plasma: 440 ± 81 ng/mL)	Method H, Table 1.1	LC-MS/MS	[9]
		10 Past smokers who were orally administered 2mg d2-nicotine 30 minutes prior	(d(2) cotinine in plasma: 10.5 ± 6.4 ng/mL) (Cotinine in plasma: 0.6 ± 0.44 ng/mL)			

9 pmol/mL	-	476	(Smokers who developed lung cancer: 13.5◊*** [12.5-14.6◊***] nmol/mg) (Smokers who remained cancer-free: 7.58◊*** [7.00-8.20◊***] nmol/mg)	Method G, Table 1.1	GC-MS	[27]
-	-	100 controls that smoked at baseline	(217 ± 111 ng/mL)		GC	[28]
0.25 ng/mL	-	12 Smokers who were orally administered 2mg d(2)-nicotine 30 minutes prior	(d(2) cotinine in plasma: 17.8 ± 6.6 ng/mL) (Cotinine in plasma: 440 ± 81 ng/mL)	Method H, Table 1.1	LC-MS/MS	[9]
-	0.1 µg/L	38 non-smokers	0.35 µg/L	**Method V** Urine was diluted with water, followed by addition of cotinine-d3 as an internal standard. The separation was performed on a C18 column.	LC–MS/MS	[29]
		7 electronic cigarette users	1530 µg/L			
		22 tobacco smokers	1772 µg/L			
2.7. trans-3'-hydroxycotinine (3HC)						
0.36 (1.94)	-	94	-	Method A, Table 1.1	LC-MS/MS	[1]

(2.85)	9.5 ng/L	240 (40 Smokers & 200 non-smokers)	6695 ng/L	Method B, Table 1.1	LC-MS/MS	[2]
-	(10.0 ng/mL)	20 smokers	(Tobacco cigarette: 4765 ± 3163 [2525-5151]* µg/g**) (Electronic cigarette: 4472 ± 4315 (1590-5862)* µg/g**)	Method N, Table 2.6	LC-MS/MS	[3]
-	0.2 ng/mL	62	[5-100 ng/mL]	Method O, Table 2.6	LC-MS/MS	[19]
-	-	22 cigarette smokers	[10-10,000 ng/mL]	**Method W** Internal standards were added to 1 mL of urine. To this, 1 mL of 50% aqueous potassium carbonate was added, followed by 2.5 mL of methylene chloride/isopropanol (50:50). After vortexing and centrifugation, the solution was cooled on a dry ice-acetone bath. The organic layer was decanted and evaporated to dryness. Methylene chloride (1 mL) was added and evaporated, followed by addition of 200 µL of a solution containing 5% (w/v) tert-butyldimethylsilyl chloride and 5% imidazole in anhydrous N,N-dimethylacetamide. This was vortexed and heated to 80 °C for 1 hour. This was cooled and 0.4 mL of toluene-butanol (90:10) and 1 mL of water added. After vortexing and centrifugation, the aqueous layer was frozen. The organic layer was collected for analysis.	GC-MS, EI	[30]
-	0.02 to 0.1 ng/mL	101 310	2440 (7030) ng/L 610 (3050) ng/L	Method C, Table 1.1	LC-MS/MS	[21]

-	18[a] ng/mL	364 African Americans	22.7 [11.5-44.4*] nmol/mL	Method E, Table 1.1	LC-MS/MS	[6]
		311 Native Hawaiians	10.6 [5.61-19.9*] nmol/mL			
		437 Caucasians	16.9 [8.77-30.1*] nmol/mL			
		453 Latinos	16.4 [7.71 – 27.8*] nmol/mL			
		674 Japanese Americans	6.98 [2.37-15.6*] nmol/mL			
-	50 ng/mL (Free)	100	-	Method F, Table 1.1	LC-MS/MS	[7]
	100 ng/mL (Total)			Method R, Table 2.6		
1-2 ng/mL	-	12	(34.7 ± 21.8 µmol/24h)	Method G, Table 1.1	GC-MS	[8]
2.8. Cotinine-N-oxide (CNO)						
1.77 (1.5)	-	94	-	Method A, Table 1.1	LC-MS/MS	[1]

(0.6)	2	240 (40 smokers & 200 non-smokers)	<1000 ng/L	Method B, Table 1.1	LC-MS/MS	[2]
-	(5.0)	20 smokers	(Tobacco cigarette: 392 ± 238 [280-466]* µg/g**) (Electronic cigarette: 345 ± 276 [122-592]* µg/g**)	Method C, Table 1.1	LC-MS/MS	[3]
2.9. Norcotinine (NorCOT)						
0.48 (0.62)	-	94	-	Method A, Table 1.1	LC-MS/MS	[1]
(0.33)	1.1	240 (40 Smokers & 200 non-smokers)	<150 ng/L	Method B, Table 1.1	LC-MS/MS	[2]
-	(5)	20 smokers	(Tobacco cigarette: 136 ± 91 [85-153]* µg/g**) (Electronic cigarette: 101 ± 97 [30-146]* µg/g**)	Method N, Table 2.6	LC-MS/MS	[3]
2.10. Cotinine glucuronide						

-	-	363 African Americans	0.48 [0.28-0.61*] nmol/mL	Method I, Table 1.2	LC-MS/MS	[6]
		311 Native Hawaiians	0.55 [0.45-0.64*] nmol/mL			
		437 Caucasians	0.58 [0.48-0.69*] nmol/mL			
		452 Latinos	0.60 [0.49-0.69*] nmol/mL			
		674 Japanese Americans	0.51 [0.41-0.60*] nmol/mL			
-	-	12	(7.67 ± 5.76 µmol/24h)	Method G, Table 1.1	GC-MS	[8]
2.11. 3'-hydroxycotinine glucuronide						
-	-	363 African Americans	0.27 [0.21-0.35*] nmol/mL	Method I, Table 1.2	LC-MS/MS	[6]
		311 Native Hawaiians	0.19 [0.14-0.25*] nmol/mL			
		437 Caucasians	0.24 [0.18-0.30*] nmol/mL			

		453 Latinos	0.23 [0.17-0.29*] nmol/mL			
		666 Japanese Americans	0.18 [0.12-0.24*] nmol/mL			
-	-	12	(8.29 ± 7.16 µmol/24h)	Method G, Table 1.1	GC-MS	[8]

* Interquartile range ** Normalized per gram creatinine *** Normalized per milligram creatinine [a] 1 µL of urine ◊ 95% Confidence interval

Table 3. Literature values and analytical methods for the minor tobacco alkaloids

LOD Free (Total) [LOQ] (ng/L)	**N**	**Median (Mean) [Range]**	**Sample Preparation**	**Instrumental Analysis**	**Ref.**
3.1. Anatabine					
0.28 (0.45)	94	(15.2) [1.2-62.3] ng/mL	Method A, Table 1.1	LC-MS/MS	[1]
(0.12) [0.4]	240 (40 Smokers & 200 non-smokers)	-	Method B, Table 1.1	LC-MS/MS	[2]
3.2. Anabasine					

0.31 (0.6)	94	(6.12) [0.6-30.0] ng/mL	Method A, Table 1.1	LC-MS/MS	[1]
(0.15) [0.5]	240 (40 Smokers & 200 non-smokers)	-	Method B, Table 1.1	LC-MS/MS	[2]

Table 4. Literature values and analytical methods for Tobacco-Specific N-nitrosamines (TSNAs)

LOD (LLOQ)	N	Median (Mean) [Range]	Sample Preparation	Instrumental Analysis	Ref.
4.1. 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL)					
0.6 ng/L	80 (50 Smokers & 30 non-smokers)	-	**Method X** Chem Elut columns were pre-treated with 5 mL of 0.1M KOH followed by washing with 2 x 10 mL of methylene chloride. Samples (5 µL) were mixed with internal standard and 10 M NaOH (100 µL). The sample was applied to the column and eluted with 2 x 7 mL aliquots of methylene chloride. HCl (3 mL, 0.1 M) was added to the eluent and mixed. After centrifugation, the HCl layer was removed and 30 µL of NaOH added. Phosphate buffer (pH 6.5, 3 mL, 0.5 M) was added and the sample loaded onto a conditioned molecularly imprinted polymer (MIP) cartridge at a rate of 0.5 mL/min. After drying, the column was washed at 10 psi with 2 x 1 mL water, 1 mL of toluene and 1 mL of toluene-methylene chloride (9:1). The analytes were then eluted with 3 x 1 mL aliquots of methylene chloride which were dried and reconstituted in 40 µL of water. A 10 µL sample was injected into the LC-MS/MS.	LC-MS/MS	[31]
(0.25 pg/mL)	20 smokers	(Tobacco cigarette: 225 ± 165 [89-340*] ng/g**)	Method N, Table 2.6	LC-MS/MS	[3]

(0.25 pg/mL)	73 Non-smokers	0.87 (5.5) [≤ 153] pg/mL	Method Q, Table 2.6	LC-MS/MS	[32]
(0.25 pg/mL, 0.0012 pmol/mL)	13	Water pipe: (247) [127-374] pmol/24h Cigarette: (770) [176-946] pmol/24h	Method Q, Table 2.6	LC-MS/MS	[5]
0.4 (100) fmol/mL	2641	(1.65 ± 2.13 pmol/mL)	**Method Y** A ninety-six well plate method was used for sample enrichment. Urine samples (80 µL) were taken with internal standards and 50 µL of 0.5 N NaOH. For total NNAL, 40 µL of urine was taken with 50 µL of β-glucuronidase (1000 units) and internal standards. The free NNAL plates were incubated at 80 °C for 30 minutes and adjusted to pH 7.0 using 250 µL of 0.1 N HCl in PBS. The total NNAL plates were incubated at 37 °C overnight. On all samples, solid-phase extraction purification was carried out on Isolute SLE+ plates. The sample was transferred to the plates and rinsed with 50 µL of PBS before eluting with 3 x 0.6 mL of dichloromethane. The eluent was concentrated to dryness. HCl (1 N, 0.2 mL) was added followed by sonication for 15 minutes. A second solid-phase extraction was performed on Oasis MCX 96-well plates that were pre-conditioned with 1 mL of MeOH and 2 mL of water. Samples were added and washed with additional 50 µL of 1 N HCl. The plates were then washed successively with 250 µL of 1 N HCl, 250 µL of MeOH, and 250 µL of 90:5:5 H_2O/MeOH/ NH_4OH (v/v/v). All of these washings were discarded. The final elution with 250 µL of 35:60:5 H_2O/MeOH/NH_4OH (v/v/v) was collected in 96-well plates and concentrated to dryness overnight and reconstituted in 20 µL of 5 mM NH_4OAc.	LC-MS/MS	[33]
-	305	Electronic cigarette: (246) [222-269◊] ng/24h	Method J, Table 1.2	LC-MS/MS	[11]

	102	Tobacco Cigarette: (244) [202-286◊] ng/24h			
-	37	(264.68 ± 144.78 ng /g**)	Method F, Table 1.1	LC-MS/MS	[13]
(50 pg/mL)	100	-		LC-MS/MS	[7]
-	160	(Conventional cigarettes (6.0 mg tar/0.5 mg nicotine) 296.87 ± 183.78 ng/g**)		LC-MS/MS	[35]
		(Conventional cigarettes (10.6 mg tar/0.84 mg nicotine) 563.60 ± 358.60 ng/g**)			
		(Test cigarettes (6.0 mg tar) 339.44 ± 228.85 ng/g**)			
		(Test cigarettes (11 mg tar) 248.48 ± 207.86 ng/g**)			

-	343 Smokers who developed lung cancer	(0.30$^{***\lozenge}$) [0.28-0.34*] pmol/mg	Method S, Table 2.6	LC-MS/MS	[24]
	392 Smokers who remained cancer-free	(0.22$^{***\lozenge}$) [0.20-0.24*] pmol/mg			
-	17	(Whilst smoking: 2.70 ± 2.03 nmol/24h)		LC-MS/MS	[34]
		(After not smoking for 56 days: 0.132 ± 0.113 nmol/24h)			
0.1 pmol/mL	41 Smokers	(2.60 ± 1.30 pmol/mg)	Method T, Table 2.6	Gas chromatography with nitrosamine selective detection	[35]
	55 Snuff-dippers	(3.25 ± 1.77 pmol/mg)			
	18 Non-smokers exposed to environmental smoke	(0.042 ± 0.020 pmol/mg)			
0.04 pmol/mL	155 Shanghai cohort cases	0.22*** [0.02-4.55***] pmol/mg	Method T, Table 2.6	Gas chromatography with nitrosamine selective detection	[25]

	152 Shanghai cohort controls	0.15*** [0.01-2.23***] pmol/mg			
	91 Singapore cohort cases	0.89*** [0.10-3.51***] pmol/mg			
	93 Singapore cohort controls	0.59*** [0.11-4.56***] pmol/mg			
-	27 people who stopped smoking	(944 ± 517) [180-2080] pmol/24h	**Method Z** Urine samples were adjusted to pH 7 and extracted with EtOAc followed by drying with Na_2SO_4. To this, 25,000 units of β-glucuronidase type IX-A was added and the solution incubated overnight at 37 °C. The solution was extracted three times with equal volumes of dichloromethane. The combined extracts were dried with Na_2SO_4 and concentrated to dryness. The residue was taken up in 40 µL of methanol, and 1 mL of H_2O was added. The resulting solution was vortexed for 1 min and the pH adjusted to 7 with concentrated NaOH solution. This was further purified by HPLC and analysed.	GC-MS	[18]
1ng/sample	61	(0.96*** ± 1.15) [0.08-4.89***] pmol/mg		LC/GC-MS	[36]
-	11	[0.23-1.0 µg/24h]	**Method AA** Urine samples were adjusted to pH 7 before extraction with 3 volumes of ethyl acetate, dried with Na_2SO_4 and concentrated to dryness. The residue was dissolved in two 0.5 mL aliquots of H_2O, which were combined and set aside for subsequent HPLC purification and analysis for NNAL. β-Glucuronidase, 5000 units, was added and the sample incubated at 37 °C for 16 h with gentle shaking. After the incubation was complete, the pH was adjusted to 2 with concentrated HCl and the resulting solution was extracted 2 times with ethyl acetate. The pH of the aqueous layer was then adjusted to 7 with concentrated NaOH, and the solution extracted 3 times with dichloromethane. The combined dichloromethane layers were dried (Na_2SO_4) and evapourated to dryness by rotary evaporation. The residue was taken up in two 0.5 mL aliquots of H_2O which were combined for analysis.	LC-MS/MS	[37]
-	5 Non-smokers exposed to cigarette smoke	(After exposure: 33.9 ± 20.0 ng/24h, 127 ± 74 pmol /24h) (At baseline: 8.4 ± 11.2 ng/24h, 31 ± 41 pmol / 24h)		LC-MS/MS	[38]

0.04 pmol/mL	476	Smokers who developed lung cancer: 0.28◊*** [0.26-0.30◊***] pmol/mg Smokers who remained cancer-free: 0.20◊*** [0.18-0.22◊***] pmol/mg	Method T, Table 2.6	GC-MS	[27]
-	100 controls that smoked at baseline	(77.4 ± 39.3 fmol/mL)	**Method BB** Urine samples (1 mL) were taken and 3 mL of saline added. The pH was adjusted to 6-7 if necessary. Internal standards and β-glucuronidase (12,000 units in 0.4 mL water) were added. This was incubated overnight at 37 °C followed by acidification to pH 2-3 using 1 N HCl. A mixed mode cation exchange cartridge was conditioned with 5 mL methanol and 10 mL water. After loading, the sample was washed with 5 mL 1N HCl and 5 mL of 1% methanol in water. The r -1, t -2,3, c -4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (PheT) fraction was eluted with 5 mL of 40% methanol in water followed by further washing with 5 mL methanol and 5 mL of 90:5:5 water/methanol/NH_4OH. The NNAL-containing fraction was eluted from the column with 30:65:5 water/methanol/NH_4OH and both fractions concentrated to dryness. The residue from the PheT-containing fraction was transferred using three 80 µL aliquots of 5:1 water/methanol to a 0.45 µm nylon filter HPLC vial and 30 µg of 2,7-dihydroxynaphthalene added as an HPLC retention time marker. HPLC eluant was collected from 7.5 to 12 minutes. The HPLC fraction was concentrated to dryness and the residue collected in 3 x 60 µL aliquots of methanol. This was concentrated to dryness once more and 1,4-benzoMethod A, Table 1.1pyrene (BaP) (2 µL of 100 fmol/µL in acetonitrile) added as an injection standard and 12 µL of N,O-Bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane (BSTFA-TMCS). The mixture was then heated at 60 °C for 60 minutes with occasional mixing before analysis. The residue from the NNAL-containing fraction was reconstituted in 0.5 mL of 15 mmol/L ammonium acetate. The solution was extracted with three 1 mL portions of methylene chloride. The methylene chloride layers were and concentrated to dryness. The residue was transferred to 250 µL polypropylene autosampler vials with two 100 µL portions of methanol and concentrated to dryness again. The sample was reconstituted in 10 µL of 2% methanol in 15 mmol/L ammonium acetate.	LC-MS/MS	[28]
	100 lung cancer cases	(92.4 ± 40.7 fmol/mL)			
(3 fmol/mL)	16 plasma samples	(36 ± 21) [13-88] fmol/mL		LC-MS	[39]
-	47 Smokers – Pack tar 10mg/cig	(487◊) [424-549◊] ng/day	Method L, Table 1.2	LC-MS/MS	[15]

	46 Smokers – Pack tar 10mg/cig	(337◊) [282-391◊] ng/day			
	45 Smokers – Pack tar 6mg/cig	(308◊) [254-363◊] ng/day			
	44 Smokers – Pack tar 6mg/cig	(259◊) [209-309◊] ng/day			
	48 Smokers – Pack tar 1mg/cig	(212◊) [178-245◊] ng/day			
0.25 pg/mL	5 Non-smokers	(0.11◊) [0.00◊-0.24] pg/mL	Method Q, Table 2.6	LC-MS/MS	[23]
		(0.28◊***) [0.00-1.43◊***] pg/mg			
	19 Hookah smokers	[0.08-242.20◊***pg/mg]			
(0.500 pg/mL)	47 (Heated cigarette)	(127◊) [111-146◊] pg/day	Method K, Table 1.2	LC-MS/MS	[12]
	23 (Conventional cigarette)	(188◊) [156-227◊] pg/day			

(10 ng/mL)	50 Non-smokers	(12) [10-14◊] ng/day	Method L, Table 1.2	LC-MS/MS	[16]
	50 Smokers of 1mg tar cigarettes	(195) [164-226◊] ng/day			
	50 Smokers of 4mg tar cigarettes	(295) [247-343◊] ng/day			
	50 Smokers of 10mg tar cigarettes	(489) [426-551◊] ng/day			
-	49 Non-smokers	(10) [9-11◊] ng/day		LC-MS/MS	[17]
	42 Smokers of 4mg tar cigarettes	(213) [178-248◊] ng/day			
	48 Smokers of 9mg tar cigarettes	(176) [147-204◊] ng/day			
	49 Smokers of 14mg tar cigarettes	(252) [220-284◊] ng/day			
-	52 exclusive adult cigarette smokers	(332.7) [4- 1407] ng/g creatinine	Method Y	LC-MS/MS	[10]

	120 exclusive adult e-vapor users	(144.4) [1-1054] ng/g creatinine			
4.2. 4-[(methylnitrosamino)-1-(3-pyridyl)but-1-yl]-beta-O-D-glucosiduronic acid (NNAL-Gluc)					
-	27 people who stopped smoking	(2200 ± 1130) [280-4970] pmol/24h	Method Z, Table 4.1	GC-MS	[18]
1ng/sample	61	(2.81*** ± 2.92) [0.16-19.0***] pmol/mg		LC-MS/MS	[36]
-	11	[0.57-6.5 µg/24h]		LC-MS/MS	[37]
4 fmol/mL	9 Non-smokers exposed to environmental tobacco smoke	(0.059 ± 0.028) [0.005-0.11] pmol/mL (23 pg/mL urine)	Method AA, Table 4.1	GC-TEA	[47]
		(0.041*** ± 0.014 pmol/mg)			
4.3. N′-nitrosonornicotine (NNN)					
0.6 ng/L	80 (50 Smokers & 30 non-smokers)	-	Method X, Table 4.1	LC-MS/MS	[31]
4.4. 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)					
10 ng/L	80 (50 Smokers & 30 non-smokers)	-	Method X, Table 4.1	LC-MS/MS	[31]

4.5. N′-nitrosoanatabine (NAT)					
0.4 ng/L	80 (50 Smokers & 30 non-smokers)	-	Method X, Table 4.1	LC-MS/MS	[31]

* Interquartile range ** Normalized per gram creatinine *** Normalized per milligram creatinine ◊ 95% Confidence interval

Table 5. Literature values and analytical methods for VOC metabolites

LOD (LOQ) [LLOQ] (ng/mL)	N	Median (Mean) [Range]	Sample Preparation	Instrumental Analysis	Ref.
5.1. N-Acetyl-S-(2-carbamoylethyl)-L-cysteine					
2.2	347 Smokers	(196±180 ng/mL)	**Method CC** Urine samples (1.8 mL) were assayed diluted 1:10 with buffer (50 µL urine + 25 µL working mixed internal standard + 425 µL 15 mM ammonium acetate pH 6.8). This dilution yielded minimal suppression of ionization while maintaining sensitivity. Nevertheless, ion suppression was observed for some analytes in <1% of urine specimens. Further dilution of the specimen overcame this problem.	UHPLC-MS/MS	[40]
	1203 Non-smokers	(82±128 ng/mL)			
[0.5]	20 smokers	Tobacco cigarette: (254 ± 148) [119-395*] µg/g**	Method C, Table 1.1	LC-MS/MS	[3]
		Electronic cigarette: (163 ± 188) [66-211*] µg/g**			
(0.5)	13	Water pipe: (44.1) [77.7-121.8] µg/24h	**Method DD** Internal standards in 50 µL of water were added to 1 mL of urine samples followed by 0.9 mL of saturated potassium bromide and 0.1 mL saturated potassium hydrogen sulfate. These were extracted with a mixture of methylene chloride, isopropyl alcohol, and ethyl acetate (1:1:1, 3 mL). The extracts were evaporated using a stream of nitrogen at 60 °C, and the analytes converted to pentafluorobenzyl ester derivatives by treatment with 120 µL acetonitrile, 15 µL 10% w/v pentafluorobenzyl bromide in acetonitrile and 15 µL N,N-diisopropylethylamine at 60° C for 30 min. Following derivatization, the tubes were dried in a stream of nitrogen to remove excess derivatizing agent, cooled, and 0.25 mL saturated aqueous potassium dihydrogen phosphate	LC-MS/MS	[5]

		Cigarette: (84.4) [96.8-181.2] µg/24h	added. The analytes were extracted with 2 mL of 50:50 (v/v) pentane/methylene chloride. The extracts were evaporated to dryness using a stream of nitrogen, and the analytes dissolved in 0.15 mL of methanol for analysis.		
0.5-20	488 Third trimester pregnant women	33.3$^{\lozenge}$ [Maximum: 582] ng/mL	Method CC, Table 5.1	UHPLC-MS/MS	[41]
(3.2)	38 non-smokers	47.9 µg/g creatinine	**Method EE** 500 µL of 0.2 M formic acid and 20 µL of internal standard solution were added to 500-µL of each urine sample. The mixture was vortexed and filtered through a 0.45-µm cellulose membrane filter into an autosampler vial	LC-MS/MS	[29]
	7 electronic cigarette users	55.8 µg/g creatinine			
	22 tobacco smokers	114.6 µg/g creatinine			
8.7 (10.0)	25 Non-smokers	11.1 [5.4-84.3] µg/g**	**Method FF** Urine samples were split into 2 x 500 µL aliquots. For HEMA, CEMA, HMPMA, CMEMA, MMA, EMA, PHEMA 1, PHEMA 2, SPMA, and SBMA internal standards were added to the sample. The pH was reduced to approximately 1 by addition of 20 µL of 37 % HCl. After 5 min of shaking, ammonium formate buffer (pH 2.5) and 10 µL of 50% NaOH were added. Samples were centrifuged, and 50 µL of the supernatant was analyzed by a column-switching LC-MS/MS. For the analysis of 3-HPMA, 2-HPMA, AAMA, GAMA, 1-MHBMA, 2-MHBMA, DHBMA, and AMCC, urine samples were taken and internal standards added. The mixture was evapourated to dryness and reconstituted in 100 µL methanol. After thoroughly shaking for approximately 20 min, the supernatant was transferred into microvials and 2 µL was injected into the LC-MS/MS system.	LC-MS/MS	[42]
	25 Light smokers (<10/day)	64.7 [32.9-98.5] µg/g**			
	25 Smokers (>10/day)	68.4 [37.1-123] µg/g**			
5.2. N-Acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine					
9.4	347 Smokers	(57±57) ng/mL	Method CC, Table 5.1	UHPLC-MS/MS	[40]

	1203 Non-smokers	(28±36) ng/mL			
0.5-20	488 Third trimester pregnant women	<9.4* [Maximum: 203] ng/mL	Method CC, Table 5.1	UHPLC-MS/MS	[41]
(1.0)	38 non-smokers	2.5 µg/g creatinine	Method EE, Table 5.1	LC-MS/MS	[29]
	7 electronic cigarette users	3.9 µg/g creatinine			
	22 tobacco smokers	5.3 µg/g creatinine			
0.36 (1.0)	25 Non-smokers	3.9 [1.6-16.5] µg/g**	Method FF, Table 5.1	LC-MS/MS	[42]
	25 Light smokers (<10/day)	12.5 [6.3-25.0] µg/g**			
	25 Smokers (>10/day)	12.7 [6.4-19.6] µg/g**			
5.3. N-Acetyl-S-(N-methylcarbamoyl)-L-cysteine					
5.5	347 Smokers	(479±410) ng/mL	Method CC, Table 5.1	UHPLC-MS/MS	[40]

	1203 Non-smokers	(122±135) ng/mL			
0.5-20	488 Third trimester pregnant women	66.6◊ [Maximum: 2950] ng/mL	Method CC, Table 5.1	UHPLC-MS/MS	[41]
(2)	38 non-smokers	142 µg/g creatinine	Method EE, Table 5.1	LC-MS/MS	[29]
	7 electronic cigarette users	243 µg/g creatinine			
	22 tobacco smokers	405 µg/g creatinine			
0.93 (2.5)	25 Non-smokers	30.6 [12.8-219] µg/g**	Method FF, Table 5.1	LC-MS/MS	[42]
	25 Light smokers (<10/day)	139 [82.7-256.4] µg/g**			
	25 Smokers (>10/day)	146 [111-292] µg/g**			
5.4. 2-Aminothiazoline-4-carboxylic acid					
15	347 Smokers	(191±340 ng/mL)	Method CC, Table 5.1	UHPLC-MS/MS	[40]

	1203 Non-smokers	(167±245 ng/mL)			
5.5. 4-Aminobiphenyl					
1.00 pg/mL	47 (Heated cigarette)	(3.93◊◊) [3.55-4.35] ng/day	Method K, Table 1.2	LC-MS/MS	[12]
	23 (Conventional cigarette)	(13.3◊◊) [11.5-15.3] ng/day			
5.6. N-Acetyl-S-(benzyl)-L-cysteine					
0.5	347 Smokers	(16±29 ng/mL)	Method CC, Table 5.1	UHPLC-MS/MS	[40]
	1203 Non-smokers	(15±32 ng/mL)			
0.5-20	488 Third trimester pregnant women	5.62◊ [Maximum: 519] ng/mL		UHPLC-MS/MS	[41]
5.7. N-Acetyl-S-(n-propyl)-L-cysteine					
1.2	347 Smokers	(21±78 ng/mL)	Method CC, Table 5.1	UHPLC-MS/MS	[40]
	1203 Non-smokers	(16±29 ng/mL)			
0.5-20	488 Third trimester pregnant women	2.61◊ [Maximum: 4260] ng/mL	Method CC, Table 5.1	UHPLC-MS/MS	[41]

5.8. N-Acetyl-S-(3-hydroxypropyl)-L-cysteine					
1.3 ng/mL	347 Smokers	(1546±1643 ng/mL)	Method CC, Table 5.1	UHPLC-MS/MS	[40]
	1203 Non-smokers	(406±487 ng/mL)			
[1.0 ng/mL]	20 smokers	Tobacco cigarette: (937 ± 700) [433-1118*] µg/g**	Method C, Table 1.1	LC-MS/MS	[3]
		Electronic cigarette: (492 ± 455) [162-680*] µg/g**			
(1 ng/mL)	13	Water pipe: (152.6) [337.6-490.2] µg/24h	Method DD, Table 5.1	LC-MS/MS	[5]
		Cigarette: (388.6) [425.3-814] µg/24h			
(20 pmol/mL)	81	(6.60□) [5.80–7.48□] nmol/mg**	**Method GG** Urine samples (0.4 mL) were placed in 96-well plates and internal standards added. After vortexing and heating to 50 °C, sample clean-up was performed using a pre-conditioned (0.7 mL methanol and 0.7 mL 2% NH_4OH) solid-phase extraction 96-well plate. This was washed with 0.7 mL 2% NH_4OH and 0.7 mL methanol and dried. After washing with 0.7 mL of 2% aqueous formic acid, the eluants were collected using 0.7 mL of 30% methanol in 2% aqueous formic acid.	LC/MS-MS	[43]

4.5 (15) pmol/mL	2613	(4800±5358 pmol/mL)	The solvent was removed under vacuum and the sample re-dissolved in 10 µL of methanol. Ammonium acetate (40 µL of 15 mM) was added and the plate centrifuged. The sample was then injected for analysis.	LC/MS-MS	[44]
-	305	Electronic cigarette: (1820◊◊) [1680-1950] µg/24h	Method J, Table 1.2	LC/MS-MS	[11]
	102	Tobacco cigarette: (1710◊◊) [1510-1900] µg/24h			
0.5-20 ng/mL	488 Third trimester pregnant women	240◊ [Maximum: 14,400] ng/mL	Method CC, Table 5.1	UHPLC-MS/MS	[41]
12.6 (25.0) ng/mL	25 Non-smokers	62.5** [39.1-284] µg/g	Method FF, Table 5.1	LC-MS/MS	[42]
	25 Light smokers (<10/day)	366** [219-3175] µg/g			
	25 Smokers (>10/day)	372** [157-606] µg/g			
-	33	(2046 ± 1060) ng/mg***	Method P, Table 2.6	LC-MS/MS	[22]

	16 (Abstinent at 4 weeks)	(343 ± 178) ng/mg ***			
	17 (Smoking at 4 weeks)	(969 ± 807) ng/mg***			
13 ng/mL	2467 Non-smokers	1089** µg/g	Method CC, Table 5.1	UHPLC-MS/MS	[45]
	601 Smokers	219** µg/g			
0.049 (0.163) ng/mL	58 Non-smokers	(607.51) [0.00-3342.5] ng/mL	**Method HH** A 200 µL aliquot of urine was taken and internal standards added. Formic acid (10 µL, 100%) was added along with 740 µL of ammonium formate buffer (50 mmol/L, adjusted to pH 2.5 with formic acid). The samples were mixed, centrifuged and filtered through a 0.22 µm polyether sulfone membrane and 10 µL injected for analysis.	Column-switching LC-MS/MS	[46]
	246 Smokers	(1481.31) [103.25-8425] ng/mL			
10 ng/mL	47 (Heated cigarette)	(1656◊◊) [1488-1835] µg/day	Method K, Table 1.2	LC-MS/MS	[12]
	23 (Conventional cigarette)	(2071◊◊) [1789-2396] µg/day			
2 pmol/mL	5 Non-smokers	(616◊◊) [339-1121] pmol/mL	Method Q, Table 2.6	LC-MS/MS	[23]
		(1600◊◊) [553-5864] pmol/mg***			

	19 Hookah smokers	(714[◊◊]) [495-1031] pmol/mL			
		(1855[◊◊]) [300-8889] ng/mg[***]			
11 pg/injection (0.9 ng/mL)	35 Smokers	2900[***] pmol/mg	Method S, Table 2.6	LC-MS/MS	[47]
	21 Non-smokers	683[***] pmol/mg			
-	37	(1039.27 ± 648.48 µg/g[**])	Method F, Table 1.1	LC-MS/MS	[13]
[35 ng/mL]	100	-		LC-MS/MS	[7]
-	160	(Conventional cigarettes (6.0 mg tar/0.5 mg nicotine) 1519.51 ± 1179.13 µg/g[**])		LC-MS/MS	[14]
		(Conventional cigarettes (10.6 mg tar/0.84 mg nicotine) 1840.07 ± 1167.73 µg/g[**])			
		(Test cigarettes (6.0 mg tar) 543.01 ± 483.97 µg/g[**])			

		(Test cigarettes (11 mg tar) 444.03 ± 381.05 µg/g**)			
2.5 pmol/mL	343 Smokers who developed lung cancer	(8882◊◊) [7726-10210] pmol/mg***	Method S, Table 2.6	LC- MS/MS	[24]
	392 Smokers who remained cancer-free	(6712◊) [5845-7707] pmol/mg***			
2.3 pmol/mL	17	(Whilst smoking: 10020 ± 5150 nmol/24h)		LC- MS/MS	[34]
		(After not smoking for 56 days: 1500 ± 1005 nmol/24h)			
-	47 Smokers – Pack tar 10mg/cig	(2050◊◊) [1780-2320] jg/day	Method L, Table 1.2	LC-MS/MS	[15]
	46 Smokers – Pack tar 10mg/cig	(1991◊◊) [1654-2329] jg/day			
	45 Smokers – Pack tar 6mg/cig	(1451◊◊) [1194-1708] jg/day			

	44 Smokers – Pack tar 6mg/cig	(1119◊◊) [916-1323] jg/day			
	48 Smokers – Pack tar 1mg/cig	(988◊◊) [816-1160] jg/day			
(35 ng/mL)	50 Non-smokers	(214) [196-232◊◊] µg/day	**Method II** Urine samples (0.5 mL) were taken and internal standards added. After adding 0.5 mL of 20 mM $HCOONH_4$ (pH 2.5), the sample was subjected to solid phase extraction on a Waters Oasis® MCX cartridge (60 mg × 3 mL) which was pre-conditioned with 2 mL of methanol and 2 mL of 20 mM $HCOONH_4$ (pH 2.5) sequentially. The sample was eluted with 2 mL of 5% NH_4OH in methanol. The eluent was evaporated to dryness and reconstituted in 200 µL of methanol/water (70/30). Ten microliters of the extracted sample was injected for analysis.	LC-MS/MS	[16]
	50 Smokers of 1mg tar cigarettes	(934) [772-1096◊◊] µg/day			
	50 Smokers of 4mg tar cigarettes	(1354) [1136-1572◊◊] µg/day			
	50 Smokers of 10mg tar cigarettes	(2028) [1761-2296◊◊] µg/day			
-	49 Non-smokers	(983) [879-1088◊◊] µg/day		LC-MS/MS	[17]
	42 Smokers of 4mg tar cigarettes	(1973) [1739-2207◊◊] µg/day			
	48 Smokers of 9mg tar cigarettes	(1868) [1614-2121◊◊] µg/day			

	49 Smokers of 14mg tar cigarettes	(2494) [2252-2735[◊◊]] µg/day			
(50 ng/mL)	-	-	**Method JJ** Urine samples (1 mL) were acidified using 1 mL of trichloroacetic acid (0.02 M) and applied to a preconditioned (1 mL methanol and 1 mL 0.02 M chloroacetic acid) ENV+ cartridge. The cartridge was rinsed with 1 mL of 0.02 M trichloroacetic acid and eluted with 1 mL of basic methanol (methanol with 0.06 M aqueous ammonium carbamate, 1:1 v/v). Each step was carried out in a centrifuge. Internal standards and 100 µL of 20% trichloroacetic acid (w/v) were added, followed by evaporation in a vacuum centrifuge at 45°C for 40 minutes. The resulting solution was used for analysis.	LC-MS/MS	[48]
(0.2 ng/mL)	38 non-smokers	160.6 µg/g creatinine	Method EE, table 5.1	LC-MS/MS	[29]
	7 electronic cigarette users	222.1 µg/g creatinine			
	22 tobacco smokers	1301.2 µg/g creatinine			
2.21 (7) ng/mL	1 Non-smoker, 3 fortified samples and 5 smokers	-	SPE (column) Phenomenex Strata-X HPLC column Waters Xterra MS C18 50 × 2.1 mm, 2.5 µm	LC-MS/MS	[49]
(25 ng/mL)			SPE (column) Isolute ENV+ HPLC column Waters HILIC-Silica 150 × 2.1 mm, 3 µm		
(50 ng/mL)			SPE (column) Waters OASIS HPLC column Waters Acquity Phenyl 100 × 2.1 mm, 1.7 µm		
(35 ng/mL)			SPE (column) Waters OASIS HPLC column Thermo BioBasic AX 50 × 3 mm, 5 µm		
-	48 exclusive adult cigarette smokers	(1878.2) [145-8962] µg/g creatinine	Method GG	LC-MS/MS	[10]

	116 exclusive adult e-vapor users	(876.8) [49-4768] µg/g creatinine			
5.9. Methyl ethylmercapturic acid					
(2)	38 non-smokers	273 µg/g creatinine	Method EE, table 5.1	LC-MS/MS	[29]
	7 electronic cigarette users	233 µg/g creatinine			
	22 tobacco smokers	400 µg/g creatinine			
1.91 (5.0)	25 Non-smokers	201 [104-756] µg/g**	Method FF, Table 5.1	LC-MS/MS	[42]
	25 Light smokers (<10/day)	226 [125-408] µg/g**			
	25 Smokers (>10/day)	226 [121-299] µg/g**			
5.10. N-Acetyl-S-(2-carboxyethyl)-L-cysteine					
8	347 Smokers	(305±294 ng/mL)	Method CC, Table 5.1	UHPLC-MS/MS	[40]
	1203 Non-smokers	(128±119 ng/mL)			

0.5-20	488 Third trimester pregnant women	71.8◊ [Maximum: 2260] ng/mL		UHPLC-MS/MS	[41]
(0.9)	38 non-smokers	0.9 µg/g creatinine	Method EE, Table 5.1	LC-MS/MS	[29]
	7 electronic cigarette users	2.7 µg/g creatinine			
	22 tobacco smokers	163.1 µg/g creatinine			
0.08 (0.25)	25 Non-smokers	0.46 [0.23-8.6] µg/g**	Method FF, Table 5.1	LC-MS/MS	[42]
	25 Light smokers (<10/day)	53.6 [33.6-138.4] µg/g**			
	25 Smokers (>10/day)	72.5 [28.6-140.4] µg/g**			
8	2467 Non-smokers	78.8 µg/g**	Method CC, Table 5.1	UHPLC-MS/MS	[45]
	601 Smokers	203 µg/g**			
0.015 (0.05)	58 Non-smokers	(3.47) [0.00-12.4] ng/mL	Method HH, Table 5.8	Column-switching LC-MS/MS	[46]

	246 Smokers	(50.69) [1.58-198.69] ng/mL			
5.11. N-Acetyl-S-(2-cyanoethyl)-L-cysteine					
0.5	347 Smokers	(187±181 ng/mL)	Method CC, Table 5.1	UHPLC-MS/MS	[40]
	1203 Non-smokers	(4.60±35 ng/mL)			
[0.5]	20 smokers	Tobacco cigarette: (212 ± 178) [103-311*] µg/g**	Method DD, Table 5.1	LC-MS/MS	[3]
		Electronic cigarette: (51 ± 58) [20-48*] µg/g**			
(0.5)	13	Water pipe: (18.7) [8.8-27.4] µg/24h		LC-MS/MS	[5]
		Cigarette: (90.1) [43-133.1] µg/24h			

0.5-20	488 Third trimester pregnant women	0.642◊ [Maximum 812] ng/mL	Method CC, Table 5.1	UHPLC-MS/MS	[41]
5.12. N-Acetyl-S-(2-hydroxyethyl)-L-cysteine					
0.6 ng/mL	347 Smokers	(1.90±3.70 ng/mL)	Method CC, Table 5.1	UHPLC-MS/MS	[40]
	1203 Non-smokers	(0.66±1.16 ng/mL)			
[0.5 ng/mL]	20 smokers	Tobacco cigarette: (3821 ± 3120) [1790-5050*] ng/g**	Method DD, Table 5.1	LC-MS/MS	[3]
		Electronic cigarette: (1400 ± 864) [770-1790*] ng/g**			
(0.2 ng/mL)	13	Water pipe: (2.39) [2.48-4.88] µg/24h		LC-MS/MS	[5]
		Cigarette: (8.58) [2.97-11.55] µg/24h			

0.5-20 ng/mL	488 Third trimester pregnant women	0.963[◊] [Maximum: 33.4] ng/mL	Method CC, Table 5.1	UHPLC-MS/MS	[41]
(0.3)	38 non-smokers	1.3 µg/g creatinine	Method EE, Table 5.1	LC-MS/MS	[29]
	7 electronic cigarette users	2.0 µg/g creatinine			
	22 tobacco smokers	3.2 µg/g creatinine			
0.06 (0.2) ng/mL	25 Non-smokers	1.1 [0.11-38.3] µg/g[**]	Method FF, Table 5.1	LC-MS/MS	[42]
	25 Light smokers (<10/day)	2.3 [1.3-6.2] µg/g[**]			
	25 Smokers (>10/day)	2.0 [1.1-4.2] µg/g[**]			
0.20 pmol/mL	343 Smokers who developed lung cancer	(18.2[*** ◊◊]) [15.9-20.7[a]] pmol/mg	Method S, Table 2.6	LC- MS/MS	[24]
	392 Smokers who remained cancer-free	(13.6[*** ◊◊]) [12.0-15.5[a]] pmol/mg			

0.24 pmol/mL	17	Whilst smoking: (102 ± 47.1 nmol/24h) After not smoking for 56 days: (19.2 ± 13.6) nmol/24h		LC- MS/MS	[34]
5.13. N-Acetyl-S-(1,2-dichlorovinyl)-L-cysteine (1,2 DCVMA) and N-Acetyl-S-(2,2-dichlorovinyl)-L-cysteine (2,2 DCVMA)					
12.6 (1,2 DCVMA) 6.5 (2,2 DCVMA)	347 Smokers 1203 Non-smokers	(<LOD) (< LOD)	Method CC, Table 5.1	UHPLC-MS/MS	[40]
5.14. N-Acetyl-S-(3,4-dihydroxybutyl)-L-cysteine					
5 ng/mL	347 Smokers 1203 Non-smokers	(440±311 ng/mL) (331±279 ng/mL)	Method CC, Table 5.1	UHPLC-MS/MS	[40]
0.5-20 ng/mL	488 Third trimester pregnant women	281[◊] [Maximum: 1730] ng/mL	Method CC, Table 5.1	UHPLC-MS/MS	[41]
4.6 (12.5) ng/mL	25 Non-smokers	76.2 [47.4-349] µg/g[**]	Method FF, Table 5.1	LC-MS/MS	[42]

	25 Light smokers (<10/day)	112 [65.5-243] µg/g**			
	25 Smokers (>10/day)	122 [52.9-244] µg/g**			
0.053 (0.177) ng/mL	58 Non-smokers	(184.61) [0.00-567.5] ng/mL	Method HH, Table 5.8	Column-switching LC-MS/MS	[46]
	246 Smokers	(230.47) [0.00-1345.0] ng/mL			
(1.0)	38 non-smokers	247.5 µg/g creatinine	Method EE, Table 5.1	LC-MS/MS	[29]
	7 electronic cigarette users	263.8 µg/g creatinine			
	22 tobacco smokers	479.1 µg/g creatinine			
12 pmol/mL	17	Whilst smoking: (1038 ± 514 nmol/24h)	Method S, Table 2.6	LC-MS/MS	[34]
		After not smoking for 56 days: (662 ± 248 nmol/24h)			

5.15. N-Acetyl-S-(1-hydroxymethyl-2-propenyl)-L-cysteine					
0.7 ng/mL	347 Smokers	(<LOD)	Method CC, Table 5.1	UHPLC-MS/MS	[40]
	1203 Non-smokers	(<LOD)			
0.09 (0.12) ng/mL	25 Non-smokers	<LOD [<LOD-0.15] µg/g**	Method FF, Table 5.1	LC-MS/MS	[42]
	25 Light smokers (<10/day)	<LOD [<LOD-0.52] µg/g**			
	25 Smokers (>10/day)	0.28 [<LOD-0.66] µg/g**			
10.0 pg/mL	47 (Heated cigarette)	(1206◊◊) [1008-1443] ng/day	Method K, Table 1.2	LC-MS/MS	[12]
	23 (Conventional cigarette)	(3247◊◊) [2526-4173] ng/day			
-	37	(2.34±1.38** µg/g)	Method F, Table 1.1	LC-MS/MS	[13]
(0.1 ng/mL)	160	Conventional cigarettes (6.0 mg tar/0.5 mg nicotine) (3.91 ± 5.34 µg/g**)		LC-MS/MS	[14]

		Conventional cigarettes (10.6 mg tar/0.84 mg nicotine) (2.06 ± 2.04 µg/g**)			
		Test cigarettes (6.0 mg tar) (1.26 ± 1.37 µg/g**)			
		Test cigarettes (11 mg tar) (0.78 ± 0.56 µg/g**)			
3.0 pmol/mL	343 Smokers who developed lung cancer	(11.3◊◊) [9.8-13.1] pmol/mg***	Method S, Table 2.6	LC- MS/MS	[24]
	392 Smokers who remained cancer-free	(8.3◊◊) [7.2-9.7] pmol/mg***			
3.2 pmol/mL	17	Whilst smoking: (66.1 ± 69.4 nmol/24h)		LC- MS/MS	[34]
		After not smoking for 56 days: (3.66 ± 2.41 nmol/24h)			

5.16. N-Acetyl-S-(2-hydroxy-3-butenyl)-L-cysteine					
0.7	347 Smokers	(1.80±2.10 ng/mL)	Method CC, Table 5.1	UHPLC-MS/MS	[40]
	1203 Non-smokers	(<LOD)			
[0.1]	20 smokers	Tobacco cigarette: (1912 ± 1283) [830-2860*] ng/g**	Method C, Table 1.1	LC-MS/MS	[3]
		Electronic cigarette: (300 ± 478) [0-430*] ng/g**			
(0.2)	13	Water pipe: (0.28) [0.27-0.55] µg/24h	Method DD, Table 5.1	LC-MS/MS	[5]
		Cigarette: (0.76) [0.96-1.72] µg/24h			
0.03 (0.13)	25 Non-smokers	<LOD [<LOD-0.11] µg/g**	Method FF, Table 5.1	LC-MS/MS	[42]

	25 Light smokers (<10/day)	0.53 [<LOD-0.96] µg/g**			
	25 Smokers (>10/day)	0.80 [0.095-1.30] µg/g**			
5.17. N-Acetyl-S-(4-hydroxy-2-buten-1-yl)-L-cysteine					
0.6 ng/mL	347 Smokers	(36±34 ng/mL)	Method CC, Table 5.1	UHPLC-MS/MS	[40]
	1203 Non-smokers	(6.40±10 ng/mL)			
0.5-20 ng/mL	488 Third trimester pregnant women	6.9◊ng/mL		UHPLC-MS/MS	[41]
0.2 pmol/mL	343 Smokers who developed lung cancer	(7915◊◊ [6906-9071] pmol/mg***	Method S, Table 2.6	LC- MS/MS	[24]
	392 Smokers who remained cancer-free	5749◊◊ [5022-6581] pmol/mg***			
0.21 pmol/mL	17	Whilst smoking: (1965 ± 1001 nmol/24h)		LC- MS/MS	[34]

		After not smoking for 56 days: (273 ± 153nmol/24h)			
5.18. N-Acetyl-S-(2,4-dimethylphenyl)-L-cysteine + N-Acetyl-S-(2,5-dimethylphenyl)-L-cysteine + N-Acetyl-S-(3,4-dimethylphenyl)-L-cysteine					
0.5	347 Smokers	(<LOD)	Method CC, Table 5.1	UHPLC-MS/MS	[40]
	1203 Non-smokers	(<LOD)			
5.19. 2-Methylhippuric acid					
5	347 Smokers	(144±265 ng/mL)	Method CC, Table 5.1	UHPLC-MS/MS	[40]
	1203 Non-smokers	(71±277 ng/mL)			
0.5-20	488 Third trimester pregnant women	21.2◊ [Maximum 3810] ng/mL		UHPLC-MS/MS	[41]
5.20. 3-Methylhippuric acid and 4- Methylhippuric acid					
8	347 Smokers	(1020±1379 ng/mL)	Method CC, Table 5.1	UHPLC-MS/MS	[40]
	1203 Non-smokers	579±3692 ng/mL)			

0.5-20	488 Third trimester pregnant women	150[◊] [Maximum: 17,800] ng/mL		UHPLC-MS/MS	[41]
5.21. N-Acetyl-S-(2-hydroxyethyl)-L-cysteine					
0.6 ng/mL	347 Smokers	(1.90±3.70 ng/mL)	Method CC, Table 5.1	UHPLC-MS/MS	[40]
	1203 Non-smokers	(0.66±1.16 ng/mL)			
[0.5 ng/mL]	20 smokers	Tobacco cigarette: (3821 ± 3120) [1790-5050[*]] ng/g[**]	Method C, Table 1.1	LC-MS/MS	[3]
		Electronic cigarette: (1400 ± 864) [770-1790[*]] ng/g[**]			
(0.2 ng/mL)	13	Water pipe: (2.39) [2.48-4.88] µg/24h	Method DD, Table 5.1	LC-MS/MS	[5]
		Cigarette: (8.58) [2.97-11.55] µg/24h			

0.5-20 ng/mL	488 Third trimester pregnant women	0.963[◊] [Maximum: 33.4] nm/mL	Method CC, Table 5.1	UHPLC-MS/MS	[41]
0.06 (0.2) ng/mL	25 Non-smokers	1.1 [0.11-38.3] µg/g[**]	Method FF, Table 5.1	LC-MS/MS	[42]
	25 Light smokers (<10/day)	2.3 [1.3-6.2] µg/g[**]			
	25 Smokers (>10/day)	2.0 [1.1-4.2] µg/g[**]			
0.20 pmol/mL	343 Smokers who developed lung cancer	(18.2[◊]) [15.9-20.7] pmol/mg[***]	Method S, Table 2.6	LC- MS/MS	[24]
	392 Smokers who remained cancer-free	(13.6[◊]) [12.0-15.5] pmol/mg[***]			
0.24 pmol/mL	17	Whilst smoking: (102 ± 47.1 nmol/24h)		LC- MS/MS	[34]
		After not smoking for 56 days: (19.2 ± 13.6 nmol/24h)			

5.22. N-Acetyl-S-(2-hydroxypropyl)-L-cysteine					
1.3	347 Smokers	(185±235 ng/mL)	Method CC, Table 5.1	UHPLC-MS/MS	[40]
	1203 Non-smokers	(81±118 ng/mL)			
[1.0]	20 smokers	Tobacco cigarette: (45 ± 24) [23-55*] µg/g**	Method C, Table 1.1	LC-MS/MS	[3]
		Electronic cigarette: (24 ± 18) [15-28*] µg/g**			
(1.0) [1.0]	13	Water pipe: (80.3) [28.7-109] µg/24h	Method DD, Table 5.1	LC-MS/MS	[5]
		Cigarette: (148.1) [50.2-198.2] µg/24h			
0.5-20	488 Third trimester pregnant women	44.6◊ [Maximum 2660] ng/mL	Method CC, Table 5.1	UHPLC-MS/MS	[41]

(0.5)	38 non-smokers	8.8 µg/g creatinine	Method EE, Table 5.1	LC-MS/MS	[29]
	7 electronic cigarette users	9.8 µg/g creatinine			
	22 tobacco smokers	28.4 µg/g creatinine			
1.3 (2.5)	25 Non-smokers	3.2 [0.93-17.8] µg/g**	Method FF, Table 5.1	LC-MS/MS	[42]
	25 Light smokers (<10/day)	13.3 [6.2-26.9] µg/g**			
	25 Smokers (>10/day)	19.0 [6.9-37.3] µg/g**			
5.23. Propylene glycol					
-	305	Electronic cigarette: (6.3) [5.4-7.2◊◊] mg/24h	Method J, Table 1.2	LC-MS/MS	[11]
	102	Tobacco Cigarette: (7.4) [5.0-9.8◊◊] mg/24h			

5.24. N-Acetyl-S-(3-hydroxypropyl-1-methyl)-L-cysteine					
2	347 Smokers	(1992±2009 ng/mL)	Method CC, Table 5.1	UHPLC-MS/MS	[40]
	1203 Non-smokers	(429±478 ng/mL)			
[1.0]	20 smokers	Tobacco cigarette: (1857 ± 1379) [936-2384*] µg/g**	Method C, Table 1.1	LC-MS/MS	[3]
		Electronic cigarette: (632 ± 387) [312-856*] µg/g**			
0.5-20	488 Third trimester pregnant women	342◊ [Maximum 17,700] ng/mL	Method CC, Table 5.1	UHPLC-MS/MS	[41]
5.25. 3-hydroxy-1-methylpropyl mercapturic acid					
(12 pmol/mL)	81	(4.62□) [4.02–5.30◊◊] pmol/mL	Method GG, Table 5.8	LC-MS/MS	[43]
3.5 (12) pmol/mL	2613	(3302 ± 3341 pmol/mL)		LC-MS/MS	[44]

0.49 (5.0) ng/mL	25 Non-smokers	18.9 [9.7-64.4] µg/g**	Method FF, Table 5.1	LC-MS/MS	[42]
	25 Light smokers (<10/day)	95.9 [55.3-268.0] µg/g**			
	25 Smokers (>10/day)	121.7 [57.0-220.0] µg/g**			
0.032 (0.107) ng/mL	58 Non-smokers	(191.9) [0.00-785.0] ng/mL	Method HH, Table 5.8	Column-switching LC-MS/MS	[46]
	246 Smokers	(1287.83) [0.00-6975.0] ng/mL			
(2)	38 non-smokers	48 µg/g creatinine	Method EE, Table 5.1	LC-MS/MS	[29]
	7 electronic cigarette users	38 µg/g creatinine			
	22 tobacco smokers	268 µg/g creatinine			
20 ng/mL	47 (Heated cigarette)	(856◊◊) [780-939] µg/day	Method K, Table 1.2	LC-MS/MS	[12]

	23 (Conventional cigarette)	(1656[◊◊]) [1454-1886] µg/day			
5.26. N-acetyl-S-methyl-L-cysteine					
(0.09)	38 non-smokers	2.57 µg/g creatinine	Method EE, Table 5.1	LC-MS/MS	[29]
	7 electronic cigarette users	4.70 µg/g creatinine			
	22 tobacco smokers	2.64 µg/g creatinine			
0.88 [2.5] ng/mL	25 Non-smokers	4.1 [1.7-16.9] µg/g[**]	Method FF, Table 5.1	LC-MS/MS	[42]
	25 Light smokers (<10/day)	3.6 [1.6-12.4] µg/g[**]			
	25 Smokers (>10/day)	3.4 [1.1-10.2] µg/g[**]			
5.27. N-acetyl-S-ethyl-L-cysteine					
(0.01)	38 non-smokers	0.03 µg/g creatinine	Method EE, Table 5.1	LC-MS/MS	[29]
	7 electronic cigarette users	0.03 µg/g creatinine			

	22 tobacco smokers	0.06 µg/g creatinine			
0.008 (0.003) ng/mL	25 Non-smokers	0.018 [LOQ-0.70] µg/g**	Method FF, Table 5.1	LC-MS/MS	[42]
	25 Light smokers (<10/day)	0.026. [LOQ-0.16] µg/g**			
	25 Smokers (>10/day)	0.028 [LOQ-0.049] µg/g **			
5.28. N-acetyl-S-benzyl-cysteine					
(0.02)	38 non-smokers	2.22 µg/g creatinine	Method EE, Table 5.1	LC-MS/MS	[29]
	7 electronic cigarette users	1.42 µg/g creatinine			
	22 tobacco smokers	1.47 µg/g creatinine			
0.027 (0.1) ng/mL	25 Non-smokers	3.1 [0.58-19.6] µg/g**	Method FF, Table 5.1	LC-MS/MS	[42]
	25 Light smokers (<10/day)	3.6 [0.79-9.9] µg/g**			

	25 Smokers (>10/day)	2.3 [1.36-4.39] µg/g**			
5.29. Mandelic acid					
12	347 Smokers	(420±357 ng/mL)	Method CC, Table 5.1	UHPLC-MS/MS	[40]
	1203 Non-smokers	(198±226 ng/mL)			
0.5-20	488 Third trimester pregnant women	208◊ [Maximum: 2190] ng/mL		UHPLC-MS/MS	[41]
5.30. Phenylglyoxylic acid					
12	347 Smokers	(330±425 ng/mL)	Method CC, Table 5.1	UHPLC-MS/MS	[40]
	1203 Non-smokers	(169±224 ng/mL)			
0.5-20	488 Third trimester pregnant women	208◊ [Maximum: 2130] ng/mL		UHPLC-MS/MS	[41]
5.31. N-Acetyl-S-(1-phenyl-2-hydroxyethyl)-L-cysteine					
0.7	347 Smokers	(<LOD)	Method CC, Table 5.1	UHPLC-MS/MS	[40]
	1203 Non-smokers	(<LOD)			

0.5-20	488 Third trimester pregnant women	<LOD [Maximum: 9.84 ng/mL]		UHPLC-MS/MS	[41]
0.03 (0.1) (PHEMA 1)	25 Non-smokers	<LOD [LOQ-0.11 µg/g**]	Method FF, Table 5.1	LC-MS/MS	[42]
	25 Light smokers (<10/day)	<LOD [LOQ-0.38 µg/g**]			
	25 Smokers (>10/day)	0.41 [LOQ-0.98] µg/g**			
0.13 (0.4) (PHEMA 2)	25 Non-smokers	<LOD [LOQ-0.71 µg/g**]		LC-MS/MS	[42]
	25 Light smokers (<10/day)	<LOD [0.763 µg/g**]			
	25 Smokers (>10/day)	0.42 [LOQ-0.66] µg/g**			
5.32. trans, trans-Muconic acid					
20	347 Smokers	(473±410 ng/mL)	Method CC, Table 5.1	UHPLC-MS/MS	[40]
	1203 Non-smokers	(358±291 ng/mL)			

0.5-20	488 Third trimester pregnant women	245[◊] [Maximum: 4090] ng/mL		UHPLC-MS/MS	[41]
10.0	47 (Heated cigarette)	(53.0[◊◊]) [47.7-58.9] µg/day	Method K, Table 1.2	LC-MS/MS	[12]
	23 (Conventional cigarette)	(76.7[◊◊]) [66.1-89.1] µg/day			
5.33. N-Acetyl-S-(phenyl)-L-cysteine					
0.3 ng/mL	347 Smokers	(0.92±2.11 ng/mL)	Method CC, Table 5.1	UHPLC-MS/MS	[40]
	1203 Non-smokers	(0.60±0.40 ng/mL)			
[0.1 ng/mL]	20 smokers	Tobacco cigarette: (792 ± 674) [249-1203[*]] ng/g[**]	Method C, Table 1.1	LC-MS/MS	[3]
		Electronic cigarette: (159 ± 193) [37-193[*]] ng/g[**]			

(0.1 ng/mL)	13	Water pipe: (5.67) [0.49-6.16] µg/24h Cigarette: (0.75) [0.35-1.09] µg/24h	Method DD, Table 5.1	LC-MS/MS	[5]
(0.15 pmol/mL)	81	(2.36□) [1.88–2.98◊◊] pmol/mg***	Method GG, Table 5.8	LC/MS-MS	[43]
(0.1 pmol/mL)	329 African Americans (GSTT1 deletion)	(3.71□ pmol/mL)		LC/MS-MS	[50]
	353 African Americans (GSTM1 deletion)	(3.73□ pmol/mL)			
	265 Native Hawaiians (GSTT1 deletion)	(2.43□ pmol/mL)			
	290 Native Hawaiians (GSTM1 deletion)	(2.46□ pmol/mL)			

	404 Caucasians (GSTT1 deletion)	(2.69□ pmol/mL)			
	417 Caucasians (GSTM1 deletion)	(2.69□ pmol/mL)			
	415 Latinos (GSTT1 deletion)	(2.88□ pmol/mL)			
	430 Latinos (GSTM1 deletion)	(2.88□ pmol/mL)			
	562 Japanese Americans (GSTT1 deletion)	(1.66□ pmol/mL)			
	597 Japanese Americans (GSTM1 deletion)	(1.66□ pmol/mL)			
-	305	Electronic cigarette usage: (3820*◊◊) [3450-4190*] mg/24h	Method J, Table 1.2	LC-MS/MS	[11]

	102	Regular cigarette usage: (3660*◊◊) [3090-4220*] mg/24h			
0.5-20 ng/mL	488 Third trimester pregnant women	0.642◊ [Maximum: 12.3] ng/mL	Method CC, Table 5.1	UHPLC-MS/MS	[41]
0.005 (0.02) ng/mL	25 Non-smokers	0.018 [LOQ-0.097] µg/g**	Method FF, Table 5.1	LC-MS/MS	[42]
	25 Light smokers (<10/day)	0.75 [0.21-1.6] µg/g**			
	25 Smokers (>10/day)	1.1 [0.20-3.5] µg/g**			
0.013 (0.043) ng/mL	58 Non-smokers	(0.36) [0.00-0.95] ng/mL	Method HH, Table 5.8	Column-switching LC-MS/MS	[46]
	246 Smokers	(0.2) [0.00-4.17] ng/mL			
(0.01)	38 non-smokers	0.06 µg/g creatinine	Method EE, Table 5.1	LC-MS/MS	[29]

	7 electronic cigarette users	0.16 µg/g creatinine			
	22 tobacco smokers	0.48 µg/g creatinine			
0.1 ng/mL	47 (Heated cigarette)	(1.84◊◊) [1.63-2.06] µg/day	Method K, Table 1.2	LC-MS/MS	[12]
	23 (Conventional cigarette)	(3.18◊◊) [2.70-3.74] µg/day			
-	37	(3.75 ± 2.99 µg/g**)	Method F, Table 1.1	LC-MS/MS	[13]
[20 pg/mL]	100	-		LC-MS/MS	[7]
-	160	Conventional cigarettes (6.0 mg tar/0.5 mg nicotine) (4.98 ± 4.54 µg/g**)		LC-MS/MS	[14]
		Conventional cigarettes (10.6 mg tar/0.84 mg nicotine) (4.55 ± 1.80 µg/g**)			

		Test cigarettes (6.0 mg tar) (1.40 ± 1.80 µg/g**)			
		Test cigarettes (11 mg tar) (0.95 ± 0.55 µg/g**)			
0.025 pmol/mL	343 Smokers who developed lung cancer	(2.96◊) [2.60-3.36] pmol/mg***	Method S, Table 2.6	LC-MS/MS	[24]
	392 Smokers who remained cancer-free	(2.46◊◊) [2.16-2.80] pmol/mg***			
0.013 pmol/mL	17	Whilst smoking: (3.20 ± 3.80 nmol/24h)		LC-MS/MS	[34]
		After not smoking for 56 days: (0.214 ± 0.214 nmol/24h)			
5.34. 2-Thioxothiazolidine-4-carboxylic acid					
3.5	347 Smokers	(37.4±170 ng/mL)	Method CC, Table 5.1	UHPLC-MS/MS	[40]
	1203 Non-smokers	(27.3±83 ng/mL)			

0.5-20	488 Third trimester pregnant women	5.91◊ [Maximum: 483] ng/mL		UHPLC-MS/MS	[41]
5.35. Thiocyanate					
0.5-20 ng/mL	488 Third trimester pregnant women	832◊ [Maximum: 19,100] ng/mL	Method CC, Table 5.1	UHPLC-MS/MS	[41]
5.0 µg/L	2818	-	**Method KK** Urine samples were thawed and mixed to suspend any particulate material. Urine (100 µL) was transferred to an autosampler vial and diluted with 900 µL of DI water containing internal standards and queued for injection into the IC–MSMS system.	IC-MS/MS	[51]
5.36. N-Acetyl-S-(trichlorovinyl)-L-cysteine					
3	347 Smokers	(<LOD)	Method CC, Table 5.1	UHPLC-MS/MS	[40]
	1203 Non-smokers	(<LOD)			

***** Interquartile range ****** Normalized per gram creatinine ******* Normalized per milligram creatinine ◊ 50th percentile ◊◊ 95% Confidence interval □ Geometric mean

Table 6 - Literature values and analytical methods for PAHs

LOD (LLOQ) (ng/mL)	N	Median (Mean) [Range]	Sample Preparation	Instrumental Analysis	Ref.
6.1. 1-Hydroxyfluorene					
(0.025)	20 Smokers	Tobacco Cigarette: (1414 ± 864) [674-2052*] ng/g**	Method C, Table 1.1	LC-MS/MS	[3]
		Electronic cigarette: (441 ± 492) [44-768*] ng/g**			
(0.025)	13	Water pipe: (194) [41-235] pmol/24h	Method DD, Table 5.1	LC-MS/MS	[5]
		Cigarette: (327) [180-507] pmol/24h			

(0.100)	21 Smokers	(0.96) [<LOQ-4.57] ng/mL (5.41) [1.54-14.7] pmol/mg***	**Method LL** Internal standards were added to urine samples (2.7 mL) buffered to pH 7 with 0.3 mL of 1 M phosphate buffer and incubated overnight at 37 °C with beta-glucuronidase (3000 units, Sigma) and sulfatase (0.6 unit, Sigma). The samples were extracted with a 90:10 mixture (v/v) of pentane/ethyl acetate (4 mL) by vortex mixing, and the phases separated by centrifuging and freezing the aqueous layers in a dry ice/acetone bath. The organic phases were collected and 150 µg of gallic acid in 30 µL of methanol added. The solvent was removed using a centrifugal vacuum evaporator at ambient temperature. To the residues were added pentafluorobenzyl bromide (100 µL of 5% in methylene chloride), aqueous tetrabutylammonium bromide (50 µL of 5%), and aqueous tripotassium phosphate (50 µL of 20%). This was vortex-mixed for 30 min, followed by further vortexing with 100 µL of ammonium hydroxide (10% in 40/60 water/methanol) to destroy excess pentafluorobenzyl bromide. After adding 1 mL of 4 M sulfuric acid, the derivatives were extracted with pentane (3 mL), vortexed, centrifuged, freeze/poured and evaporated to dryness. The residues were dissolved in 120 µL of methanol, and 20 µL was injected into the LC/MS/MS system.	LC-MS/MS	[52]
	22 Non-smokers	(<LOQ) [<LOQ] ng/mL (0.97) [0.17-2.75] pmol/mg***Δ			
6.2. 2-Hydroxyfluorene					
(0.025)	20 smokers	Tobacco cigarette: (1029 ± 463) [609-1401*] ng/g**	Method C, Table 1.1	LC-MS/MS	[3]
		Electronic cigarette: (738 ± 315) [417-1003*] ng/g**			
(0.025)	13	Water pipe: (366) [34-400] pmol/24h	Method DD, Table 5.1	LC-MS/MS	[5]

		Cigarette: (513) [222-735] pmol/24h			
(0.025)	21 Smokers	(1.60) [0.20-6.62] ng/mL (9.50) [3.22-24.4] pmol/mg***	Method LL, Table 6.1	LC-MS/MS	[52]
	22 Non-smokers	(0.11) [<LOQ-0.33] ng/mL (0.90) [0.36-2.18] pmol/mg***Δ			
6.3. Sum of 1-Hydroxyfluorene and 2-Hydroxyfluorene					
-	21 Smokers	(14.9) [5.29-39.1] pmol/mg***	Method LL, Table 6.1	LC-MS/MS	[52]
	21 Non-smokers	(1.86) [0.53-3.75] pmol/mg***			
6.4. 3-Hydroxyfluorene					

(0.025)	20 smokers	Tobacco cigarette: (679 ± 312) [407-878*] ng/g**	Method C, Table 1.1	LC-MS/MS	[3]
		Electronic cigarette: (367 ± 192) [181-524*] ng/g**			
(0.025)	13	Water pipe: (45) [32-77] pmol/24h	Method DD, Table 5.1	LC-MS/MS	[5]
		Cigarette: (292) [101-393] pmol/24h			
6.5. Sum of 3-, 4-Hydroxyphenanthrenes					
(0.05)	20 smokers	Tobacco cigarette: (1314 ± 669) [808-1720*] ng/g**	Method C, Table 1.1	LC-MS/MS	[3]
		Electronic cigarette: (1098 ± 544) [630-1464*] ng/g**			

(0.020)	21 Smokers	(0.450) [0.066-2.13] ng/mL (2.31) [0.86-6.48] pmol/mg***	Method LL, Table 6.1	LC-MS/MS	[52]
	22 Non-smokers	(0.063) [<LOQ-0.23] ng/mL (0.52) [0.24-1.92] pmol/mg***Δ			
6.6. 2-Hydroxyphenanthrene					
(0.025)	20 smokers	Tobacco cigarette: (655 ± 333) [339-933*] ng/g**	Method C, Table 1.1	LC-MS/MS	[3]
		Electronic cigarette: (755 ± 492) [375-947*] ng/g**			

(0.010)	21 Smokers	(0.16) [0.017-0.70] ng/mL (0.85) [0.35-1.91] pmol/mg***	Method LL, Table 6.1	LC-MS/MS	[52]
	22 Non-smokers	(0.04) [<LOQ-0.15] ng/mL (0.29) [0.14-0.76] pmol/mg***Δ			
6.7. 1-Hydroxyphenanthrene					
(0.025)	20 smokers	Tobacco Cigarette: (488 ± 211) [316-678*] ng/g**	Method C, Table 1.1	LC-MS/MS	[3]
		Electronic cigarette: (407 ± 196) [235-561*] ng/g**			

(0.025)	21 Smokers	(0.28) [0.029-1.34] ng/mL (1.54) [0.36-4.86] pmol/mg***	Method LL, Table 6.1	LC-MS/MS	[52]
	22 Non-smokers	(0.095) [<LOQ-0.46] ng/mL (0.69) [0.16-1.62] pmol/mg***Δ			
6.8. r-1, t-2, 3,c-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene					
(100 fmol/mL)	2613	(1.43 ± 2.16 pmol/mL)	Method Y, Table 4.1	GC-MS/MS	[33]
-	343 Smokers who developed lung cancer	(29.0*◊◊) [26.9-31.2***] pmol/mg	Method S, Table 2.6	LC-MS/MS	[24]
	392 Smokers who remained cancer-free	(25.4*◊◊) [23.6-27.3***] pmol/mg			

0.1 fmol/mL	476	Smokers who developed lung cancer: (32.1◊◊) [30.5-33.8] pmol/mg*** Smokers who remained cancer-free: (28.1◊◊) [26.7-29.5] pmol/mg***	**Method MM** PheT concentrations were determined by spiking 2 mL of urine with internal standards. The pH was adjusted to 5 with 1.5 mL of sodium acetate buffer (0.5 M, pH 5). β-glucuronidase (3,500 units) and arylsulfatase (28,000 units) were added, and the mixture incubated overnight with shaking at 37 °C. A Sep-pak cartridge was prewashed with 10 mL of methanol and 10 mL of H_2O. The sample was applied slowly. The cartridge was washed with 15 mL of 0.15 M NH_4OH. The cartridge was then eluted with 12 mL of 25% methanol. Solvents were removed by overnight concentration on a Speedvac. The residue was transferred with three 65 µL portions of methanol/H_2O (1:1). HPLC eluant was collected from 6.5–14.5 min. The collected HPLC fraction was concentrated to dryness overnight on the Speedvac. The residue was transferred using three 60µL portions of methanol. The solution was concentrated to dryness again. To the residue was added 10 µL of acetonitrile and 30 µL of bis-trimethylsilyltrifluoroacetamide, followed by heating at 60 °C for 60 min. The sample was then analysed by GC. 1-Hydroxpyrene analysis was performed using 25 mL of urine adjusted to pH 5.0 with 1.0 N HCI, buffered with 10 mL 0.1 M acetate (p H 5.0) and incubated overnight with 25 µL Beta-glucuronidase/aryl sulphatase (2500 U) at 37 °C. A sep-pak C18 cartridge was used for the separation of the metabolites of PAH. After priming the cartridge with 5 mL methanol and 10 mL of water, the sample was passed through the cartridge at a rate of approximately 10 mL/min. Subsequently the cartridge was washed with 8 mL water. Retained solutes were eluted using 10 mL of methanol. The solvent was evaporated at 60 °C under a constant flow of nitrogen and the residue dissolved in 2.0 mL methanol.	GC-MS	[27]
0.2 fmol/mL	20 Psoriasis patients treated with PAH containing ointment	(791 ± 363 pmol/mg***)		GC-MS	[53]
	32 Coke oven workers exposed to PAH	(25.7 ± 16.8 pmol/mg***)			
	31 Smokers	(4.58 ± 2.95 pmol/mg***)			
	30 Non-smokers	(1.51 ± 1.15 pmol/mg***)			

-	100 controls that smoked at baseline	(217 ± 111 ng/mL)	Method BB, Table 4.1	GC- MS	[28]
	100 lung cancer cases	(227 ± 93 ng/mL)			
(13 fmol/mL)	16 plasma samples	(95 ± 71 fmol/mL)		GC-MS	[39]
6.9. Sum of hydroxyphenanthrenes					
-	21 Smokers	(4.69) [1.78-11.5] pmol/mg***	Method LL, Table 6.1	LC-MS/MS	[52]
	21 Non-smokers	(1.49) [0.62-4.17] pmol/mg***			
6.10. 1-Hydroxypyrene					
(0.025)	20 smokers	Tobacco cigarette: (778 ± 338) [556-1000*] ng/g**	Method C, Table 1.1	LC-MS/MS	[3]
		Electronic cigarette: (606 ± 279) [378-817*] ng/g**			

(0.025)	13	Water pipe: (108) [87-194] pmol/24h Cigarette: (48) [61-109] pmol/24h	Method DD, Table 5.1	LC-MS/MS	[5]
(0.025)	21 Smokers	(0.33 ng/mL) [0.029-2.00] (1.59) [0.32-4.04] pmol/mg***	Method LL, Table 6.1	LC-MS/MS	[52]
	22 Non-smokers	(0.061) [<LOQ-0.23] ng/mL (0.39) [0.093-0.77] pmol/mg***Δ			
(0.025)	47 (Heated cigarette)	(183◊◊) [167-202] ng/day	Method K, Table 1.2	LC-MS/MS	[12]
	23 (Conventional cigarette)	(306◊◊) [268-350] ng/day			
-	37	(113.25 ± 57.45 ng/g**)	Method F, Table 1.1	LC-MS/MS	[13]

(10 pg/mL)	100	-		LC-MS/MS	[7]
-	160	Conventional cigarettes (6.0 mg tar/0.5 mg nicotine) (149.23 ± 73.32 ng/g**)		LC-MS/MS	[14]
		Conventional cigarettes (10.6 mg tar/0.84 mg nicotine) (233.51 ± 143.01 ng/g**)			
		Test cigarettes (6.0 mg tar) (220.21 ± 107.18 ng/g**)			
		Test cigarettes (11 mg tar) (252.93 ± 163.87 ng/g**)			
-	17	Whilst smoking: (1.36 ± 0.776 nmol/24h)	Method S, Table 2.6	LC-MS/MS	[34]
		After not smoking for 56 days: (1.09 ± 1.97 nmol/24h)			

0.05 pmol/mL	10 Non-smokers	-	**Method NN** Urine (1 mL) was taken and internal standards added. 2,000 units of β-glucuronidase and 16,000 units of sulfatase were added and incubated overnight at 37°C. SPE was performed using a Versaplate 96-well extraction system using C18 packing. The cartridges were primed with 1 mL of methanol and 2 mL of water followed by addition of the urine sample. The cartridges were washed with 1 mL of methanol-1% aqueous formic acid (1:1) and eluted with 2 x 0.4 mL methanol. The eluent was concentrated to dryness at 40 °C using a Speedvac. Fifty microliters of methanol were added to each well and the plate was sonicated briefly. K_2HPO_4 buffer (50 µL of 50 mmol/L, pH 7) was added to each well. The 96-well plate was briefly sonicated and analysed.	LC with fluorescence detection	[54]
0.2 fmol/mL	20 Psoriasis patients treated with PAH containing ointment	(9390*** ± 10600 pmol/mg)	Method MM, Table 6.8	GC-MS	[53]
-	32 Coke oven workers exposed to PAH	(100*** ± 62.0 pmol/mg)			
	31 Smokers	(1.33***± 1.00 pmol/mg)			
	30 Non-smokers	(0.58*** ± 0.53 pmol/mg)			
-	47 Smokers – Pack tar 10mg/cig	(326) [285-367◊◊] ng/day	Method II, Table 5.8	LC-MS/MS	[15]

	46 Smokers – Pack tar 10mg/cig	(292) [250-334◊◊] ng/day			
	45 Smokers – Pack tar 6mg/cig	(271) [233-309◊◊] ng/day			
	44 Smokers – Pack tar 6mg/cig	(185) [162-209◊◊] ng/day			
	48 Smokers – Pack tar 1mg/cig	(164) [145-184◊◊] ng/day			
(10 pg/mL)	50 Non-smokers	(79) [69-89◊◊] ng/day		LC-MS/MS	[16]
	50 Smokers of 1mg tar cigarettes	(156) [139-173◊◊] ng/day			
	50 Smokers of 4mg tar cigarettes	(262) [229-295◊◊] ng/day			

	50 Smokers of 10mg tar cigarettes	(331) [287-374◊◊] ng/day			
-	49 Non-smokers	(91) [72-109◊◊] ng/day		LC-MS/MS	[17]
	42 Smokers of 4mg tar cigarettes	(334) [292-375◊◊] ng/day			
	48 Smokers of 9mg tar cigarettes	(276) [244-309◊◊] ng/day			
	49 Smokers of 14mg tar cigarettes	(350) [315-385◊◊] ng/day			
6.11. 1-Naphthol					
0.04 µg/L	18 smokers before cessation	(30.5) µg/g**	**Method OO** Urine (1 mL) was adjusted to pH 5.0 using 0.2M sodium acetate buffer (100 µL), in a dark room. 10 µL of β-glucuronidase sulfatase activity was added, and it was incubated at 37°C for 16 hours in a shaking water bath. Acetonitrile (1.5 mL) was added, followed by centrifuge at 10,000 g for 10 minutes.	HPLC-fluorescence	[55]

	18 smokers after cessation	(5.7) µg/g**			
1.5 µg/L	63 non-smokers	5.0 (6.7) [LOD-29.4] µg/L	**Method PP** Urine (2 mL) was diluted using sodium acetate buffer (4 mL, 0.1 mol/L, pH 5.0). To this, β-glucuronidase/arylsulfatase (25 µL) was added and the sample incubated for 16 hours at 37°C. After centrifuging at 1500 g for 10 minutes, 350 µL of the supernatant was injected into the HPLC with fluorescence monitored at 227/430 nm.	HPLC-fluorescence	[56]
	9 smokers	20.6 (21.7) [3.6-56.3] µg/L			
6.12. 2-Naphthol					
(0.25)	20 smokers	Tobacco cigarette: (24 ± 13) [12-34*] µg/g**	Method C, Table 1.1	LC-MS/MS	[3]
		Electronic cigarette: (15 ± 8) [11-18*] µg/g**			
(0.25)	13	Water pipe: (3354) [2100-5453] pmol/24h	Method DD, Table 5.1	LC-MS/MS	[5]

		Cigarette: (8015) [3158-11173] pmol/24h			
(0.500)	21 Smokers	(14.3) [1.7-51.1] ng/mL (110) [41-190] pmol/mg***	Method LL, Table 6.1	LC-MS/MS	[52]
	22 Non-smokers	(2.44) [<LOQ-17.5] ng/mL (19.0) [3.5-88.5] pmol/mg***ΔΔ			
0.13 ng/mL	18 smokers before cessation	(6.1) µg/g**	Method OO, Table 6.11	HPLC-fluorescence	[55]
	18 smokers after cessation	(1.6) µg/g**			
0.5 µg/L	63 non-smokers	3.6 (6.1) [LOD-23.6] µg/L	Method PP, Table 6.11	HPLC-fluorescence	[56]

	9 smokers	19.5 (20.9) [2.2-48.3] µg/L			

* Interquartile range ** Normalized per gram creatinine *** Normalized per milligram creatinine ◊ 50th percentile ◊◊ 95% Confidence interval □ Geometric mean Δ 21 Non-smokers

Table 7 - Literature values and analytical methods for aromatic amines

LOD (LLOQ) (ng/mL)	N	Median (Mean) [Range]	Sample Preparation	Instrumental Analysis	Ref.
7.1. *o*-toluidine					
0.1	-	-	Method QQ To urine samples (2 mL) was added internal standard using Hamilton STAR liquid handling system, followed by hydrolysis using 50 µL of NaOH (10 M) and incubation for about 15 hours at 90°C. The samples were cooled to room temperature, and loaded onto Isolute™ SLE cartridges. Elution was performed with 3 x 3mL dichloromethane, and the eluents were concentrated to about 250 µL, to which 3 µL of trimethylamine (1.0 M) and 3 µL of pentafluoropropionic anhydride were added for derivatization (room temperature, 30 minutes). The samples were then transferred, evaporated and reconstituted in toluene (10 µL).	GC–MS/MS	[57]
0.6	-	-	Method RR To urine (4 mL) was added NaOH to make it 4.7 M, and kept at 80 °C for 2 hours. Extraction of neutral and basic compounds was performed using butyl chloride, followed by extracting the basic compounds from this butyl chloride solution using HCl solution (0.1 N).	HPLC-ECD	[58]
1	8 smokers	1.5 ng/mL (1.7 ng/mL) [0.0-4.1 ng/mL]	Method SS Hydrolysis was performed by adding concentrated sulfuric acid (1 ml) to urine (4 ml) and heating at 80 °C for 2 hours. 32% NaOH was then used to basify the hydrolysate, followed by and saturating with NaCl. Aromatic amines were reduced using sodium borohydrate, extracted with toluene, and reextracted with 0.2 M aqueous sulfuric acid. A final extraction was performed using toluene, which was then dried over sodium sulfate. Detivatization was performed	GC-ECD	[59]

	8 non-smokers	0.0 ng/mL (0.0 ng/mL) [0.0-0.0 ng/mL]	using heptafluorobutyric anhydride.		
0.05	20 persons without known exposure	0.12 ng/mL [<0.05–3.1 ng/mL]	Method TT To urine (5 ml) was added internal standard and concentrated hydrochloric acid (37%, 1 mL), followed by heating at 80 °C for 1 hour. After cooling in an ice bath, NaOH (10 M, 600 µL), 2-(N-morpholino)ethanesulfonic acid buffer (3 ml), and NaOH (10 M, 550 µL) were added in order. The extraction was performed with 2 x 5 ml n-hexane, followed by addition of dried pyridine (25 µL) and pentafluoropropionic anhydride (50 µL) and heating at 80 °C for 1 hour. After cooling to room temperature, the samples were extracted with 3 ml phosphate buffer (pH 8) for 5 min, followed by centrifugation. To the separated organic layer was added 200 µL toluene, which was then evaporated to 40 µL.	GC–MS	[60]
1.88	-	-	Method UU Solid-phase microextraction (SPME): To a glass vials was added a magnetic stir bar, salt, and a strong base solution (1 mL), followed by the urine sample (5 mL). After stirring at room temperature and incubating at the required temperature for 3.0 minutes using a water bath, the SPME fiber was inserted into the headspace for the required extraction time period, and introduced into the GC injector.	GC–MS	[61]
1	13 e-cigarette users	(2.33) [0.94–4.23 ng/ml]	Method VV Dilute hydrochloric acid (37%, 1mL) was added to urine (5ml) and heated at 80 °C for 1 hour, followed by adding NaOH (10M) and 2-(N-morpholino)ethanesulfonic acid buffer (3 mL) to adjust the pH to 6.1–6.4. Extraction was performed using dichloromethane, using sodium sulfate to eliminate residual water. Pentafuoropropionic anhydride (50 µL) and pyridine (25 µL) were added before heating the extract at 80 °C for 1 hour. The sample was then reconstituted in dichloromethane to the final volume of 100 µL.	LC-MS	[62]
	9 non e-cigarette users (controls)	(1.00) [0.42–1.67 ng/ml]			
0.01	1 non-smoker	(0.9 ng/mL)	Method WW To each urine sample (20 mL) was added ith poly(para-phenylenediamine) modified with Fe3O4 nanoparticles (20 mg), followed by ultrasonication (2 minutes). After decanting the supernatant solution, the analytes were desorbed from the nanoparticles using dichloromethane/chloroform (3:1 v/v, 250 µL) and ultrasonication (30 seconds). A 1 µL sample was injected into the GC–FID.	GC–FID	[63]
	1 smoker	(14.5 ng/mL)			
0.02	unexposed workers	[0.17 µg//L-2.46 µg/g creatinine]	Method XX To urine sample (5 mL) was added internal standard, followed by extraction with hexane. Derivatization was	GS-MS	[64]

	exposed workers	[26.14-462.00 µg/g creatinine]	performed using anhydrous pentafluoropropionic acid (60 °C, 30 minutes).		
0.004	9 non-smokers	(105.2) [70.1-139.6] ng/24h	Method YY Internal standard and hydrochloric acid (37%, 1 mL) were added to urine (5 mL), followed by heating to 80 °C for 1 hour. After cooling to room temperature, NaOH (10M, 1.15 mL) and 2-(N-morpholino)ethanesulfonic acid buffer (3 mL) were added. Extraction was performed using 2 x 5 mL n-hexane. Sodium sulfate was used to eliminate residual water, followed by derivatization using pentafluoropropionic anhydride (50 µL) and pyridine (25 µL) and heating at 80 °C for 1 hour. The sample was washed with phosphate buffer (pH 8.0, 3 mL), and toluene (200 µL) was added after. The sample was then concentrated to 70 µL.	GC-MS	[65]
	10 smokers	(204.2) [107.9-258.7] ng/24h			
50-100 ng/24 h	12 non-smokers	(4.1 µg/24 h)	Method ZZ Urine sample (200 mL) hydrolysis was performed using NaOH (2N, 15 ml,) heated under reflux for 2.5 hours. The sample was then saturated with NaCl and extracted with 3 x 40 mL chloroform. The extract was evaporated and reconstituted 1 ml benzene, to which was added triethylamine in benzene (0.05 M, 3 mL) and pentafluoropropionic anhydride (1 mL). The extract was heated at 55 °C 20 minutes, cooled to room temperature, followed by addition of NH4OH (5%, 10 mL).	GC-EC	[66]
	16 smokers	(6.3 µg/24 h)			
0.6	16 pre-shift non-smokers	(1.3 ng/mL)	Method AAA Urine sample (4 mL) was hydrolyzed using NaOH (4.7 M) and heatint at 80°C for 2 hours. Extraction was performed using butyl chloride, followed by back extraction using aqueous hydrochloric acid (0.1 M).	HPLC-ECD	[67]
	16 post-shift non-smokers	(2.8 ngm/L)			
	10 pre-shift smokers	(0.9 ng/mL)			
	9 post-shift smokers	(2.8 ng/mL)			
7.2. m-toluidine					

1	8 smokers	0.8 ng/mL (0.7 ng/mL) [0.0-1.9 ng/mL]	Method SS	GC-ECD	[59]
	8 non-smokers	0.0 ng/mL (0.0 ng/mL) [0.0-0.0 ng/mL]			
0.05	20 persons without known exposure	0.17 ng/mL [<0.05–2.8 ng/mL]	Method TT	GC–MS	[60]
7.3. *p*-toluidine					
1	8 smokers	1.9 ng/mL (2.2 ng/mL) [0.0-6.3 ng/mL]	Method SS	GC-ECD	[59]
	8 non-smokers	1.0 ng/mL (1.3 ng/mL) [0.0-3.2 ng/mL]			
0.05	20 persons without known exposure	0.11 ng/mL [<0.05–0.55 ng/mL]	Method TT	GC–MS	[60]
7.4. 4-Chloro-*o*-toluidine					
1	8 smokers	2.2 ng/mL (3.0 ng/mL) [0.0-8.0 ng/mL]	Method SS	GC-ECD	[59]

	8 non-smokers	1.8 ng/mL (2.2 ng/mL) [0.0-6.3 ng/mL]			
0.004	-	-	Method BBB	GCxGC-qMS	[68]
7.5. Aniline					
1	8 smokers	1.1 ng/mL (1.4 ng/mL) [0.0-5.1 ng/mL]	Method SS	GC-ECD	[59]
	8 non-smokers	0.0 ng/mL (0.2 ng/mL) [0.0-1.2 ng/mL]			
0.05	20 persons without known exposure	3.5 ng/mL [0.4–8.8 ng/mL	Method TT	GC–MS	[60]
3.39	-	-	Method UU	GC–MS	[61]
0.02	-	-	Method BBB To urine sample (20 mL) was added concentrated hydrochloric acid (37%, 10 mL) and heated (12 hours, 110 °C). After cooling to room temperature, sodium hydroxide (10 M, 20 mL) was added. The sample was filtered and extracted with 3 x 5 mL diethylether. The organic phase was separated and washed with sodium hydroxide solution (0.1M, 2 mL), after which acidified water (5 mL, with 100 µL concentrated hydrochloric acid) was used to extract the aromatic amines into aqueous. The residual diethylether in the aqueous sample was then evaporated. % mL of the extract was used in derivatization, for which hydriodic acid (55 %, 100 µL) and sodium nitrite (50 g/L, 200 µL) were added followed by shaking for 20 minutes. Amidosulfonic acid (50 g/L, 0.5 mL) was then added. After shaking for 45 minutes, the sample was heated (95 °C, 5 minutes), and then cooled to room temperature. Saturated sodium sulfite (125 µL), 1 alizarinsulfonic acid (100 µL), and saturated sodium acetate (0.5 mL) were added.	GCxGC-qMS	[68]

0.007	1 non-smoker	(1.2 ng/mL)	Method WW	GC–FID	[63]
	1 smoker	(9.8 ng/mL)			
50-100 ng/24 h	12 non-smokers	(2.8 µg/24 h)	Method ZZ	GC-EC	[66]
	16 smokers	(3.1 µg/24 h)			
1.4	16 pre-shift non-smokers	(1.6 ng/mL)	Method AAA	HPLC-ECD	[67]
	16 post-shift non-smokers	(2.6 ngm/L)			
	10 pre-shift smokers	(4.2 ng/mL)			
	9 post-shift smokers	(6.2 ng/mL)			
7.6. 2-Chloroaniline					
1.05	-	-	Method UU	GC–MS	[61]
0.004	-	-	Method BBB	GCxGC-qMS	[68]
7.7. 3-Chloroaniline					

0.05	20 persons without known exposure	<0.05 ng/mL [<0.05–2.5 ng/mL	Method TT	GC–MS	[60]
0.01	1 non-smoker	(<0.06 ng/mL)	Method WW	GC–FID	[63]
	1 smoker	(4.5 ng/mL)			
7.8. 4-Chloroaniline					
1	8 smokers	0.0 ng/mL (0.1 ng/mL) [0.0-0.8 ng/mL]	Method SS	GC-ECD	[59]
	8 non-smokers	0.0 ng/mL (0.0 ng/mL) [0.0-0.0 ng/mL]			
0.05	20 persons without known exposure	0.11 ng/mL [<0.05–1.1 ng/mL	Method TT	GC–MS	[60]
7.9. 2,6-Dichloroaniline					
0.006	-	-	Method BBB	GCxGC-qMS	[68]
7.10. 3,4-Dichloroaniline					

0.05	20 persons without known exposure	<0.05 ng/mL [<0.05–0.15 ng/mL	Method TT	GC–MS	[60]
7.11. 3,5-Dichloroaniline					
0.05	20 persons without known exposure	0.18 ng/mL [<0.05–2.0 ng/mL	Method TT	GC–MS	[60]
7.12. 3-Chloro-4-fluoroaniline					
0.02	-	-	Method BBB	GCxGC-qMS	[68]
7.13. 3-Chloro-2,6-dimethylaniline					
0.01	-	-	Method BBB	GCxGC-qMS	[68]
7.14. 3-Chloro-4-methoxyaniline					
0.01	-	-	Method BBB	GCxGC-qMS	[68]
7.15. 2-Bromoaniline					
0.01	-	-	Method BBB	GCxGC-qMS	[68]
7.16. 4-Ethylaniline					
0.01	-	-	Method BBB	GCxGC-qMS	[68]
7.17. N,N-Dimethylaniline					

0.007	1 non-smoker	(0.5 ng/mL)	Method WW	GC–FID	[63]
	1 smoker	(2 ng/mL)			
7.18. 2,6-Dimethylaniline					
0.016	-	-	Method QQ	GC–MS/MS	[57]
0.81	-	-	Method UU	GC–MS	[61]
0.009	-	-	Method BBB	GCxGC-qMS	[68]
7.19. 2,4-Dimethylaniline					
0.009	-	-	Method BBB	GCxGC-qMS	[68]
7.20. 2,4,6-trimethylaniline					
0.40	-	-	Method UU	GC–MS	[61]
0.02	-	-	Method BBB	GCxGC-qMS	[68]
7.21. o-anisidine					
0.007	-	-	Method QQ	GC–MS/MS	[57]

0.05	20 persons without known exposure	0.22 ng/mL [<0.05–4.2 ng/mL	Method TT	GC–MS	[60]
7.22. 4-aminophenylthioether					
1.1	-	-	Method CCC Phosphate buffer (2 mL, pH 9) was added to filtered urine sample (20 mL), followed by addition of 50 mg of C18-functional ultrafine magnetic silicananoparticles. After stirring for 20 minutes, the solution was decanted, the C18-UMS NPs were washed using deionized water (1 mL), and added to acetonitrile (3 mL). After stirring for 1 minute, elution was performed using acetonitrile. The eluate was dried and reconstituted in methanol (0.2 mL).	UFLC-UV/vis	[69]
7.23. 1-aminonaphthalene					
0.0015	-	-	Method QQ	GC–MS/MS	[57]
1.3	-	-	Method CCC	UFLC-UV/vis	[69]
0.005	40 smokers	(67.02 ng/24 h)	Method DDD For acid analysis, urine sample (5 mL) was mixed with concentrated HCl (1 mL) and kept at 80 °C for 1 hour. For enzyme hydrolysis, sodium acetate buffer (10 mmol/L, pH 5 ± 0.1, 10 mL) and β-glucuronidase arylsulfatase (10 μL) were added to the urine (5 mL) and kept at 37 °C for 16 hours. After cooling to room temperature, NaOH (10 mol/L, 1.2 mL) and ammonium acetate buffer solution (0.5 mol/ L, pH 6.0, 5 mL), as well as internal standard were added. Purification was performed using a PAHs MIPs SPE cartridge, preconditioned with 1 mL cyclohexane. After loading the urine sample on the cartridge, it was washed with 1 mL cyclohexane, and elution was performed using 10 mL ethyl acetate. The eluate was evaporated and reconstituted in 100 μL methanol.	LC–MS/MS	[70]
	10 non-smokers	(12.32 ng/24 h)			
0.01 (ng/24h)	12 smokers	(506.7 ng/24 h)	Method EEE To urine sample (200 mL) was added benzene (100 mL) and β-glucuronidase/arylsulfatase, followed by shaking at 37 °C for 16 hours. The benzene layer was then washed with water (10 mL) and evaporated to 2 mL, to which was added methanol (5 mL). A column was prepared by placing aromatic sulfonic acid (0.5 g) between two Teflon frits, and was activated with 1 N phosphoric acid (5 mL) and was washed with methanol (30 mL). After passing the solution through	GC–MS	[71]

	14 non-smokers	(68.9 ng/24 h)	the prepared column, nitroarenes were eluted with methanol (30 mL). Aromatic amines were eluted with ammonium acetate solution in 90% methanol (0.2 M, 20 mL). To the eluate was added water (30 mL) and the amines were extracted benzene (50 mL). Aromatic amines were acylated using pentafluoropropionyl-imidazol and purified on Florisil.		
	22 passive smokers	(79.7 ng/24 h)			
7.24. 2-aminonaphthalene					
0.0028	-	-	Method QQ	GC–MS/MS	[57]
0.02	-	-	Method BBB	GCxGC-qMS	[68]
0.001	10 non-smokers	(10.7) [3.7-30.2] ng/24h	Method YY	GC-MS	[65]
	10 smokers	(20.8) [6.2-46.9] ng/24h			
0.003	40 smokers	(47.40 ng/24 h)	Method DDD	LC–MS/MS	[70]
	10 non-smokers	(10.18 ng/24 h)			
0.01 (ng/24h)	12 smokers	(84.5 ng/24 h)	Method EEE	GC–MS	[71]
	14 non-smokers	(120.8 ng/24 h)			
	22 passive smokers	(94.9 ng/24 h)			

1	8 smokers	3.1 ng/mL (3.1 ng/mL) [0.0-7.4 ng/mL]	Method SS	GC-ECD	[59]
	8 non-smokers	0.0 ng/mL (0.5 ng/mL) [0.0-1.6 ng/mL]			
7.25. 2-aminobiphenyl					
0.01 (ng/24h)	12 smokers	(66.5 ng/24 h)	Method EEE	GC–MS	[71]
	14 non-smokers	(45.5 ng/24 h)			
	22 passive smokers	(48.1 ng/24 h)			
7.26. 3-aminobiphenyl					
0.003	40 smokers	(5.39 ng/24 h)	Method DDD	LC–MS/MS	[70]
	10 non-smokers	(1.11 ng/24 h)			
7.27. 4-aminobiphenyl					
0.0018	-	-	Method QQ	GC–MS/MS	[57]
0.88	-	-	Method CCC	UFLC-UV/vis	[69]

0.001	47 heated cigarette users	(3.93) [3.55-4.35] ng/day	Method K, Table 1.2	LC-MS/MS	[12]
	23 conventional cigarette smokers	(13.3) [11.5-15.3] ng/day			
0.001	10 non-smokers	(9.6) [3.7-19.5] ng/24h	Method YY	GC-MS	[65]
	10 smokers	(15.3) [4.3-32.9] ng/24h			
0.0009	41 non-smokers	(1.64) [1.30–2.07] pg/mg creatinine	Method FFF To each urine sample (5 mL) was added internal standard and NaOH (10 M, 50 µL) and kept at 90 °C for 15 hours. After cooling to room temperature, extraction was performed using 8 mL hexane on a rugged rotator (60 rpm) for 1 hour. The hexane layer was extracted into aqueous phase using 1 mL of HCl (0.1 N) and was subsequently neutralized using NaOH (10 M). Sample clean-up was performed on an HLB cartridge, which was conditioned with 1 mL methanol and 1 mL water. After loading the sample, it was washed with 1 mL water, followed by eluting the analytes with 2 mL of methylene chloride. The residual water was eliminated using a sodium sulfate cartridge. The eluate was concentrated to about 300 µL, and derivatization was performed after by adding trimethylamine (0.1 M, 3 µL) followed by of pentafluoropropionic acid anhydride (2 µL) and keeping the sample at room temperature for 30 minutes. The sample was evaporated and rexonstituted in 10 µL toluene.	GC–MS/MS	[72]
	89 smokers	(8.69) [7.43–10.16] pg/mg creatinine			
0.0015	40 smokers	(17.27 ng/24 h)	Method DDD	LC–MS/MS	[70]
	10 non-smokers	(5.58 ng/24 h)			
0.01 (ng/24h)	12 smokers	(78.6 ng/24 h)	Method EEE	GC–MS	[71]

	14 non-smokers	(68.1 ng/24 h)			
	22 passive smokers	(49.6 ng/24 h)			
7.28. Benzidine					
1	8 smokers	0.0 ng/mL (0.2 ng/mL) [0.0-1.4 ng/mL]	Method SS	GC-ECD	[59]
	8 non-smokers	0.0 ng/mL (0.0 ng/mL) [0.0-0.0 ng/mL]			
7.29. 1,5-Diaminonaphthaline					
1	8 smokers	0.0 ng/mL (0.0 ng/mL) [0.0-0.0 ng/mL]	Method SS	GC-ECD	[59]
	8 non-smokers	0.0 ng/mL (0.0 ng/mL) [0.0-0.0 ng/mL]			
7.30. 4,4'-Diaminodiphenylmethane					
1.1	-	-	Method CCC	UFLC-UV/vis	[69]
7.31. 4-Methyl-1,3-phenylenediamine					
2	8 smokers	1.6 ng/mL (2.3 ng/mL) [0.0-6.7 ng/mL]	Method SS	GC-ECD	[59]

	8 non-smokers	0.0 ng/mL (0.0 ng/mL) [0.0-0.0 ng/mL]			

Supplementary Information References

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