

Supplementary files

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Table:1S. Group statistics of the exposed sample (100) and the control (50)

Table:S2 Data of 100 exposed and 50 control samples with their six DAP metabolite concentrations.

Figure S1: The chemical structure of six DAP metabolites

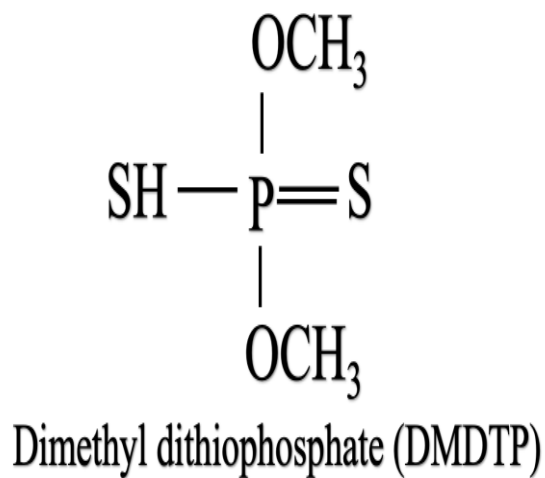
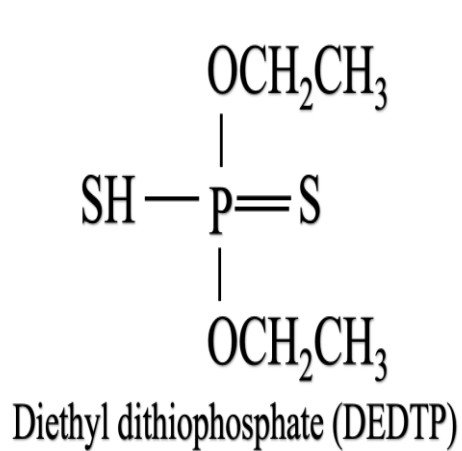
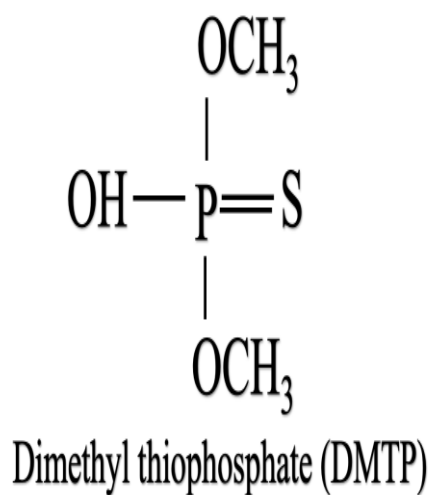
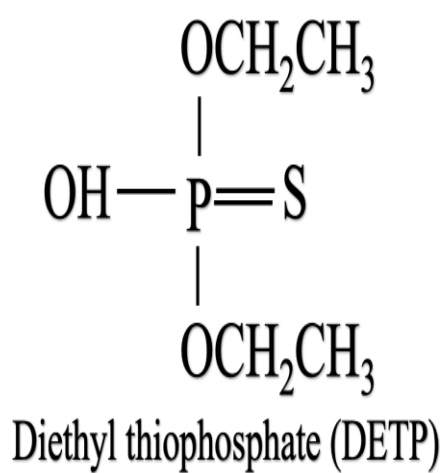
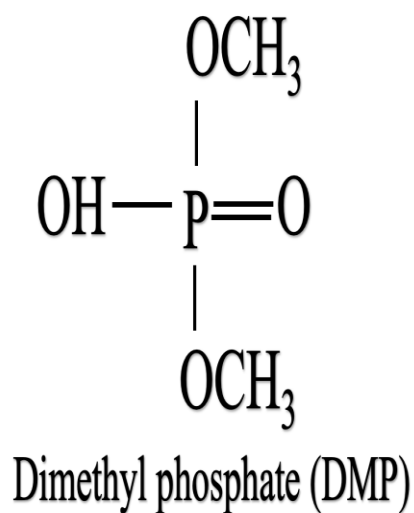
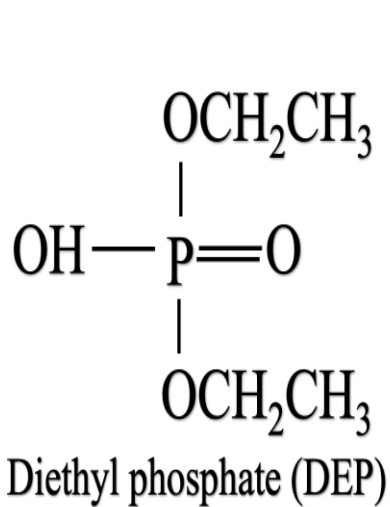


Figure S2: The linearity diagram of all DAP metabolites

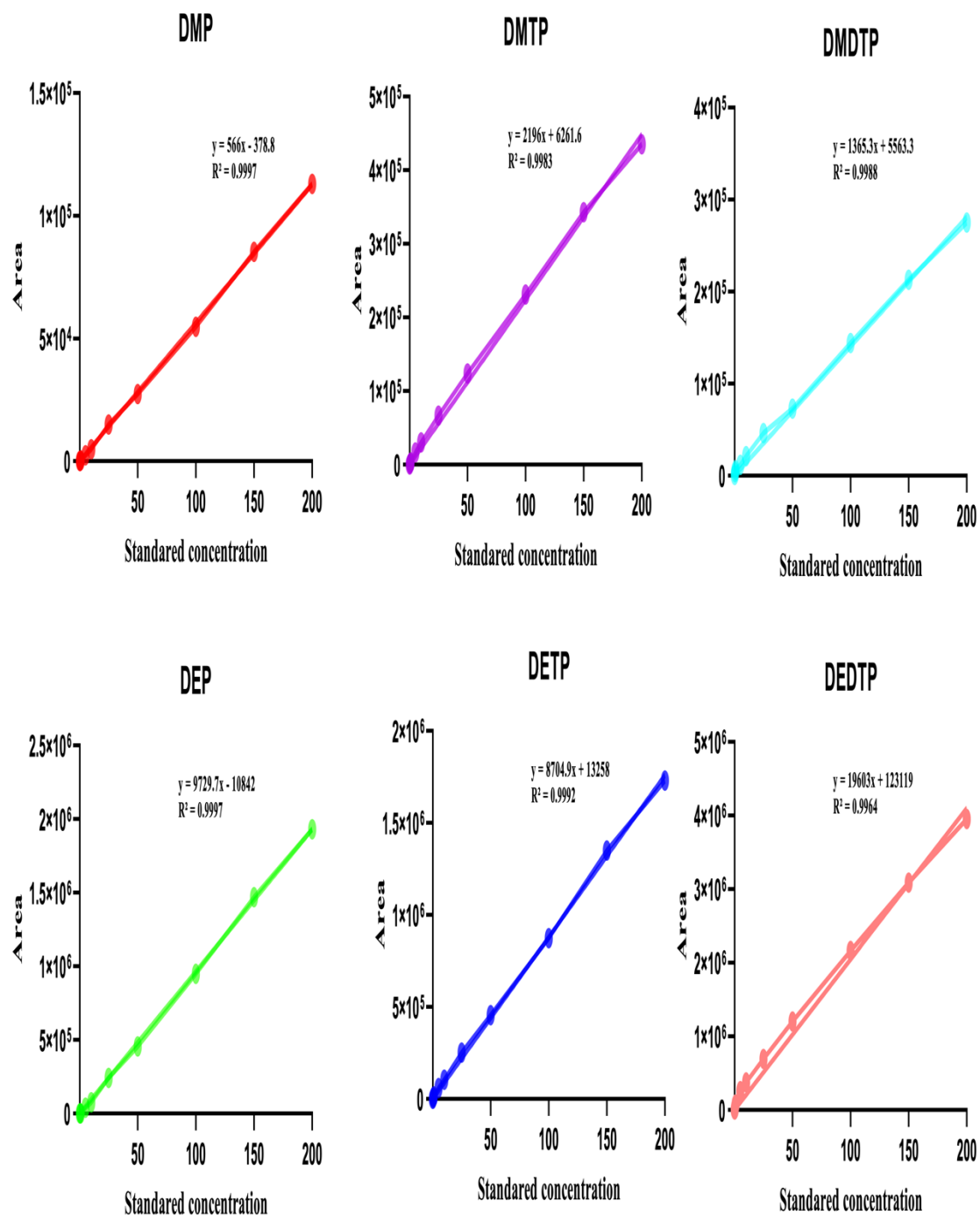
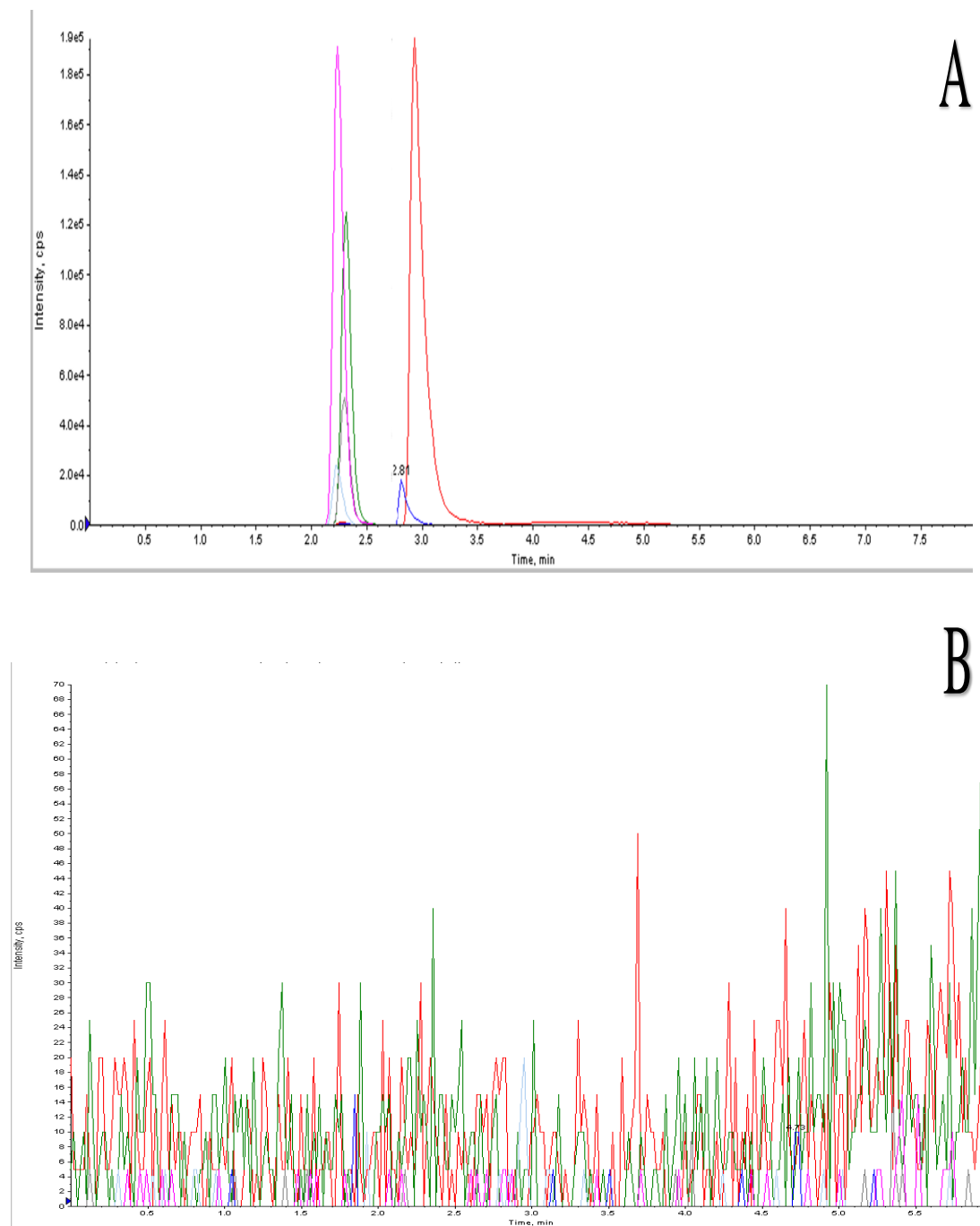
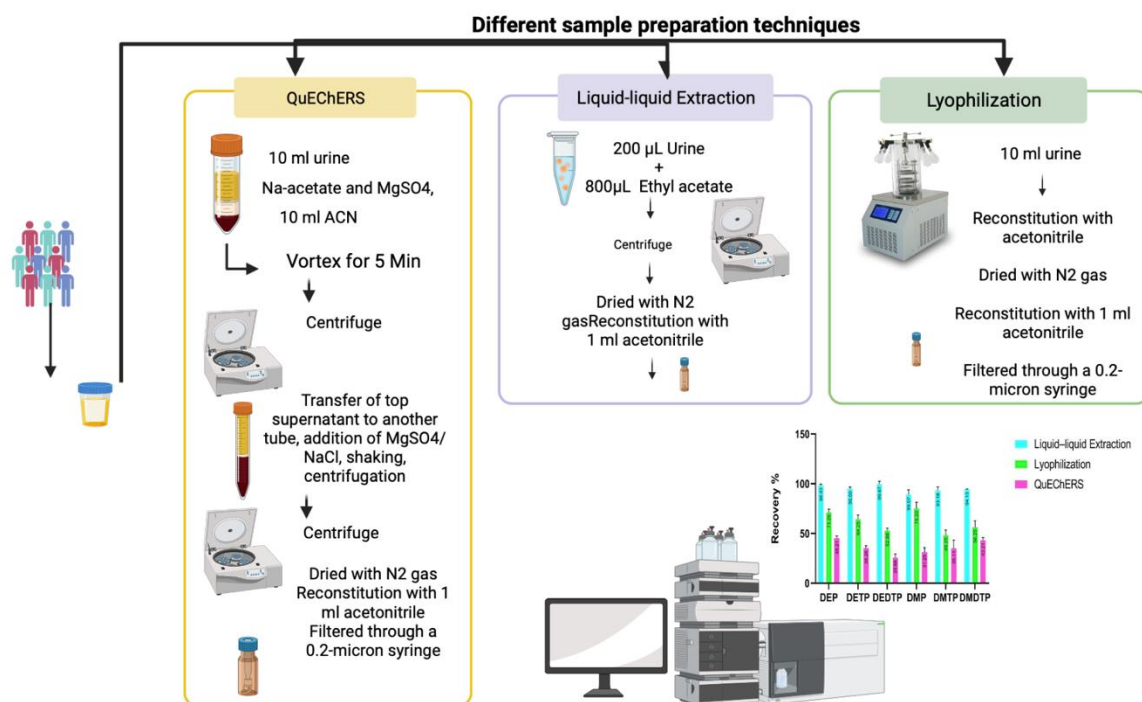


Figure S3: The carry-over study



Based on the carryover study, which involved the analysis of a 200 ng/mL standard sample, it was observed that after B examined the blank (mobile phase), there were no other interfering analytes carried forward to the subsequent chromatographic run.

Figure S4: Comparative extraction procedures (QuEChERS, lyophilisation and liquid-liquid extraction)



For each extraction process in the lyophilization method, a series of procedures were carried out. The dried sample was then combined with different solvents such as H₂O, methanol, and a mixture of H₂O and ACN (acetonitrile). The best solvent for extraction was chosen for the subsequent recovery study. In the official AOAC QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method, the prescribed procedures were followed strictly to ensure accurate and reliable results. Various solvents including hexane, ethyl ether, methanol, and benzene were used for liquid-liquid extraction (LLE). Ethyl acetate was found to be the most favourable solvent for extraction after comparative analysis.

Figure S5: The chromatogram of the real urine sample analysed for DAP metabolites

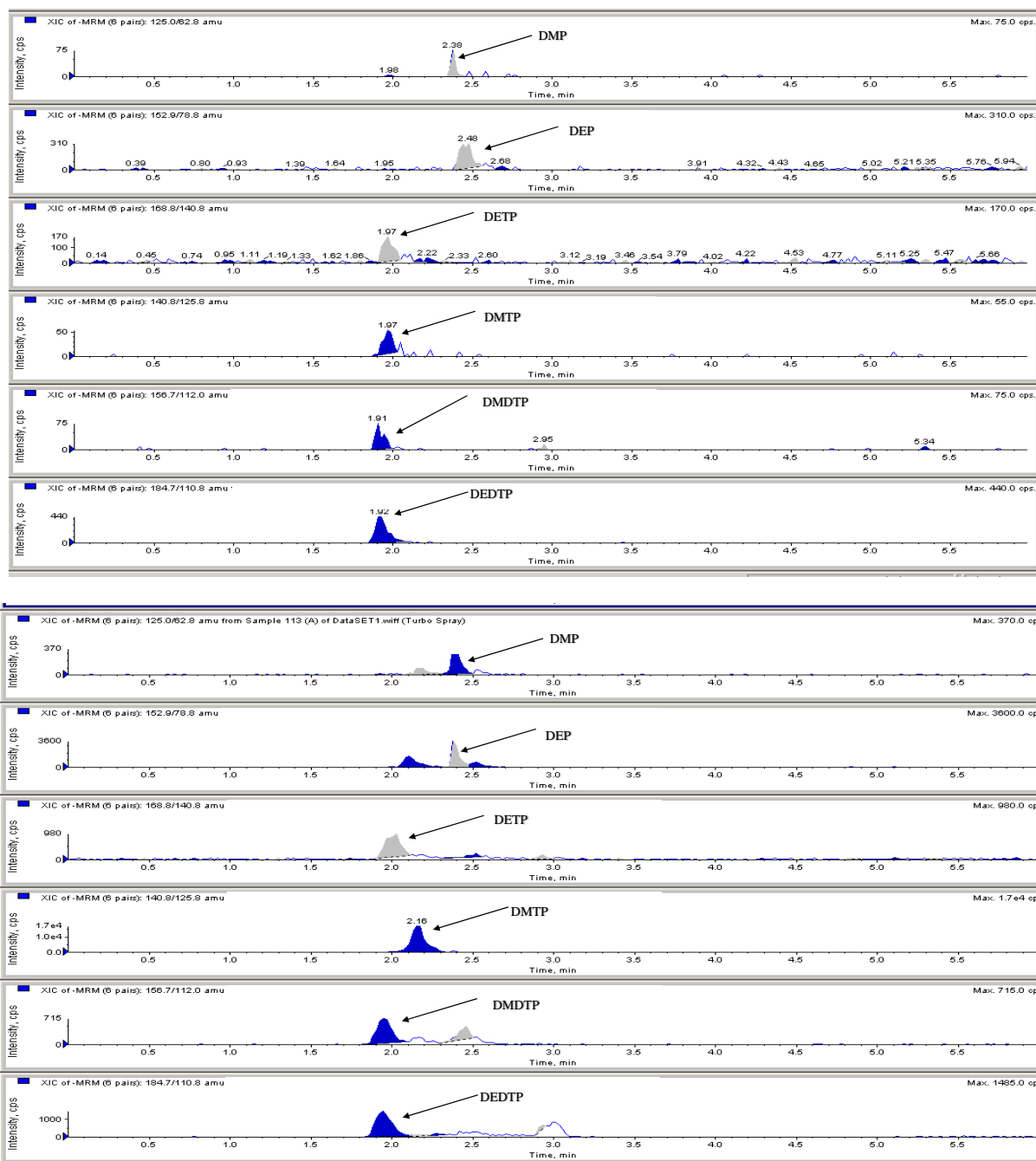


Table:1S. Group statistics of the exposed sample (100) and the control (50)

Group Statistics					
SAMPLE TYPE		N	Mean	Std. Deviation	Std. Error Mean
DEP	exposed	100	13.03515	5.932902	0.593290
	control	50	11.69200	4.972661	0.703240
DETP	exposed	100	1.247381	2.2372394	0.2237239
	control	50	1.288408	1.5132391	0.2140043
DEDTP	exposed	100	0.58306	0.430734	0.043073
	control	50	0.73642	0.622833	0.088082
DMP	exposed	100	16.8787	54.32874	5.43287
	control	50	8.9518	4.40802	0.62339
DMTP	exposed	100	20.833630	21.6048938	2.1604894
	control	50	18.194852	12.8176958	1.8126959
DMDTP	exposed	100	4.37326	7.265589	0.726559
	control	50	2.84300	2.484496	0.351361

Independent Samples Test											
		Levene's Test for Equality of Variances		t-test for Equality of Means							
		F	Sig.	t	df	Significance		Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
						One-Sided p	Two-Sided p			Lower	Upper
DEP	Equal variances assumed	1.033	0.311	1.377	148	0.085	0.171	1.343150	0.975688	-0.584930	3.271230
	Equal variances not assumed			1.460	114.792	0.074	0.147	1.343150	0.920076	-0.479379	3.165679
DETP	Equal variances assumed	0.039	0.845	-0.117	148	0.454	0.907	-0.0410270	0.3509803	-0.7346071	0.6525531
	Equal variances not assumed			-0.133	134.888	0.447	0.895	-0.0410270	0.3095969	-0.6533190	0.5712650
DEDTP	Equal variances assumed	4.409	0.037	-1.762	148	0.040	0.080	-0.153360	0.087041	-0.325364	0.018644
	Equal variances not assumed			-1.564	73.166	0.061	0.122	-0.153360	0.098050	-0.348765	0.042045

DMP	Equal variances assumed	2.959	0.087	1.028	148	0.153	0.305	7.92690	7.70874	-7.30651	23.16031
	Equal variances not assumed			1.450	101.588	0.075	0.150	7.92690	5.46852	-2.92042	18.77422
DMTP	Equal variances assumed	2.417	0.122	0.796	148	0.214	0.428	2.6387780	3.3164438	-3.9149213	9.1924773
	Equal variances not assumed			0.936	143.634	0.176	0.351	2.6387780	2.8202094	-2.9356979	8.2132539
DMDTP	Equal variances assumed	7.178	0.008	1.446	148	0.075	0.150	1.530260	1.058609	-0.561681	3.622201
	Equal variances not assumed			1.896	135.722	0.030	0.060	1.530260	0.807058	-0.065775	3.126295

Table:S2 Data of 100 exposed and 50 control samples with their six DAP metabolite concentrations.

SAMPLE CODE	Age	Sex	EDUCATION	Tobacco	ALCOHOL	DMP ng/mL	DEP ng/mL	DETP ng/mL	DMTP ng/mL	DMDTP ng/mL	DEDTP ng/mL
EXP 1	50	Male	Illiterate	No	Yes	2.81	10.700	1.4600	20.3000	2.120	0.629
EXP 2	34	Female	5-10 std	No	Yes	4.96	6.540	0.3440	3.3200	2.050	0.342
EXP 3	37	Male	Illiterate	Yes	Occasionally	3.98	18.400	1.5600	25.1000	2.140	0.785
EXP 4	48	Male	1-4 std	No	Yes	4.62	19.200	0.3870	20.3000	2.120	0.405
EXP 5	29	Male	College	No	No	3.47	7.890	0.2350	11.1000	2.120	0.362
EXP 6	74	Male	5-10 std	No	Yes	8.75	8.150	0.1660	6.9400	2.100	0.415
EXP 7	32	Female	1-4 std	No	Yes	2.87	11.400	0.9240	25.5000	2.070	0.529
EXP 8	45	Female	Illiterate	No	Toddy	4.28	9.830	1.3900	0.2330	2.090	0.404
EXP 9	35	Female	Illiterate	No	No	3.63	15.200	0.4930	11.6000	2.110	0.421
EXP 10	48	Female	Illiterate	Yes	No	4.89	15.300	0.9520	6.5400	2.050	0.429
EXP 11	54	Female	Illiterate	No	Yes	2.58	2.770	0.0670	7.7200	2.110	0.363
EXP 12	58	Male	5-10 std	No	Yes	7.38	15.700	1.2700	15.8000	1.093	0.576
EXP 13	44	Female	Illiterate	No	Yes	7.99	14.600	0.5280	11.0000	2.150	0.412
EXP 14	46	Male	5-10 std	No	Yes	5.54	10.300	3.3100	11.9000	2.030	0.890
EXP 15	42	Male	College	No	No	20.80	12.200	1.1300	11.2300	2.120	0.676
EXP 16	35	Female	5-10 std	No	Yes	6.75	12.900	0.4930	22.4000	2.080	0.388
EXP 17	70	Male	Illiterate	No	No	2.67	20.400	0.3480	15.5000	15.500	0.345
EXP 18	49	Male	5-10 std	No	Yes	346.00	37.500	0.4490	10.8000	10.800	0.481

EXP 19	40	Male	11-12 std	Yes	Yes	4.74	21.100	3.0560	6.4200	64.200	0.040
EXP 20	28	Female	5-10 std	Yes	Yes	4.26	16.000	0.3630	11.7000	11.700	0.406
EXP 21	38	Female	1-4 std	No	No	7.58	16.300	0.4150	20.0000	20.000	0.373
EXP 22	42	Male	11-12 std	No	Toddy	6.11	21.500	0.8160	11.9000	1.100	0.786
EXP 23	50	Male	5-10 std	No	No	1.92	6.680	0.2080	3.4600	3.460	0.421
EXP 24	52	Male	1-4 std	No	Yes	5.54	7.240	0.3500	9.5500	1.950	0.357
EXP 25	57	Female	Illiterate	No	No	7.16	7.480	0.3600	7.9500	7.950	0.412
EXP 26	35	Female	Illiterate	No	No	10.80	4.400	0.2720	6.5100	6.510	0.383
EXP 27	52	Male	1-4 std	No	Yes	2.26	11.750	0.0692	7.2600	7.260	0.367
EXP 28	32	Female	Illiterate	No	No	5.75	9.970	0.9800	22.3000	22.300	0.358
EXP 29	67	Male	5-10 std	No	No	9.09	6.750	0.3440	10.6000	10.600	0.386
EXP 30	51	Male	5-10 std	Yes	Yes	4.35	22.600	2.2600	16.9000	1.690	0.639
EXP 31	40	Male	5-10 std	No	Yes	16.90	15.700	0.6850	14.1000	14.100	0.445
EXP 32	38	Male	5-10 std	No	No	3.08	20.200	1.2900	28.2000	2.280	0.422
EXP 33	60	Male	Illiterate	No	Yes	1.40	13.300	0.9320	21.5000	2.110	0.373
EXP 34	40	Male	5-10 std	No	No	7.51	7.950	0.4890	8.5800	8.580	0.465
EXP 35	40	Male	1-4 std	No	Yes	7.11	10.700	0.2900	2.6800	2.680	0.394
EXP 36	35	Male	College	No	No	3.82	17.300	0.2050	11.5000	11.500	0.380
EXP 37	23	Male	College	No	Yes	434.00	11.000	0.9100	4.9500	4.950	0.564
EXP 38	57	Male	Illiterate	Yes	yes	6.25	13.900	1.2600	5.9600	5.600	0.846
EXP 39	25	Male	College	No	Yes	22.40	4.520	0.4320	14.6000	13.600	0.372

EXP 40	36	Male	College	No	Yes	10.60	17.200	0.3010	7.0000	7.030	0.665
EXP 41	29	Male	11-12 std	No	Yes	28.50	13.200	0.6000	31.1000	2.140	0.518
EXP 42	54	Male	Illiterate	Yes	Occasionally	8.66	6.410	0.2500	11.8000	2.120	0.392
EXP 43	40	Male	5-10 std	No	Yes	12.98	14.000	0.3470	12.4000	2.080	0.369
EXP 44	30	Male	11-12 std	No	Yes	8.10	16.990	0.1770	19.1000	2.150	0.388
EXP 45	60	Male	Illiterate	Yes	Toddy	6.25	9.770	1.0500	5.8600	2.140	0.579
EXP 46	50	Male	1-4 std	No	Occasionally	10.30	8.150	0.2930	16.9000	2.120	0.400
EXP 47	48	Male	Illiterate	No	Yes	9.51	9.840	0.0741	2.9600	2.160	0.425
EXP 48	36	Male	11-12 std	Yes	Occasionally	7.67	10.400	1.0700	44.4000	2.150	0.770
EXP 49	40	Male	5-10 std	No	Toddy	3.34	10.200	0.6910	10.1000	2.080	0.395
EXP 50	65	Male	Illiterate	No	Yes	3.08	16.200	0.3240	13.2000	2.150	0.359
EXP 51	70	Male	Illiterate	No	Toddy	8.45	13.400	0.2510	3.7700	2.080	0.412
EXP 52	62	Male	Illiterate	Yes	Occasionally	11.10	8.820	0.5330	7.7000	2.050	0.445
EXP 53	30	Male	5-10 std	Yes	Occasionally	9.66	11.800	0.8220	6.9100	2.130	0.383
EXP 54	55	Male	Illiterate	No	Yes	6.24	5.040	0.9830	12.7000	2.100	0.351
EXP 55	37	Male	5-10 std	No	Yes	7.41	5.570	1.0930	30.4000	2.110	0.386
EXP 56	27	Male	Illiterate	No	Yes	14.90	21.000	1.2600	26.4000	2.100	0.749
EXP 57	55	Male	Illiterate	No	Yes	6.24	15.750	3.8700	144.0000	2.180	0.660
EXP 58	70	Male	Illiterate	Yes	Yes	7.98	11.740	0.2820	17.4000	2.110	0.491
EXP 59	65	Male	Illiterate	Yes	Yes	7.33	11.900	0.7740	25.6000	2.110	0.672
EXP 60	54	Male	Illiterate	Yes	Yes	7.28	16.700	0.3690	29.3000	2.170	0.692

EXP 61	70	Male	1-4 std	Yes	Yes	6.93	15.300	0.1480	37.4000	2.110	0.399
EXP 62	60	Male	Illiterate	Yes	Yes	6.58	1.640	0.7750	13.3000	2.090	1.920
EXP 63	70	Male	Illiterate	No	Yes	10.50	12.100	1.0200	36.0000	2.110	0.750
EXP 64	70	Male	Illiterate	Yes	Yes, Toddy	13.00	14.700	0.3350	34.5000	2.070	0.377
EXP 65	35	Male	11-12 std	No	Yes	6.84	6.990	0.3880	12.8000	2.030	0.425
EXP 66	58	Male	Illiterate	No	Yes	10.85	8.750	0.4960	57.2000	2.170	0.384
EXP 67	39	Male	5-10 std	No	Occasionally	10.96	4.870	1.0280	37.3000	2.090	1.130
EXP 68	50	Male	5-10 std	No	Yes	8.28	15.000	1.2500	74.3000	2.140	0.437
EXP 69	28	Male	5-10 std	No	Occasionally	12.08	17.600	2.3200	103.0000	2.130	1.450
EXP 70	65	Male	College	Yes	Occasionally	14.10	8.580	0.8220	32.3000	2.070	0.457
EXP 71	65	Male	5-10 std	Yes	Stopped	17.45	8.720	2.7600	63.8000	2.150	1.790
EXP 72	60	Male	Illiterate	Yes	Yes	31.90	14.100	0.1190	8.1600	2.190	0.355
EXP 73	66	Male	11-12 std	No	No	6.37	9.300	0.2950	21.3000	2.130	0.439
EXP 74	50	Male	5-10 std	No	Yes	7.85	13.500	2.0400	55.0000	2.170	2.940
EXP 75	41	Male	5-10 std	No	Occasionally	9.29	6.450	0.9380	12.0000	2.110	0.756
EXP 76	52	Male	11-12 std	Yes	Occasionally	34.80	10.200	6.2900	73.4000	2.130	1.080
EXP 77	50	Male	5-10 std	Yes	Toddy	11.94	10.900	1.5400	33.1000	2.140	0.980
EXP 78	45	Male	Illiterate	Yes	Yes	9.53	13.600	0.7520	10.1000	2.160	0.366
EXP 79	37	Male	Illiterate	Yes	Yes	16.50	15.800	0.0733	31.9000	2.160	0.349
EXP 80	40	Male	5-10 std	Yes	Occasionally	7.98	24.700	0.5900	29.1000	2.050	0.444
EXP 81	52	Male	5-10 std	No	No	9.19	10.500	2.4500	34.9000	12.190	2.580

EXP 82	42	Male	Illiterate	No	Occasionally	7.37	9.450	0.1370	56.0000	12.120	0.746
EXP 83	45	Male	College	No	Occasionally	7.19	11.200	6.7800	19.6000	2.070	1.650
EXP 84	65	Male	Illiterate	No	Yes	6.83	20.700	0.4680	44.0000	2.190	0.477
EXP 85	56	Male	5-10 std	Yes	everyday	9.93	30.500	1.0100	10.9000	2.140	0.508
EXP 86	45	Male	1-4 std	Yes	Yes	13.30	9.830	1.3000	12.9000	2.160	0.581
EXP 87	35	Female	1-4 std	No	No	29.60	11.200	0.6010	13.9000	2.150	0.561
EXP 88	30	Male	College	Yes	Occasionally	26.80	10.520	0.4090	20.0000	2.180	0.363
EXP 89	52	Male	5-10 std	Yes	everyday	29.30	22.050	2.9100	3.9500	2.130	0.466
EXP 90	32	Male	5-10 std	No	Occasionally	6.90	16.600	0.1935	8.5000	2.100	0.364
EXP 91	64	Male	5-10 std	No	No	6.15	3.045	0.4105	0.5950	2.150	0.370
EXP 92	50	Male	5-10 std	No	No	8.65	9.900	0.4010	14.8500	2.090	0.394
EXP 93	43	Male	1-4 std	Yes	Yes	7.40	9.550	0.7550	13.3000	2.120	0.401
EXP 94	52	Male	5-10 std	No	Occasionally	6.50	14.365	1.6550	1.6700	2.160	0.384
EXP 95	60	Male	Illiterate	No	No	7.45	13.755	0.4685	3.7350	2.110	0.462
EXP 96	80	Male	1-4 std	No	No	8.20	25.050	2.2300	6.7700	2.120	0.522
EXP 97	42	Male	5-10 std	Yes	Toddy	8.85	17.650	17.6000	47.0000	2.100	0.397
EXP 98	70	Male	Illiterate	No	Toddy	9.50	25.150	11.3000	9.2200	2.013	0.497
EXP 99	55	Male	Illiterate	Yes,	No	7.45	15.250	1.5200	19.8500	2.050	0.383
EXP 100	50	Female	Illiterate	No	No	6.00	15.050	1.2540	2.8600	2.150	0.430
CONT 1	36	Male			Yes	7.85	9.740	0.9260	22.5000	2.090	0.614
CONT 2	70	Male	1-4 std	Yes	Yes	13.32	10.200	0.7510	7.2000	2.140	0.449

CONT 3	45	Female	Illiterate	No	No	12.94	5.280	0.1460	4.2000	2.150	0.335
CONT 4	52	Male	College	Yes	Yes	3.35	4.010	6.7940	21.1000	2.160	0.649
CONT 5	54	Male	Illiterate	No	Yes	5.62	18.200	0.6850	33.1000	2.150	0.586
CONT 6	35	Female	Illiterate	No	No	4.95	10.720	0.2920	8.5500	2.150	0.356
CONT 7	45	Female	Illiterate	No	Yes	4.21	12.330	0.2300	4.3300	2.110	0.350
CONT 8	57	Male	5-10 std	Yes	Yes	2.54	20.700	0.0942	16.3000	2.070	0.395
CONT 9	27	Female	11-12 std	No	No	4.02	4.420	0.7020	5.2800	2.240	0.734
CONT 10	43	Female	5-10 std	No	Yes	4.89	16.200	0.3510	19.0000	2.190	0.374
CONT 11	50	Female	Illiterate	Yes	Occasionally	5.97	10.100	0.9550	40.0000	2.050	0.396
CONT 12	56	Male	5-10 std	No	Yes	5.89	13.400	0.7790	28.7000	2.030	0.665
CONT 13	60	Female	Illiterate	No	Yes	3.14	4.910	6.0682	1.5900	2.170	0.505
CONT 14	28	Female	5-10 std	No	No	11.74	5.830	0.3770	20.0000	2.130	0.525
CONT 15	45	Female	Illiterate	Yes	yes	12.44	9.120	0.6190	16.4000	16.400	1.500
CONT 16	55	Male	Illiterate	Yes	Toddy	6.54	3.570	0.1370	9.5700	9.570	0.326
CONT 17	45	Female	Illiterate	Yes	Yes	18.35	10.500	1.1200	9.6100	9.610	0.599
CONT 18	46	Male	5-10 std	Yes	Yes	4.91	12.200	0.4920	13.2000	2.530	0.357
CONT 19	28	Female	5-10 std	No	No	5.64	16.580	0.6660	19.9000	1.090	0.447
CONT 20	25	Male	College	No	Yes	13.43	16.100	0.7040	34.9000	4.140	0.678
CONT 21	33	Male	College	No	Yes	5.02	9.650	0.3040	10.7000	2.800	0.415
CONT 22	70	Male	College	Yes	Occasionally	14.60	10.200	0.6570	15.2000	3.150	0.520
CONT 23	60	Male	Illiterate	No	No	11.92	8.890	0.3310	18.9000	2.160	0.354

CONT 24	38	Male	Illiterate	No	No	5.23	23.940	1.7400	28.3000	3.360	0.671
CONT 25	48	Male	Illiterate	No	Yes	12.35	18.800	3.4900	43.4000	2.120	1.661
CONT 26	45	Male	5-10 std	No	No	13.52	15.000	0.6430	9.9400	2.050	0.497
CONT 27	65	Male	1-4 std	Yes	No	12.82	3.910	1.0900	21.6000	3.010	0.642
CONT 28	60	Male	Illiterate	Yes	Yes	5.19	10.950	0.8070	28.9000	2.110	0.587
CONT 29	55	Female	Illiterate	No	No	12.37	16.100	0.3170	15.5000	2.110	0.366
CONT 30	55	Female	Illiterate	No	No	4.69	12.600	0.3170	7.4100	2.140	2.413
CONT 31	60	Male	Illiterate	No	Yes	14.53	3.040	0.1350	12.7000	2.100	0.368
CONT 32	50	Female	Illiterate	No	No	4.01	13.700	5.6610	31.1000	2.100	0.437
CONT 33	45	Female	5-10 std	No	No	15.28	10.000	1.6200	48.2000	2.130	0.475
CONT 34	37	Male	5-10 std	No	No	4.80	9.920	2.1400	48.1000	2.090	0.431
CONT 35	52	Male	5-10 std	Yes	Yes	13.55	8.050	1.2900	0.2530	2.130	0.471
CONT 36	40	Male	5-10 std	No	Yes	13.43	8.080	1.4100	8.1500	2.110	0.348
CONT 37	30	Male	Illiterate	Yes	Yes	14.43	7.880	1.3080	22.4000	2.070	1.361
CONT 38	51	Male	11-12 std	Yes	Yes	6.46	13.800	0.4490	12.8000	2.040	0.468
CONT 39	39	Female	5-10 std	No	No	9.83	12.500	0.6240	0.0396	2.110	0.545
CONT 40	50	Male	Illiterate	No	Yes	13.68	12.200	2.5900	3.6400	4.080	2.530
CONT 41	65	Male	Illiterate	No	No	5.99	11.900	2.7090	37.5000	2.100	0.407
CONT 42	47	Male	5-10 std	Yes	Yes	12.05	11.900	1.3100	27.2000	2.130	0.551
CONT 43	39	Male	College	No	No	5.77	13.800	0.4450	10.6300	2.130	0.742
CONT 44	50	Male	Illiterate	Yes	Yes	12.18	15.040	0.5980	2.8000	2.130	1.586

CONT 45	35	Male	5-10 std	No	Yes	14.56	18.100	2.2780	25.3000	2.110	0.416
CONT 46	70	Male	Illiterate	Yes	Yes	5.61	20.900	0.8680	28.3000	2.090	0.578
CONT 47	60	Male	5-10 std	Yes	Yes	5.20	13.200	4.1000	1.4100	2.090	0.677
CONT 48	65	Male	Illiterate	No	Yes	5.93	4.140	0.2180	18.0000	2.120	1.362
CONT 49	48	Male	1-4 std	No	Yes	15.70	19.700	0.6930	3.8400	2.090	0.710
CONT 50	30	Male	College	Yes	Toddy	5.15	12.600	1.3900	32.1000	2.020	3.392

- EXP = exposed
- CONT = control