NMR untargeted and HPLC-MS/MS targeted metabolomic approaches for evaluating styrene exposure in the urine of shipyard workers

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Supplementary Material
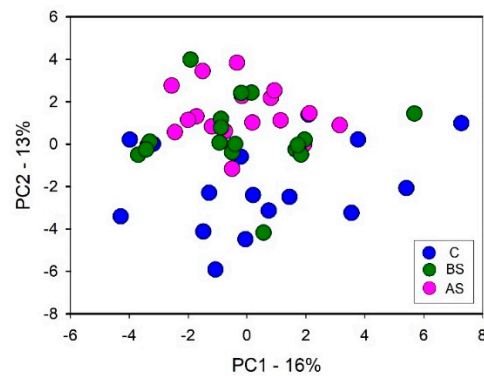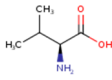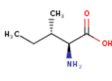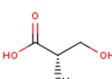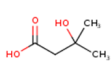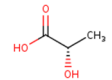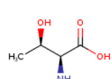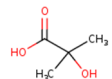
Supplementary Figure S1. Scores plot for PCA performed on non-exposed subjects (C, blue) and exposed subjects BS (green), AS (magenta). Even if the total variance explained is di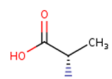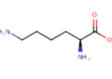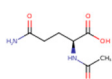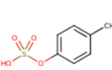stributed over 8 components, from the analysis of the first two components (29% of the total variance) a tendency towards separation along PC2 between all the samples of subject exposed (BS, AS) and C can be observed.
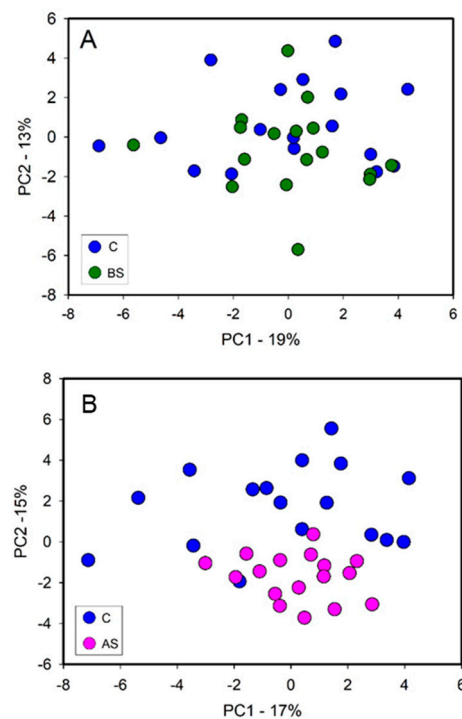
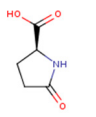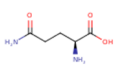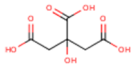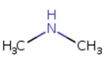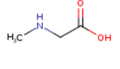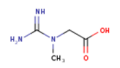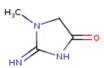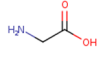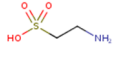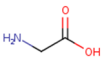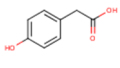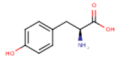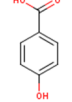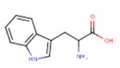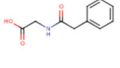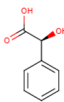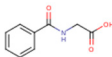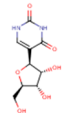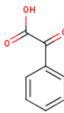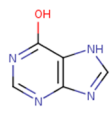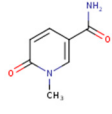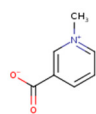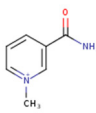Supplementary Figure S2. PCA scores plots performed on A) subjects BS (green) and C (blue), B) subjects AS (magenta) and C (blue). No obvious groupings are observed between BS subjects and controls, which is mostly observable between AS subjects and controls.

Supplementary Table 1. ^{1}H chemical shifts of metabolite signals in urines. The resonances choose for the quantification are reported in bold.

bs: broad singlet; d: doublet; dd: doublet of doublets; m: multiplet; q: quadruplet; s: singlet; t: triplet.

Metabolite	Structure	^{1}H δ ppm	Multiplicity	Assignment
1. Valine (Val)		0.99 **1.05**	d **d**	CH$_3$ **CH$_3$'**
2. Isoleucine (Ile)		0.92 **1.01** 1.99	t **d**	CH$_3$ **CH$_3$** CH
3. 3-Hydroxyisobutyrate (3-HIB)		**1.07** 2.49 3.54 3.71	d	CH$_3$
4. 3-Hydroxy-3-methylbutyrate (3-H-3-MB)		**1.27**	**s**	**CH$_3$, CH$_3$'**
5. Lactate		**1.33** 4.11	**d** q	**CH$_3$** CH
6. Threonine (Thr)		**1.33** 3.59 4.26	**d** d m	**CH$_3$** α-CH β-CH
7. 2-Hydroxyisobutyrate (2-HIB)		**1.36**	**s**	**CH$_3$, CH$_3$'**
8. Alanine (Ala)		**1.49** 3.78	**d** q	**CH$_3$** α-CH
9. Lysine (Lys)		1.48 **1.71** 1.89 3.02 3.74	m **m** m m m	CH$_2$ **CH$_2$** CH$_2$ CH$_2$ CH
10. N-acetylglutamine (NAcGln)		1.95 2.12 **2.27** 4.18 7.97	**m** bs	**CH$_2$**
11. p-Cresol sulphate		**2.35** 7.21 7.28	**bs** dd dd	**CH$_3$** CH, CH' CH, CH'

12. Pyroglutamate (pyro-Glu)		2.03 **2.40** 2.50 4.19	m **m** m dd	CH$_2$ **CH** CH CH
13. Glutamine (Gln)		2.13 **2.46** 3.78	m **m** t	CH$_2$ **CH$_2$** CH
14. Citrate		**2.54** 2.69	**d** d	**CH, CH'** CH, CH'
15. Dimethylamine (DMA)		**2.73**	**s**	**CH$_3$, CH$_3$'**
16. Sarcosine (Sar)		**2.78**	**s**	**CH$_3$**
17. Creatine		**3.05** 3.95	**s** s	**CH$_3$** CH$_2$
18. Creatinine		3.03 **4.05**	s **s**	CH$_3$ **CH$_2$**
19. Trimethylamine N-Oxide (TMAO)		**3.27**	**s**	**CH$_3$** CH$_2$
20. Taurine (Tau)		3.27 **3.43**	t **t**	CH2 **CH2**
21. Glycine (Gly)		**3.57**	**s**	**CH$_2$**
22. 4-Hydroxyphenylacetate (4-HPA)		**6.87** 7.17	**dd** dd	**CH,CH** CH,CH
23. Tyrosine (Tyr)		**6.90** 7.18	**dd** dd	**2-CH,6-CH** 3-CH,5-CH
24. 4-Hydroxybenzoate (4-HBz)		6.97 **7.76**	dd **dd**	2-CH,6-CH **3-CH,5-CH**
25. Tryptophan (Trp)		7.20 7.27 7.29 **7.50** 7.70	m m bs **d** d	5-CH 6-CH 2-CH **4-CH** 7-CH
26. Phenylacetylglycine (PAGly)		**7.36** 7.42	**m** m	**2,4,6-CH** 3,5-CH

27. Mandelic acid (MA)		5.00	s	CH
		7.38-7.43	m	2-5-CH
		7.42	**s,s**	OH, OH'
28. Hippurate (Hipp)		3.97	d	CH$_2$
		7.55	**m**	**3,5-CH**
		7.64	m	4-CH
		7.83	m	2,6-CH
29. Pseudouridine (PSI)		**7.67**	**bs**	**CH**
30. Phenylglyoxylic acid (PGA)		7.60	m	5,3-CH
		7.76	m	**4-CH**
		7.96	m	6,2-CH
31. Hypoxanthine (Hyp)		**8.19**	s	2-CH
		8.21	s	7-CH
32. N1-Methyl-2-pyridone-5-carboxamide (2PY)		3.64	s	N-CH$_3$
		6.67	d	3-CH
		7.98	d	4-CH
		8.33	**dd**	**6-CH**
33. Formate		**8.46**	**s**	**CH**
34. U01(2-Methylnicotinamide?)		8.06		
		8.54		
		8.78	**d**	**6-CH**
35. Trigonelline (Trig)		4.44	s	N-CH$_3$
		8.08	m	5-CH
		8.84	m	4,6-CH
		9.12	**s**	**2-CH**
36. 1-Methylnicotinamide (1-MNA)		8.17	t	5-CH
		8.89	d	4-CH
		8.96	d	6-CH
		9.28	**s**	**2-CH**