Electronic Supplementary Material (ESI) for Toxicology Research.

Supplementary Data

^{1}H NMR-based Urine Metabolomics for Evaluation of Kidney Injury on Wistar Rat by 3-MCPD

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MATERIALS AND METHODS

1.1 Histopathology

The largest lobe of the liver and testis from the control and treated groups was excised, fixed in 10 % formalin, processed with standard histological protocol, and cut into 4-μm serial sections using a microtome. The deparafinized sections were stained with haematoxylin and eosin for histopathological examination.

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Fig.S1 Box plots and kernel density plots before and after normalization, selected methods: Row-wise normalization: Probabilistic Quotient Normalization; Data transformation: N/A; Data scaling: Pareto scaling.

Fig. S2 Organ coefficient comparison between controls and 3-MCPD treated rat (mean±SD, $^{*}P<0.05$, $^{**}P<0.01$)

Fig. S3 Photomicrographs of kidney and testis sections with haematoxylin-eosin observed by light microscope. Control group rats showing normal kidney (G, H) and testis (K, L) (magnification, G, K: 200×; H, L: 400×) and 3-MCPD treated rats showing testis with lesion (I, J) and testis (M, N) (magnification, I, M: 200×; J, N: 400×).

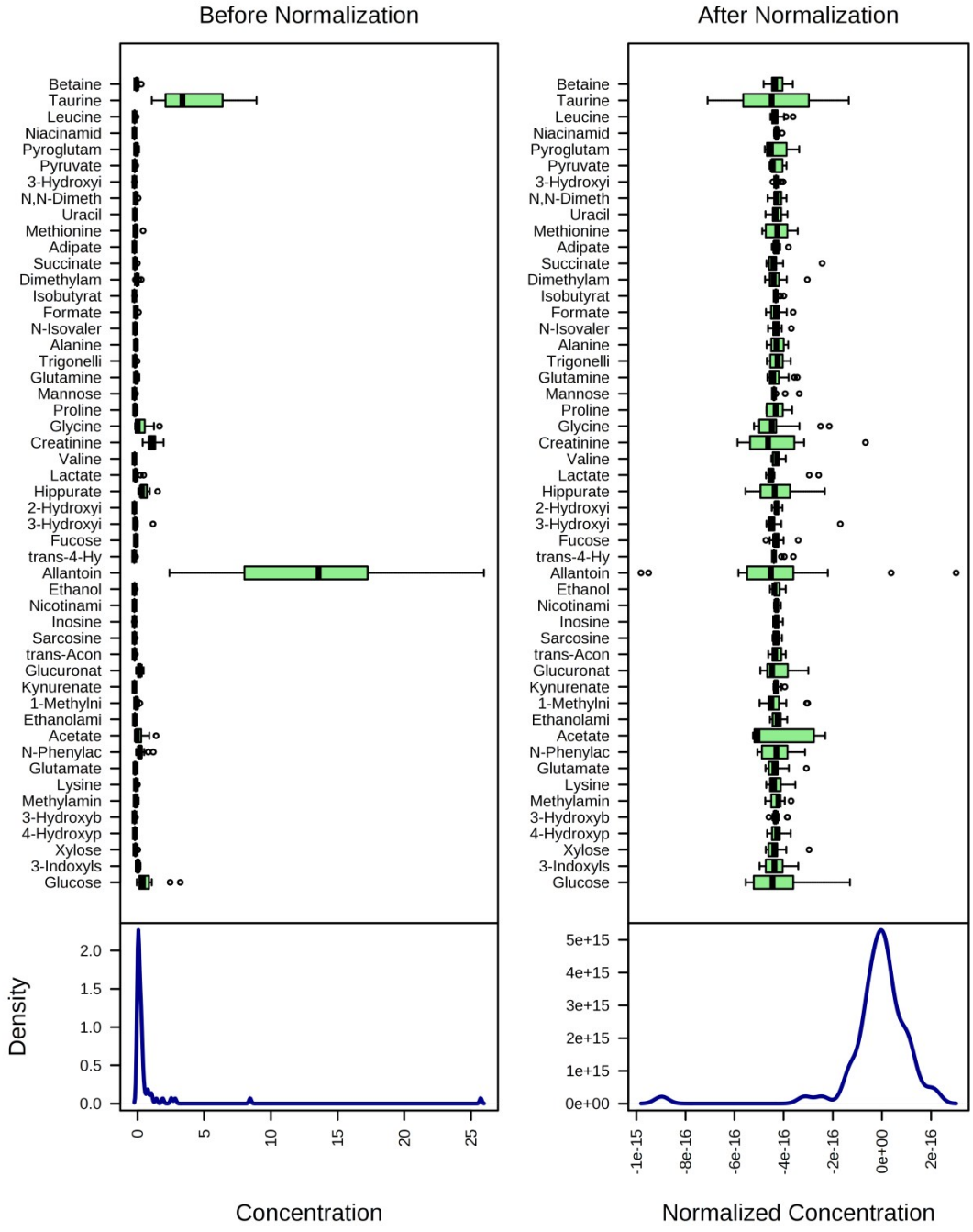
Fig. S4 Clinical chemistry comparison between controls and 3-MCPD treated rat for GAL and NAG (mean±SD, $^{*}P<0.05$, $^{**}P<0.01$).

Fig. S5 the permutations plot was applied for assess the risk of the current OPLS-DA model, (A) 7 days, (B) 21 days, (C) 35 days and (D) the group of 35 day VS. the groups of control, 7 days and 21 days.

Table Titles List:

Table S1 the pool of 68 metabolites identified in rat urine by NMR

Table S2 The fold change value selected potential biomarkers



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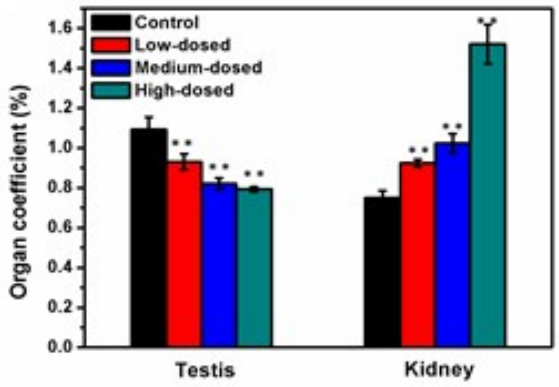

Fig. S2 Organ coefficient comparison between controls and 3-MCPD treated rat (mean±SD, $^{*}P<0.05$, $^{**}P<0.01$)

We observed that the testis coefficient decreased and kidney coefficient increased significantly by the 35th day for the treated group, as shown in Fig. S2.

Fig. S3 Photomicrographs of kidney and testis sections with haematoxylin-eosin observed by light microscope. Control group rats showing normal kidney (G, H) and testis (K, L) (magnification, G, K: 200×; H, L: 400×) and 3-MCPD treated rats showing testis with lesion (I, J) and testis (M, N) (magnification, I, M: 200×; J, N: 400×).

The kidney histopathology of the control rat, shown in Fig. S3 G and Fig. S3 H, revealed glomerulus and kidney tubules; the complete afferent artery entering the glomerular from the vascular pole and the smooth muscle cells specialized through the granulosa cells near afferent artery walls of juxtaglomerular was observed, consistent with normal kidney[1]. In Fig. S3 I and Fig. S3 J, the kidney histopathology of high-dose 3-MCPD treated rat, we observed many small vesicas, elongated radiated or cystic arrangement, salient features of hydropic degeneration. Additionally, residual glomeruli with abnormal shape and incomplete form were observed in the renal cortex, surrounded by disorderedgranulosa cells and capillaries[1]. Together, these findings reveal

that 3-MCPD had significant toxic effects on rat kidney. The testis coefficient evaluation showed that the 3-MCPD caused damage to rat testis, supported by the testis histopathology. The testis histopathology of control rats showed seminiferous tubules with a large number of germ cells; sertoli cells without the central tubules and testis leydig cells within the interstitial space between seminiferous tubules, consistent with healthy testis (Fig. S3K and Fig S3L). In contrast, the testis histopathology of high-dose 3-MCPD treated rat (Fig. S3M and Fig S3 N) revealed testicular atrophy, mainly as atrophy of focal seminiferous tubules, uneven distribution of leydig cells and the disordered phenotype of the remaining seminiferous tubules, consistent with 3-MCPD having potential reproductive toxicity.

(1) Klatt, E. C. *Robbins and Cotran atlas of pathology*; Elsevier Health Sciences, 2014.

Fig. S4 Clinical chemistry comparison between controls and 3-MCPD treated rat, (D) for GAL, (E) for NAG (mean±SD, $^{*}P<0.05$, $^{**}P<0.01$).

The changes in serum biochemical parameters are presented in Fig. 4D and Fig. 4E. At 7th day, the GAL (β-galactosidase) and NAG (N-acetyl β-D amino glycosidase enzymes) levels, which were related to kidney function, were significantly increased in all the treated groups, most obviously in the high-dose treated rats[2]

(2) Wellwood, J.; Lovell, D.; Thompson, A.; Tighe, J. *The Journal of pathology* **1976**, *118*, 171-182.

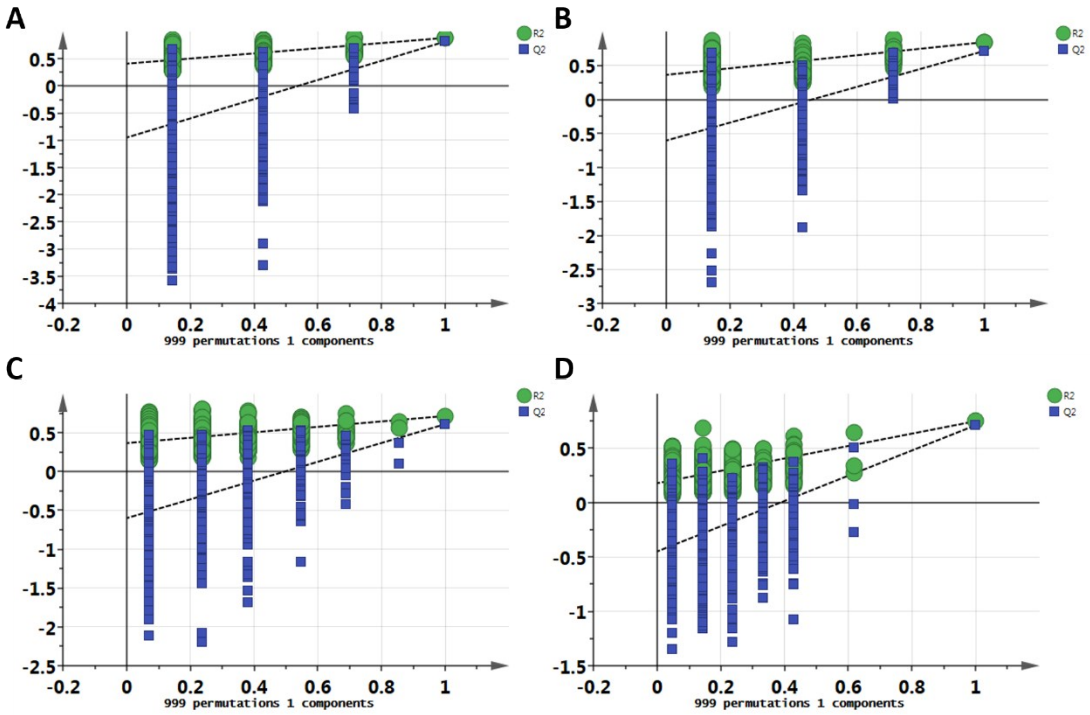

Fig. S5 the permutations plot was applied for assess the risk of the current OPLS-DA model, (A) 7 days, (B) 21 days, (C) 35 days and (D) the group of 35 day VS. the groups of control, 7 days and 21 days.

Table S1 The pool of 68 metabolites identified in rat urine by NMR

Classification	Metabolites	$_1$H chemical shift (ppm)	Formula	SMILES
alcohols	Ethanol	1.162, 1.174, 1.186	C2H6O	CCO
	Methanol	3.352	CH4O	CO
amides	Allantoin	6.035	C4H6N4O3	NC(=O)NC1NC(=O)NC1=O
	N-Isovaleroylglycine	0.921, 0.932	C7H13NO3	CC(C)CC(=O)NCC(O)=O
	N-Phenylacetylglycine	3.665, 3.741. 3.751. 7.335-7.360, 7.395-7.420	C10H11NO3	OC(=O)CNC(=O)CC1=CC=CC=C1
	Creatinine	3.024,3.920	C4H7N3O	CN1CC(=O)NC1=N
	Dimethylamine	2.713	C2H7N	CNC
	Ethanolamine	3.127, 3.135, 3.144	C2H7NO	NCCO
	Methylamine	2.595	CH5N	CN
amino acid derivatives	3-Indoxylsulfate	7.480, 7.494, 7.680, 7.694	C8H7NO4S	OS(=O)(=O)OC1=C[NH]C2=CC=CC=C12
	Creatine	3.029, 4.042	C4H9N3O2	CN(CC(O)=O)C(N)=N
	Hippurate	3.954,3.964,7.524,7.537,7.611,7.624,7.636,7.815,7.828	C9H9NO3	OC(=O)CNC(=O)C1=CC=CC=C1
	Kynurenate	6.927	C10H7NO3	OC(=O)C1=NC2=CC=CC=C2C(=C1)O
	Pyroglutamate	4.158,4.168,4.173,4.183	C5H7NO3	OC(=O)[C@@H]1CCC(=O)N1
	Urea	5.740-5.860	CH4N2O	NC(N)=O
amino acids	Alanine	1.464,1.476	C3H7NO2	C[C@H](N)C(O)=O
	Betaine	3.252,3.891	C5H11NO2	C[N+](C)(C)CC([O-])=O
	Glutamate	2.330,2.335,2.342,2.348,2.355	C5H9NO4	N[C@@H](CCC(O)=O)C(O)=O
	Glutamine	2.425,2.436,2.451,2.462	C5H10N2O3	N[C@@H](CCC(N)=O)C(O)=O
	Glycine	3.554	C2H5NO2	NCC(O)=O
	Isoleucine	0.994,1.005	C6H13NO2	CC[C@H](C)[C@H](N)C(O)=O
	Leucine	0.939,0.950,0.961	C6H13NO2	CC(C)C[C@H](N)C(O)=O
	Lysine	1.688,1.700,1.714,1.727	C6H14N2O2	NCCCC[C@H](N)C(O)=O
	Methionine	3.125	C5H11NO2	CSCC[C@H](N)C(O)

			S	=O
	N,N-Dimethylglycine	2.914,3.714	C4H9NO2	CN(C)CC(O)=O
	Proline	3.315,3.324,3.335	C5H9NO2	OC(=O)[C@@H]1CCCN1
	Sarcosine	1.29	C3H7NO2	CNCC(O)=O
	Taurine	3.409-3.431	C2H7NO3S	NCCS(O)(=O)=O
	Threonine	1.314,1.324	C4H9NO3	C[C@@H](O)[C@H](N)C(O)=O
	Valine	0.972,0.984	C5H11NO2	CC(C)[C@H](N)C(O)=O
	trans-4-Hydroxy-L-proline	4.324-4.354	C5H9NO3	O[C@H]1CN[C@@H](C1)C(O)=O
	β-Alanine	3.174	C3H7NO2	NCCC(O)=O
ammoniums compounds	Choline	3.187	C5H14NO	C[N+](C)(C)CCO
	Succinate	2.399	C4H6O4	OC(=O)CCC(O)=O
	Trimethylamine N-oxide	3.257	C3H9NO	C[N+](C)(C)[O-]
	sn-Glycero-3-phosphocholine	3.213	C8H21NO6P	C[N+](C)(C)CCOP([O-])(=O)OC[C@H](O)CO
food and components	Dimethyl sulfone	3.141	C2H6O2S	C[S](C)(=O)=O
	Trigonelline	4.424,8.810-8.835,8.810-8.835,9.112	C7H7NO2	C[N+]1=CC=CC(=C1)C([O-])=O
nucleic acid components	5,6-Dihydrouracil	2.659,2.671,2.682	C4H6N2O2	O=C1CCNC(=O)N1
	Cytosine	5.956,5.968	C4H5N3O	NC1=NC(=O)NC=C1
	Inosine	8.213,8.332	C10H12N4O5	OC[C@H]1O[C@H]([C@H](O)[C@@H]1O)[N]2C=NC3=C2N=CNC3=O
	Uracil	7.513,7.526	C4H4N2O2	O=C1NC=CC(=O)N1
organic acids	2-Hydroxyisobutyrate	1.349	C4H8O3	CC(C)(O)C(O)=O
	2-Oxoglutarate	2.421,2.432,2.444	C5H6O5	OC(=O)CCC(=O)C(O)=O
	3-Hydroxybutyrate	1.185,1.196	C4H8O3	C[C@@H](O)CC(O)=O
	3-Hydroxyisobutyrate	1.054,1.066	C4H8O3	CC(CO)C(O)=O

	3-Hydroxyisovalerate	1.26	C5H10O3	CC(C)(O)CC(O)=O
	4-Hydroxyphenylacetate	6.841,6.855	C8H8O3	OC(=O)CC1=CC=C(O)C=C1
	Acetate	1.912	C2H4O2	CC(O)=O
	Acetoacetate	2.27	C4H6O3	CC(=O)CC(O)=O
	Adipate	1.54	C6H10O4	OC(=O)CCCCC(O)=O
	Formate	8.447	CH2O2	OC=O
	Fumarate	6.512	C4H4O4	OC(=O)/C=C/C(O)=O
	Glycolate	3.941	C2H4O3	OCC(O)=O
	Isobutyrate	1.036,1.048	C4H8O2	CC(C)C(O)=O
	Lactate	1.316,1.327	C3H6O3	C[C@H](O)C(O)=O
	Pyruvate	4.364	C3H4O3	CC(=O)C(O)=O
	Sebacate	1.29	C10H18O4	OC(=O)CCCCCCCCC(O)=O
	Sucrose	4.202,4.217	C12H22O11	OC[C@H]1O[C@H](O[C@]2(CO)O[C@H](CO)[C@@H](O)[C@@H]2O)[C@H](O)[C@@H](O)[C@@H]1O
	trans-Aconitate	6.586	C6H6O6	OC(=O)C/C(=C\C(O)=O)C(O)=O
sugars	Fucose	1.231,1.242,4.541,4.554	C6H12O5	C[C@@H]1OC(O)[C@@H](O)[C@H](O)[C@@H]1O
	Glucose	3.466,3.482,3.497,4.633,4.646,5.224,5.231	C6H12O6	OC[C@H]1O[C@@H](O)[C@H](O)[C@@H](O)[C@@H]1O
	Glucuronate	5.235,5.241	C6H10O7	OC1O[C@@H]([C@@H](O)[C@H](O)[C@H]1O)C(O)=O
	Mannose	4.893,5.186,5.189	C6H12O6	OC[C@H]1OC(O)[C@@H](O)[C@@H](O)[C@@H]1O
	Xylose	4.563,4.576	C5H10O5	O[C@@H]1COC(O)[C@H](O)[C@H]1O
vitamin/cofactors	1-Methylnicotinamide	4.452,8.860,8.875，9.250	C7H9N2O	C[N+]1=CC=CC(=C1)C(N)=O

	Niacinamide	8.694-8.703	C6H6N2O	NC(=O)C1=CC=CN=C1
	Nicotinamide N-oxide	8.729	C6H6N2O2	NC(=O)C1=CC=C[N+](=C1)[O-]

Table S2 The fold change value of the selected potential biomarkers

Metabolites name	Fold change
Creatine	0.010633
Glycine	0.12392
Threonine	0.1362
Betaine	0.46056
Taurine	0.50295
Glutamate	1.8758
1-Methylnicotinamide	0.52155

The fold change value of the selected potential biomarker were calculated on a web-based Metaboanalyst 3.0. (http://www.metaboanalyst.ca/MetaboAnalyst/faces/ModuleView.xhtml)