Supplementary Methods

Animals

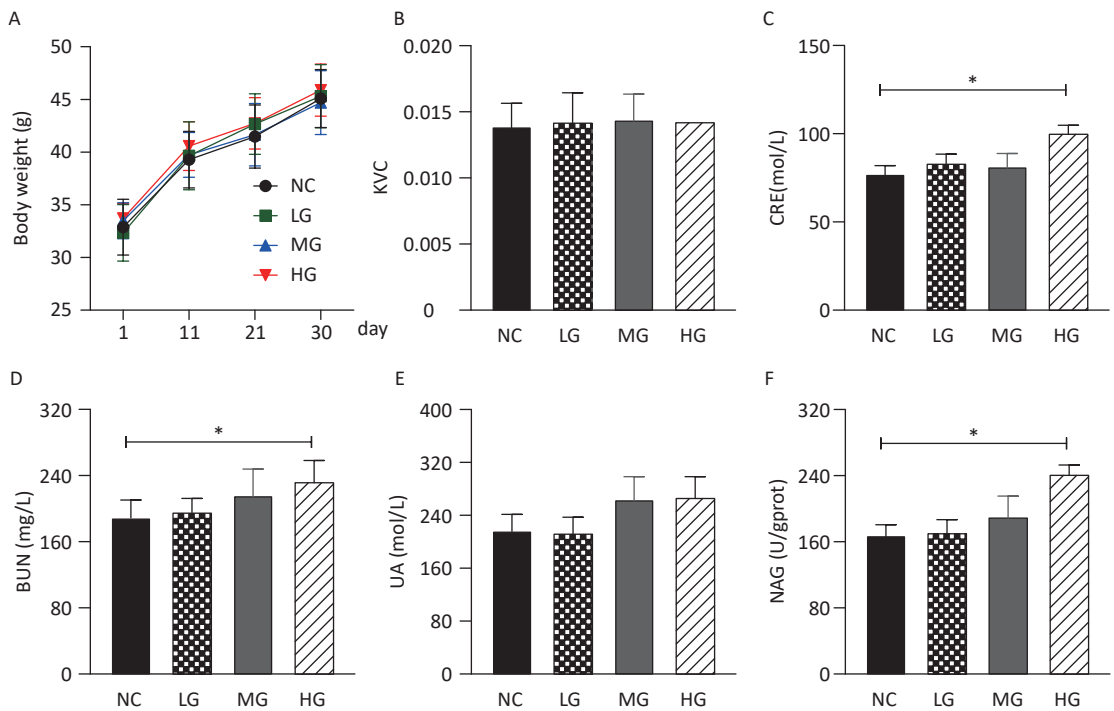
Forty male Kunming mice, weighing between 27 and 34 g, were purchased from Weitong Lihua Laboratory Animal Technology Co. Ltd. (Beijing, China) and maintained in standard environmental conditions as described in our previous study[1]. All the animal experiments were approved by the Committee on the Ethics of Animal Experiments of the Qiqihar Medical University (Qiqihar, China; Approval number QMU-AECC-2022-136).

Study Design

The test animals were administered RoundUp® (41% isopropyl amine salt, Monsanto, St, Louis, MO, USA) via gavage for 30 days. Thereafter, the mice were randomly assigned to 4 groups (10 mice/group) as described in Supplementary Table S5. The GBH dosage for mice in the high-dose GBH (HG) group was approximately equal to 1/10 the LD50 (oral pathway, 5,600 mg/kg)[2] or the NOAEL of RoundUp® in mice (EPA, 1993). Moreover, it has been confirmed that the 500 mg/kg dose induces sub-chronic to chronic toxicity in mice[3]. The medium and low doses were 1/2 and 1/10 the dosage administered to mice in the HG group, as described in our previous studies[1,4]. After the last herbicide administration, the animals were euthanized via anesthesia using pentobarbital, and their kidneys were collected, weighed, separated, fixed and stored at −80 °C until further analysis.

Measurement of Kidney Function Parameters

Creatinine (CRE), urea nitrogen (BUN), β-N-acetylaminoglucosidase (NAG), and uric acid (UA) were assayed using commercial reagent kits purchased from Jiancheng Bio-Technology Co., Ltd. (Nanjing, China).



Supplementary Figure S1. The effects of GBH exposure on body weight and kidney function parameters in the serum. A, body weight; B, kidney viscera coefficient; C, CRE; D, BUN; E, UA; F, NAG. Compared with the control group, $^{*}P < 0.05$; $n = 6$. NC, normal control group, 0 mg/kg per day; LG, low dose group, 50 mg/kg per day; MG, middle dose group, 250 mg/kg per day; HG, high dose group, 500 mg/kg per day. Data are presented as means ± SD.

Histological Analysis

Kidney tissue samples were fixed in 4% paraformaldehyde, dehydrated with alcohol, stained with hematoxylin-eosin, and observed *via* light microscopy.

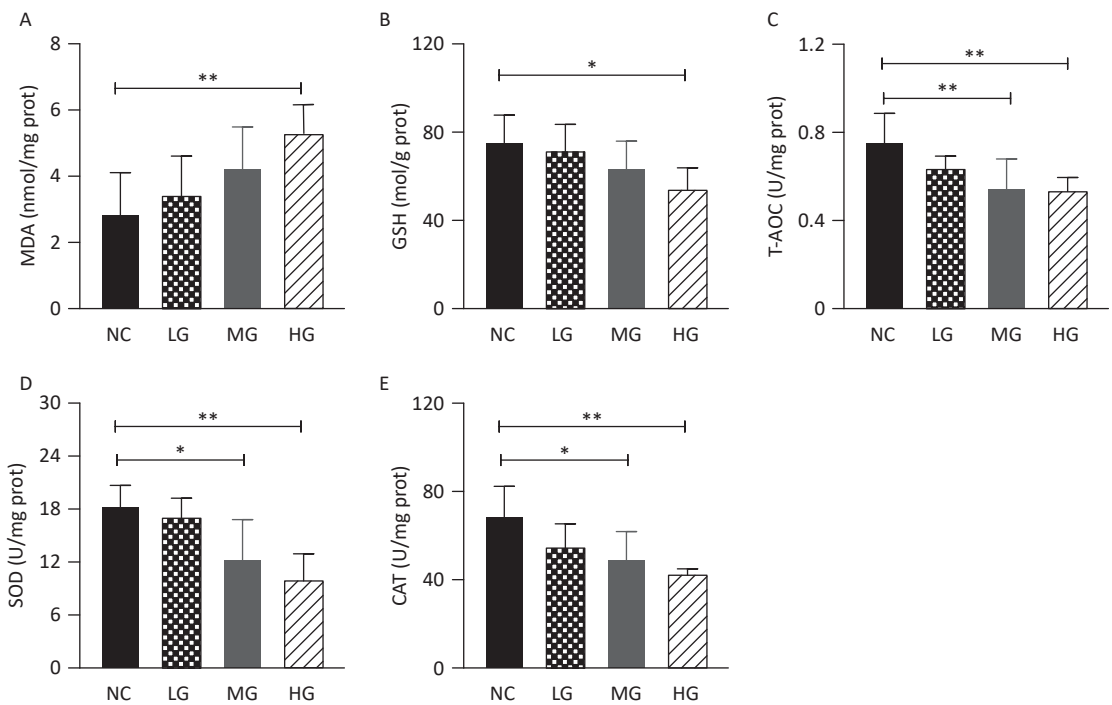
Measurement of Oxidative Stress Indicators of Kidney Samples

OS level in the kidneys of mice from the different groups were assessed using glutathione (GSH), superoxide dismutase (SOD), MDA, catalase (CAT), and total antioxidant capacity (T-AOC) assay kits purchased from Jiancheng Bio-Technology Co., Ltd.

Chromatography, Mass Spectrometry and Data Processing

Six kidney samples from NC and HG groups were randomly selected to perform untargeted metabonomics at Biotree Ltd. (Shanghai, China). Briefly, all samples were pretreated and detected based on a UPLC system (Vanquish, Thermo Fisher Scientific) with a UPLC BEH Amide column (2.1 mm × 100 mm, 1.7 μm) coupled to Q Exactive HFX mass spectrometer (Orbitrap MS, Thermo). The QE HFX mass spectrometer was used to acquire MS/MS spectra based on information-dependent acquisition software (Xcalibur, Thermo), which evaluates the full scan MS spectrum. ProteoWizard was used to convert the raw data to the mzXML format. Further, the data was processed with a new program which was developed using R and based on XCMS, for peak detection, extraction, alignment, and integration. Peaks were pretreated through relative standard deviation de-noising. Then, the missing values were filled up by half of the minimum value. Also, total ion current normalization method was employed in this data analysis. Then an in-house MS2 database (BiotreeDB) was applied in metabolite annotation. The cutoff for annotation was set at 0.3.

SIMCA16.0.2 software package (Sartorius Stedim Data Analytics AB, Umea, Sweden) was used to perform a multivariate analysis of the final dataset. Supervised orthogonal projections to latent structures-discriminate analysis (OPLS-DA) was created to visualize the separation between two groups and identified significantly

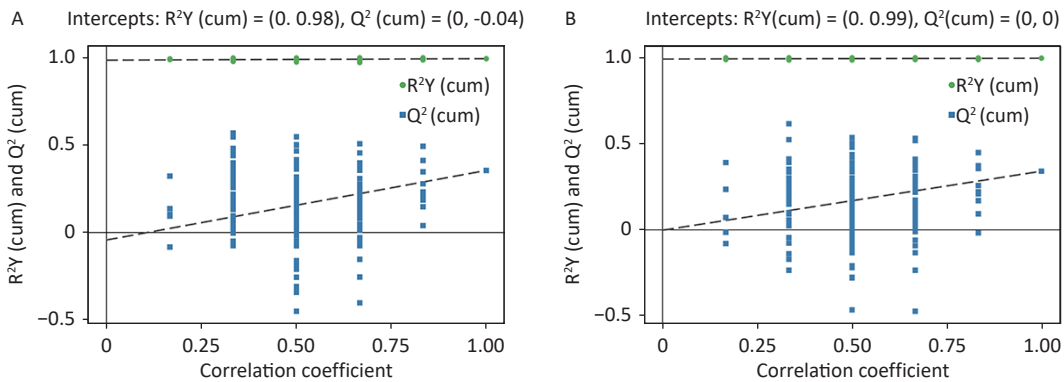


Supplementary Figure S2. The effect of GBH exposure on histopathology in kidneys of male mice. A-H, HE-stain magnification; A B, C and D, 100×; E, F, G and H, 400×. Black arrow: water degeneration; blue arrow: protein deposition in renal tubules. *n* = 6. NC, normal control group, 0 mg/kg per day; LG, low dose group, 50 mg/kg per day; MG, middle dose group, 250 mg/kg per day; HG, and high dose group, 500 mg/kg per day.

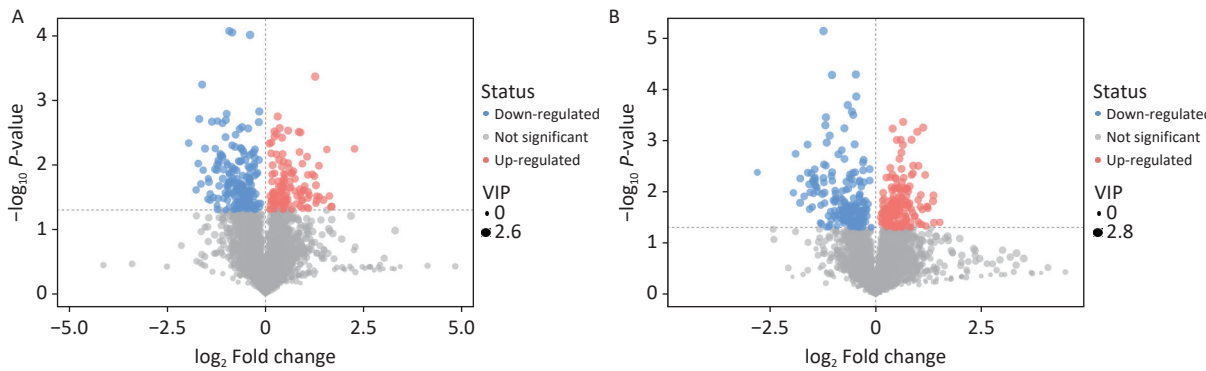
changed metabolites (SCMs). Moreover, 200 times permutations were conducted to check the robustness and predictive ability of the OPLS-DA model. The compound with $P < 0.05$ (student t-test) and the value of variable importance in the projection (VIP) > 1 was considered as SCM. All SCMs were annotated to the Kyoto Encyclopedia of Genes and Genomes databases (KEGG, http://www.genome.jp/kegg/) for pathway enrichment analysis.

Statistical Analysis

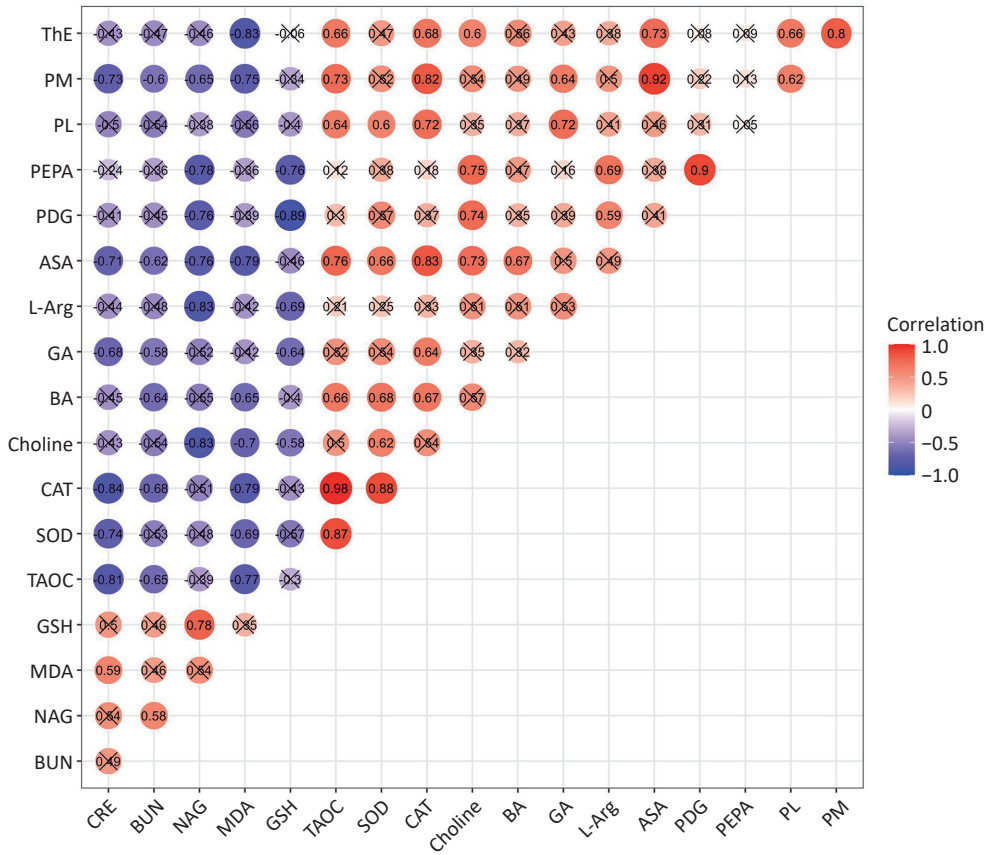
Group comparisons to identify significant differences were realized via one-way analysis of variance and post-hoc Dunnett's test based using SPSS software version 17.0 (Beijing Stats Data Mining Co. Ltd., Beijing, China). Pearson's correlation analyses were also performed to identify correlations between kidney function indices, SCMs and OS indices. Statistical significance was set at $P < 0.05$.



Supplementary Figure S3. Permutation test of OPLS-DA model in the positive and negative ion modes. A, permutation test of OPLS-DA model in the positive ion mode; B, permutation test of OPLS-DA model in the negative ion mode. $n = 6$.



Supplementary Figure S4. Volcano plots in the positive and negative ion modes. A, volcano plot in the positive ion mode; B, volcano plot in the negative ion mode. $n = 6$.



Supplementary Figure S5. Correlation analysis between kidney function indexes and oxidative stress indicators and SCMs. Red indicates that the correlation coefficient greater than 0, and blue indicates that the correlation coefficient lower than 0; the number represents the value of the correlation coefficient; the dot without × indicates that there is statistical difference, the dot with × indicate that there is no statistical difference of correlation. PM, pyridoxamine; PL, pyridoxal; ASA, argininosuccinic acid; GA, D-Glucuronic acid; BA, betaine aldehyde; PDG, 2-phospho-D-glyceric acid; PEPA, phosphoenolpyruvic acid; L-Arg, L-arginine; CRE, creatinine; BUN, urea nitrogen; NAG, β-N-acetylaminoglucosidase; MDA, malondialdehyde; GSH, glutathione; CAT, catalase; T-AOC, total antioxidant capacity; SOD, superoxide dismutase. n = 6.

Supplementary Table S1. SCMs in the positive mode between NC and HG groups (*n* = 6)

Compounds	rt	mz	KEGG ID	VIP	*P*-value	Fold change
Argininosuccinic acid	485.52	291.13	C03406	2.43	0.010	0.43
Choline	299.16	104.11	C00114	2.34	0.002	0.89
Deoxyuridine	180.59	229.08	C00526	2.32	0.003	1.29
Purine	105.16	121.05	C15587	2.02	0.036	0.71
L-Gulose	324.54	203.05	C15923	1.91	0.036	1.92
Histamine	417.27	112.09	C00388	1.90	0.021	0.50
Betaine aldehyde	376.90	102.09	C00576	1.89	0.033	0.74
3-Dehydrosphinganine	85.62	300.29	C02934	1.84	0.034	1.16
Thiamine	386.22	265.11	C00378	1.82	0.047	0.70
Glycitein	43.42	285.08	C14536	1.80	0.035	0.61
N-Acetylhistidine	367.64	198.09	C02997	1.80	0.033	2.11
Queuine	286.50	278.12	C01449	1.77	0.027	0.63
3-Methylguanine	207.89	166.07	C02230	1.77	0.020	0.60
Galactosylhydroxylysine	548.73	325.16	C05547	1.75	0.016	0.75
5-Amino-6-ribitylamino uracil	313.06	277.12	C04732	1.73	0.048	1.35
Pyridoxamine	325.71	169.10	C00534	1.64	0.048	0.59
p-Aminobenzoic acid	41.83	138.05	C00568	1.58	0.048	0.74
Pyridoxal	41.66	168.07	C00250	1.35	0.039	0.59

Supplementary Table S2. SCMs in the negative mode between NC and HG groups (*n* = 6)

Compounds	rt	mz	KEGG ID	VIP	*P*-value	Fold change
Argininosuccinic acid	486.86	289.11	C03406	2.56	0.001	0.45
2-Phospho-D-glyceric acid	477.03	184.98	C00631	2.33	0.006	0.37
Phosphoenolpyruvic acid	491.77	166.97	C00074	2.21	0.037	0.62
Xanthosine	240.10	283.07	C01762	2.15	0.006	0.77
D-Glucuronic acid	403.82	193.03	C00191	1.99	0.020	0.74
Eicosadienoic acid	40.56	307.26	C16525	1.94	0.016	0.77
D-Malic acid	416.64	133.01	C00497	1.93	0.017	0.43
L-Arginine	542.01	173.10	C00062	1.87	0.039	0.77
Deoxyribose 5-phosphate	184.19	213.02	C00673	1.87	0.033	1.18
Terephthalic acid	373.64	165.02	C06337	1.81	0.026	1.10
Beta-D-Galactose	319.55	179.06	C00962	1.78	0.038	2.01
Pentadecanoic acid	42.26	241.22	C16537	1.77	0.027	0.81
3-(3,4-Dihydroxy-5-methoxy)-2-propenoic acid	386.63	209.04	C05619	1.74	0.041	1.10
D-Ornithine	540.85	131.08	C00515	1.15	0.042	0.59

Supplementary Table S3. The information of pathways in the positive mode (NC *vs*. HG) (*n* = 6)

Pathway	Total	Hits	*P*	Impact	Hits Cpd
Vitamin B6 metabolism	9	2	0.005	0.569	Pyridoxal; Pyridoxamine
Glycine, serine and threonine metabolism	31	2	0.051	< 0.001	Choline; Betaine aldehyde
Thiamine metabolism	7	1	0.081	0.400	Thiamine
Riboflavin metabolism	11	1	0.125	< 0.001	5-Amino-6-ribitylamino uracil
Histidine metabolism	15	1	0.166	0.220	Histamine
Sphingolipid metabolism	21	1	0.225	0.083	3-Dehydrosphinganine
Alanine, aspartate and glutamate metabolism	24	1	0.253	0.022	Argininosuccinic acid
Glycerophospholipid metabolism	30	1	0.306	0.023	Choline
Pyrimidine metabolism	41	1	0.395	0.021	Deoxyuridine
Arginine and proline metabolism	44	1	0.417	0.024	Argininosuccinic acid

Supplementary Table S4. The information of pathways in the negative mode (NC *vs.* HG, *n* = 6)

Pathway	Total	Hits	P	Impact	Hits Cpd
Glycolysis or Gluconeogenesis	26	2	0.022	0.201	Phosphoenolpyruvic acid ; 2-Phospho-D-glyceric acid
D-Arginine and D-ornithine metabolism	4	1	0.036	< 0.001	D-Ornithine
Arginine and proline metabolism	44	2	0.059	0.106	Argininosuccinic acid; L-Arginine
Ascorbate and aldarate metabolism	9	1	0.080	0.400	D-Glucuronic acid
Pentose and glucuronate interconversions	16	1	0.138	< 0.001	D-Glucuronic acid
Starch and sucrose metabolism	19	1	0.162	< 0.001	D-Glucuronic acid
Pentose phosphate pathway	19	1	0.162	0.068	Deoxyribose 5-phosphate
Citrate cycle (TCA cycle)	20	1	0.169	< 0.001	Phosphoenolpyruvic acid
Pyruvate metabolism	23	1	0.192	< 0.001	Phosphoenolpyruvic acid
Alanine, aspartate and glutamate metabolism	24	1	0.200	0.022	Argininosuccinic acid
Inositol phosphate metabolism	28	1	0.229	< 0.001	D-Glucuronic acid
Biosynthesis of unsaturated fatty acids	42	1	0.325	< 0.001	Icosadienoic acid
Purine metabolism	68	1	0.474	< 0.001	Xanthosine
Aminoacyl-tRNA biosynthesis	69	1	0.479	< 0.001	L-Arginine

Supplementary Table S5. Glyphosate doses for all treatment groups

Group	Abbreviation	Dose of glyphosate
Control group	NC	0 mg/kg per day
Low-dose GBH group	LG	50 mg/kg per day
Medium-dose GBH group	MG	250 mg/kg per day
High-dose GBH group	HG	500 mg/kg per day

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2. FAO/WHO, *Pesticide Residues in Food* 2016, FAO/WHO: Rome, Italy, .
3. Bali YA, Kaikai NE, Ba-M'hamed S, et al. Learning and memory impairments associated to acetylcholinesterase inhibition and oxidative stress following glyphosate based-herbicide exposure in mice. Toxicology, 2019; 415, 18–25.
4. Qi L, Dong YM, Chao H, et al. Glyphosate based-herbicide disrupts energy metabolism and activates inflammatory response through oxidative stress in mice liver. Chemosphere, 2023; 315, 137751.