

Supplemental Information for
Occurrence and removal of four artificial sweeteners in wastewater
treatment plants of China

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Text S1. Sample extraction

The volume of sample collection is 2 L for influent, 4 L for effluent, and 1 L for slurry mixture in each WWTP. The Artificial sweeteners were extracted from wastewater samples (500 mL of influent and 1 L of effluent) and sludge samples (0.5 g/dw) according to the method reported in previous study ¹. Briefly, filtered water samples were adjusted to pH 3.0 using 0.1 M hydrochloric acid and were spiked at 100 μ L of the internal standards (1 mg L⁻¹) before extraction. For sludge samples, homogenized and sieved (100-mesh) sludge (0.5 g) was put into 30 mL glass centrifuge tubes. 10 mL of ethyl acetate was added to glass centrifuge tubes, and a Vortex Mixer was used to mix the solution for about 30 s after extraction. The tubes with samples were put into an ultrasonic bath for 15 min, and then centrifuged at 3500 rpm for 10 min. The clear supernatant from each tube was transferred to a 100 mL pear-shaped flask. The extraction procedure was repeated twice using 10 mL and 5 mL of ethyl acetate. Then three extracts (25 mL) were mixed, evaporated at 45 °C using a rotary evaporator, re-dissolved with 1 mL of methanol and filtered through a 0.2 μ m membrane filter (Agilent Technologies, CA, USA) prior to purification using HLB SPE cartridges. For purification with HLB SPE cartridges, the supernatants were diluted with 200 mL of methanol with a concentration lower than 10%. Then, diluted extract was purified using an Oasis HLB cartridge (200 mg, 6 mL). The preconditioned conditions were the same as the SPE procedure for wastewater samples. The eluents were evaporated to dryness under a gentle N₂ stream. The dried residue was immediately reconstituted with 1 mL of methanol and filtered through a 0.2 μ m membrane filter (Agilent Technologies, CA, USA) for UPLC-MS/MS detection.

Table S1 Basic information and influent parameters of 12 municipal WWTPs.

WWTPs	Sampling cities	Secondary treatment	Treatment capacity ($\times 10^4$ m ³ /d)	Population equivalent ($\times 10^4$)	Population Density ($\times 10^4$ /km ²)	COD (mg/L)	TP (mg/L)	TN (mg/L)	NH ₄ ⁺ -N (mg/L)	SS (mg/L)	pH
WWTP-A	Harbin	AO	32.5	113	1.71	235.2	3.77	42.5	32.15	230	7.9
WWTP-B	Beijing	AAO	75	240	2.48	319	6.5	48	30.7	310	8.4
WWTP-C	Shijiazhuang	AAO	54.6	200	1.60	315	4.5	60.9	52	/	7.6
WWTP-D	Qingdao	AAO	23	80	0.65	885	25.5	106	36.5	127.7	7.6
WWTP-E	Lanzhou	AAO	15.2	80	1.90	728	/	62.5	47.21	220	7.1
WWTP-F	Xi'an	AAO	25	90	2	480	6.1	60	40	372	7.8
WWTP-G	Hefei	AAO	21	56	0.57	278	4	38.39	/	/	7.49
WWTP-H	Shanghai	AAO	30	90	2.41	230.2	4.28	35	19	278	7.4
WWTP-I	Ningbo	AAO	27.5	110	1.24	215	2.25	22.6	17	0.5	7.5
WWTP-J	Fuzhou	AAO	50	100	1.72	243	2.8	28.9	21.5	331.4	7.24
WWTP-K	Kunming	AAO	11.8	72.92	1.62	180	3.76	26.4	16.3	93.5	7.12
WWTP-L	Guangzhou	AAO	120	303	2.46	305	4.54	25.81	23.93	151	7.17

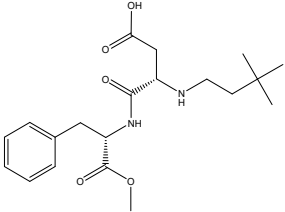
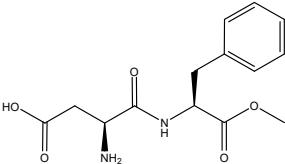
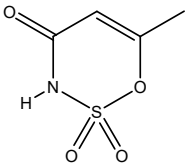
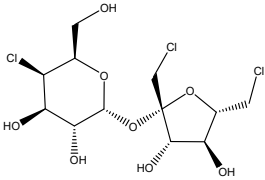
Table S2 Artificial sweeteners concentrations in influents, effluents, and excess sludge of 12 China's WWTPs.

Artificial sweeteners	Influents ($\mu\text{g/L}$, n = 12)				Effluents ($\mu\text{g/L}$, n = 12)				Excess sludge (ng/g , n = 12)			
	min	max	median	Freq (%)	min	max	median	Freq (%)	min	max	median	Freq (%)
SUC	1.76	8.62	3.85	100	0.98	3.47	2.55	100	<LOD ^a	56.9	11.34	91.7
ACE	0.25	2.43	1.29	100	0.07	3.06	0.52	100	<LOD	8.31	<LOD	41.7
APM	0.02	1.23	0.560	100	0.03	0.71	0.15	100	<LOD	51.8	<LOD	41.7
NTM	4.79E-04	0.56	0.0300	100	<LOD	0.04	9.34E-03	91.7	<LOD	0.87	<LOD	25

a: Concentration of individual Artificial sweeteners less than respective LOD.

LOD: Method limit of detection

Table S3 Major physicochemical characteristics of target Artificial sweeteners.

Compound	Structure	Acronym	CAS No.	Formula	$\log K_{ow}^d$
Neotame		NTM	165450-17-9	C ₂₀ H ₃₀ N ₂ O ₅	2.88
Aspartame		ASP	22839-47-0	C ₁₄ H ₁₈ N ₂ O ₅	0.070
Acesulfame		ACE	33665-90-6	C ₄ H ₄ NO ₄ SK	-2.67
Sucralose		SUC	56038-13-2	C ₁₂ H ₁₉ Cl ₃ O ₈	-1.00

^d K_{ow} : the octanol-water partition coefficient, calculated based on EPI Suite 4.11 from U.S. EPA

Table S4 Multi-selected reaction monitoring (MRM) conditions, recoveries, LOD and LOQ of the 4 Artificial sweeteners.

Compound Name	Parent (m/z)	Product ions (m/z)	Cone Voltage (V)	Influents			Effluents			Sludge		
				Recovery (%) (n=6)	LOD ^b (ng·L ⁻¹)	LOQ ^c (ng·L ⁻¹)	Recovery (%) (n=6)	LOD (ng·L ⁻¹)	LOQ (ng·L ⁻¹)	Recovery (%) (n=5)	LOD (ng·g ⁻¹ dw)	LOQ (ng·g ⁻¹ dw)
NTM	377.2	199.9/345	40	88 ± 2.4 ^a	4	2.1	90 ± 1.2 ^a	4	3.1	92 ± 2.2 ^a	0.6	0.18
ASP	293.2	200.1/261	38	87 ± 5.3	10	40	89 ± 5.1	3	10	87 ± 5.3	0.1	0.5
ACE	162	78/82	38	95 ± 5.1	10	20	93 ± 3.7	10	20	92 ± 2.5	0.3	0.1
SUC	395.01	359.08	30	89 ± 3.8	10	100	93 ± 3.1	10	100	82 ± 3.6	0.25	0.75

^a mean ± standard deviation; ^b LOD: method limit of detection; ^c LOQ: method limit of quantitation

Table S5 Gradient conditions of mobile phase for HPLC.

Retention (min)	Flow (mL/min)	%A (0.1% ammonia in water)	%B (Methanol)
Initial	0.25	95.0	5.0
0.5	0.25	95.0	5.0
1.0	0.25	5.0	95.0
3.0	0.25	5.0	95.0
3.5	0.25	95.0	5.0
4.0	0.25	95.0	5.0

Table S6 Summary of the aquatic toxicity data and PNEC values for 4 Artificial sweeteners.

Artificial sweeteners	Specie	Endpoint	Values ($\mu\text{g L}^{-1}$)	Duration	Effects	PNEC ($\mu\text{g L}^{-1}$)	Comment	References
ACE	<i>Cyprinus carpio</i>	NOEC	0.05	12-96 h	oxidative stress	20	NOEC	2
	Zebrafish	NOEC	1000	60 days	Neuro-behavior		(fish) AF=50 ^a	3
SUC	<i>Cyprinus carpio</i>	LOEC	0.05	48-96 h	oxidative damage	0.23	EC50	4
	<i>Cyprinus carpio</i>	LOEC	0.05	12-96 h	DNA damage		(fish) AF=1000	5
ASP	Algae	EC50	236	/	/	2280		ECOSAR
	Fish	pLC50	2.28*10 ⁶	/	/		LC50	DSSTox

NEO	Fish	pLC50	1.63*10 ⁶	/	/	1630	(fish) AF=1000	DSSTox
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^a AF: artificial sweeteners assessment factor. The artificial sweeteners assessment factor chosen according to the European Technical Guidance Document (European Commission, 2003)

Table S7 Per capita mass loads of artificial sweeteners in influents of 12 WWTPs

WWTPs	SUC (mg/(d·p))	ACE (mg/(d·p))	APM (mg/(d·p))	NTM (mg/(d·p))
WWTP-A	7.21	3.61	1.14	0.04
WWTP-B	14.35	7.59	2.63	0.02
WWTP-C	10.25	3.32	3.34	1.52
WWTP-D	13.90	0.73	0.06	0.01
WWTP-E	7.49	2.90	1.50	0.27
WWTP-F	9.70	4.61	0.28	0.04
WWTP-G	6.61	2.33	2.70	0.19
WWTP-H	9.98	6.06	3.40	0.10
WWTP-I	5.04	1.74	1.93	0.07
WWTP-J	43.08	6.59	0.54	0.19
WWTP-K	13.65	2.01	0.16	0.00
WWTP-L	18.99	8.70	1.17	0.43

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