Long-overlooked coumarins in swimming pool water and their contributions to the formation of disinfection byproducts

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Text S1: Materials.

Chloroacetic acid (MCAA), dichloroacetic acid (DCAA), trichloroacetic acid (TCAA), bromoacetic acid (MBAA), dibromochloroacetic acid (DBCA), tribromoacetic acid (TBAA), dibromoacetic acid (DBAA), bromochloroacetic acid (BCAA), iodoacetic acid (IAA) were provided from Shanghai Macklin Co., Ltd (Shanghai, China). 1,2,3-trichloropropanone (1,2,3-TCP), trichloromethane (TCM), dibromochloromethane (DBCM), dichlorobromomethane (BDCM), trichloroacetaldehyde (TCA), tribromomethane (TBM), tribromoacetaldehyde (TBA), m-bromofluorobenzene (MBF), 7-hydroxy-4-methylcoumarin (7-OH-4-CH_3-coumarin), 6-hydroxy-4-methylcoumarin (6-OH-4-CH_3-coumarin), 7-hydroxycoumarin (7-OH-coumarin), 6,7-dihydroxycoumarin (6,7-di-OH-coumarin), 3-aminocoumarin (3-NH_2-coumarin), and coumarin were purchased from Shanghai BiDe Pharmaceutical Technology Co., Ltd. (Shanghai, China). Moreover, 5,7-dihydroxy-4-methylcoumarin (5,7-di-OH-4-CH_3-coumarin) and 7-methoxy-4-methylcoumarin (7-OCH_3-4-CH_3-coumarin) were obtained from Shanghai Yien Chemical Technology Co., Ltd. (Shanghai, China). Humic acid and ascorbic acid were directly obtained from Shanghai Xianding Biological Technology. The solvents used in the experiments, including analytical-grade methanol and acetonitrile were obtained from Taicang Hu's Reagent Co., Ltd. (Shanghai, China), while LC/MS grade methanol and acetonitrile were procured from Tediai Company (Anhui, China). Sodium hypochlorite (analytical grade, available chlorine ≥6%) was purchased from Tianjin Kemeier Chemical Reagent Co., Ltd. The free chlorine content in the solution was measured to be 72.5 mg/mL using a chlorine colorimeter (Thermo Fisher Scientific, AQUAfast AQ3170). Sodium hydrogen phosphate dodecahydrate and disodium hydrogen phosphate dihydrate were obtained from Guangdong Xilong Technology Co., Ltd. (Guangdong, China). The deionized water used in the experiments was obtained using a Millipore Milli-Q system (Millipore-Sigma Inc., Milford, MA, USA). Methyl tert-butyl ether (MTBE), Na_2SO_4, $NaHCO_3$ were obtained from Sinopharm Chemical Reagent Co., Ltd. NanoMicro HLB solid-phase extraction cartridges (6 mL, 500 mg per cartridge) were acquired from NanoChrom Technologies (Suzhou) Co., Ltd. The

sand was purchased from the water treatment plant.

Text S2: High-Resolution Mass Spectrometry Analysis

The UltiMate 3000 liquid chromatography-tandem Q Exactive hybrid quadrupole-orbitrap mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) equipped with a heated electrospray ionization (H-ESI II) source was used to acquire the accurate full scan mass spectra and the MS/MS spectra of the analytes. The separation was carried out by a Luna C18 column (100 mm × 2.0 mm, 3 μm, Phenomenex) at a flow rate of 0.3 mL/min with the column temperature at 40 °C. The mobile phase consisted of ultrapure water containing 0.1% FA (solvent A), and acetonitrile (ACN) with 0.1% FA (solvent B). A gradient elution program was performed by increasing solvent B from 20% to 90% within 0-5 minutes, held for 2.5 minutes, then returning to 20% solvent B in 0.1 minutes and held for 2.4 minutes for column equilibration. The auto-sampler was set at 8 °C, and the resolution of full mass scan (m/z 50-600) was set to 60,000. Data dependent acquisition ($ddMS^2$) was performed under both positive and negative mode with a resolution of 15000. HESI parameters were set as follows: static spray voltage, positive voltage at 3.5 kV, negative voltage at 2.5 kV, sheath gas flow rate at 50, auxiliary gas flow rate at 10, sweep gas flow rate at 1, ion transfer tube temperature at 325 °C, and vaporizer temperature at 350 °C. Data analysis was conducted using Xcalibur software (version 2.2).

Text S3: HPLC-MS/MS (MRM) Method

Quantification of coumarins was performed on a Triple Quad 7500+ (SCIEX, USA) operated in MRM mode. The mobile phase and gradient program were identical to those described in **Text S2**. The optimized mass spectrometer parameters included a spray voltage of 1500 V for positive mode, and 2000 V for negative mode; curtain gas, 40 psi; gas 1, 50; gas 2, 50 psi; source temperature, 500 ℃, and an accumulation time of 430 ms for each ion pair. The MRM transition ions, along with optimized values for DP, collision energy (CE), and cell exit potential (CXP), are detailed in Table S1. Data analysis was performed using the analysis software SCIEX OS for the Sciex QTRAP 7500+. The MRM mode detection conditions for coumarins are shown in **Table S1**.

Text S4: GC-MS/MS (MRM) Method

THMs, HALs, and HAAs in actual water samples were determined using GC-MS (Thermo Fisher Scientific, TSQ8000) equipped with a DB-5 capillary column (30 m × 0.25 mm × 0.25μm). The parameters for detection of THMs, and HALs were as follows: injection port temperature, 200 ℃; ion source temperature, 200 ℃; interface temperature, 230 ℃; split ratio, 5:1; carrier gas, ammonia; scan mode, MRM; solvent delay, 1 min. The temperature programming started at 35 ℃, held for 3 minutes, ramped to 60 ℃ at 15 ℃/min and held for 5 min, then ramped to 150 ℃ at 30 ℃/min and held for 3 min, and finally ramped to 220℃ at 50℃/min and held for 3 min.

The parameters for detection of haloacetic acid methyl ester included injection port temperature, 230 ℃; ion source temperature, 250 ℃; interface temperature, 240 ℃; splitless; carrier gas, helium; scan mode: MRM; solvent delay, 1 min. The temperature programming started at 35 ℃, held for 5 min, ramped to 85°C at 10°C/min and held for 5 minutes, then ramped to 250°C at 30°C/min and held for 5 minutes. The MRM mode detection conditions for volatile DBPs are shown in **Table S2**.

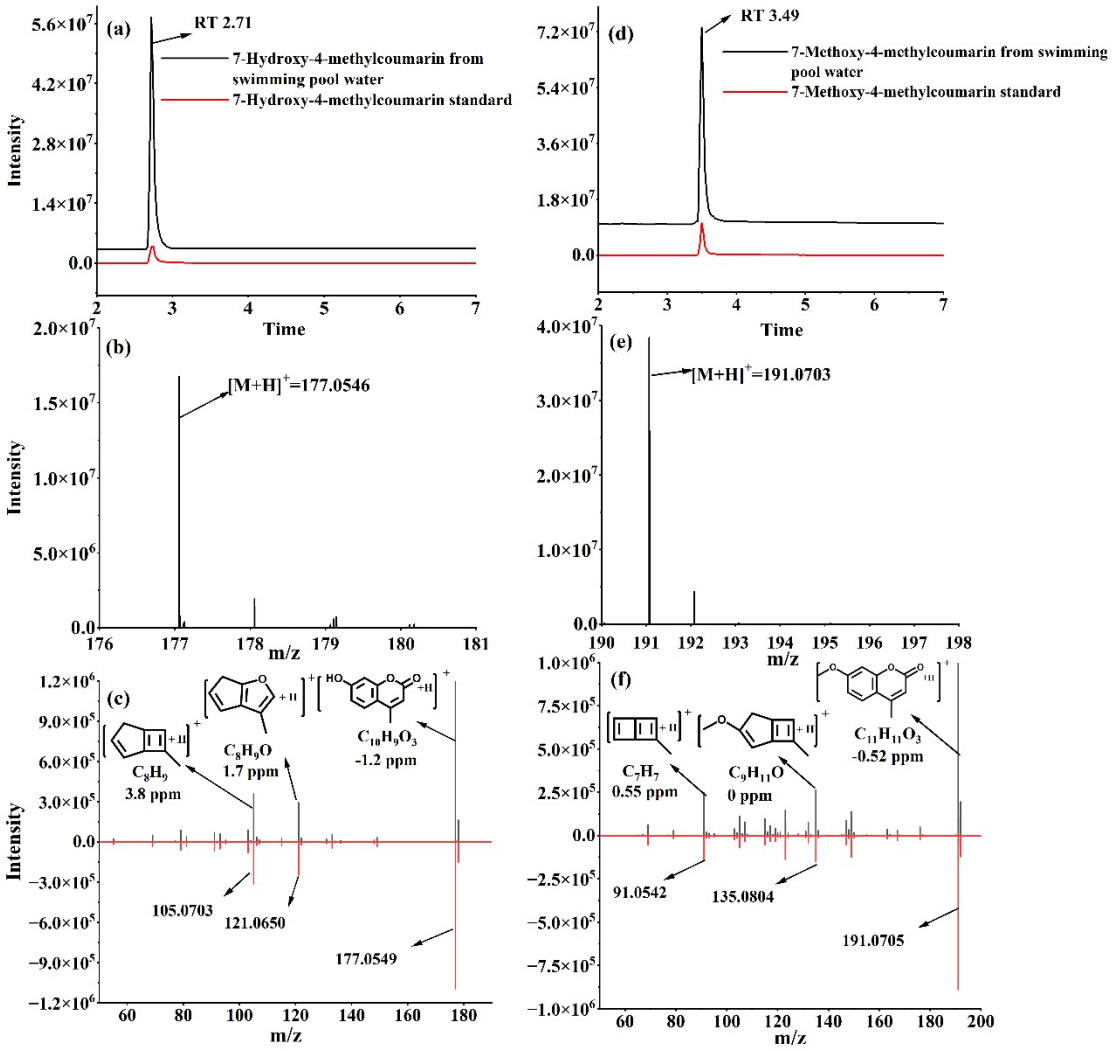


Figure S1. Mass spectra of commonly detected 7-OH-4-CH_3-coumarin and 7-OCH_3-4-CH_3-coumarin in two different sources of swimming pool water: (a) extracted ion chromatogram (EIC), (b) full mass scan, and (c) MS^2 spectrum of 7-OH-4-CH_3-coumarin in swimming pool water; (d) EIC, (e) full mass scan, and (f) MS^2 spectrum of 7-OCH_3-4-CH_3-coumarin in swimming pool water.

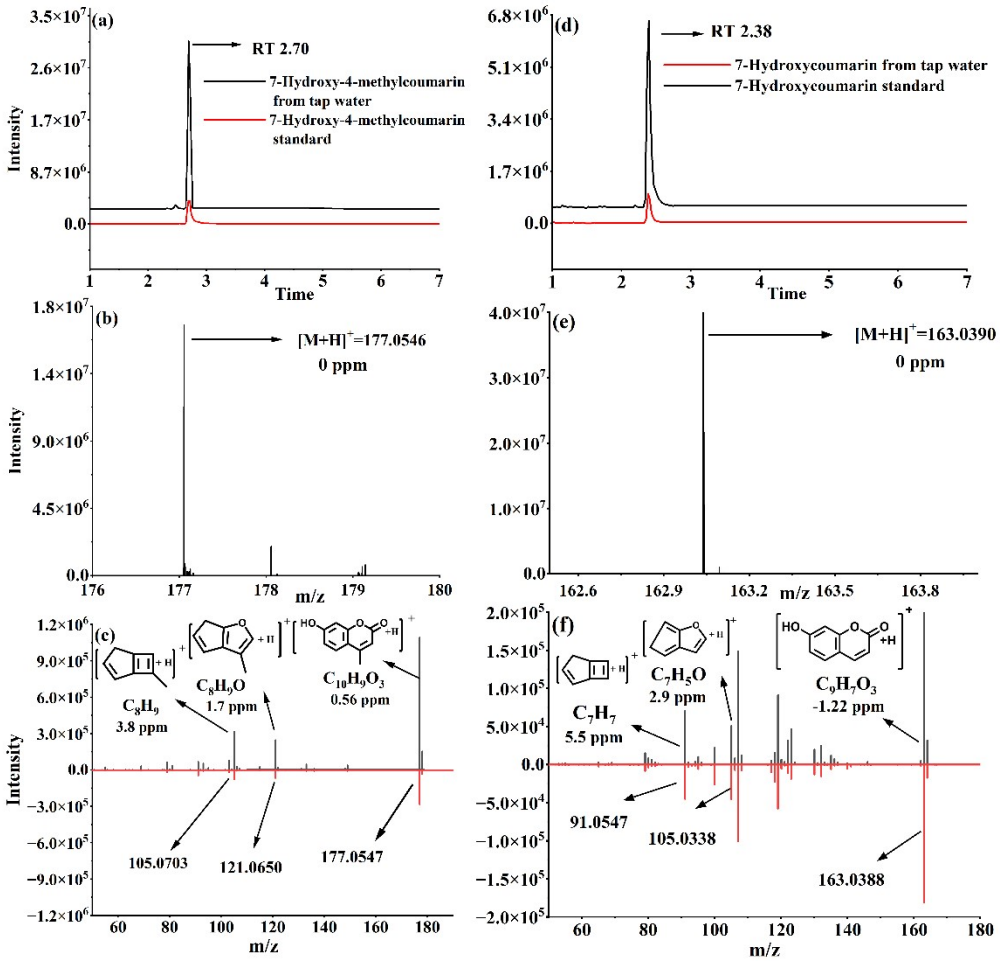


Figure S2. Mass spectra of commonly detected 7-OH-4-CH_3-coumarin and 7-OH-coumarin in two different sources of tap water: (a) extracted ion chromatogram (EIC), (b) full mass scan, and (c) MS^2 spectrum of 7-OH-4-CH_3-coumarin in tap water; (d) EIC, (e) full mass scan, and (f) MS^2 spectrum of 7-OCH_3-4-CH_3-coumarin in tap water.

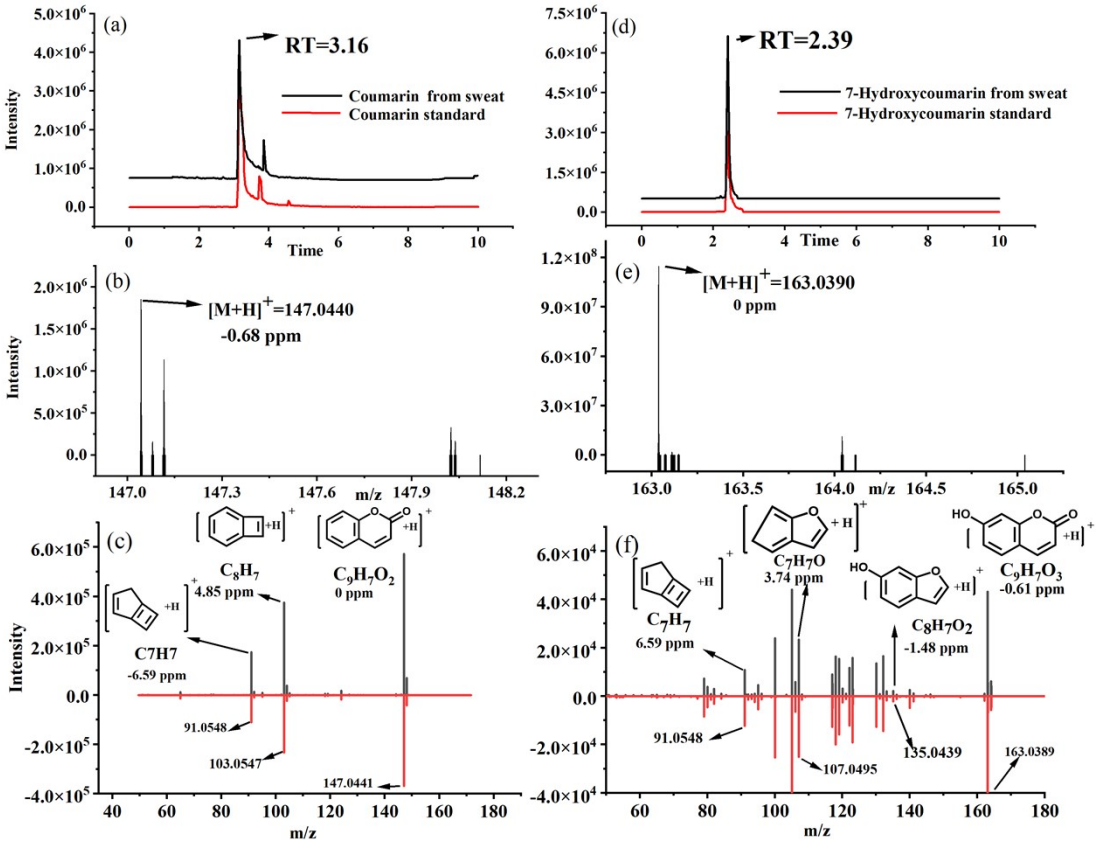


Figure S3. Mass spectra of commonly detected coumarin and 7-OH-coumarin in two different sources of sweat samples: (a) extracted ion chromatogram (EIC), (b) full mass scan, and (c) MS^2 spectrum of coumarin in sweat samples; (d) EIC, (e) full mass scan, and (f) MS^2 spectrum of 7-OH-coumarin in sweat samples.

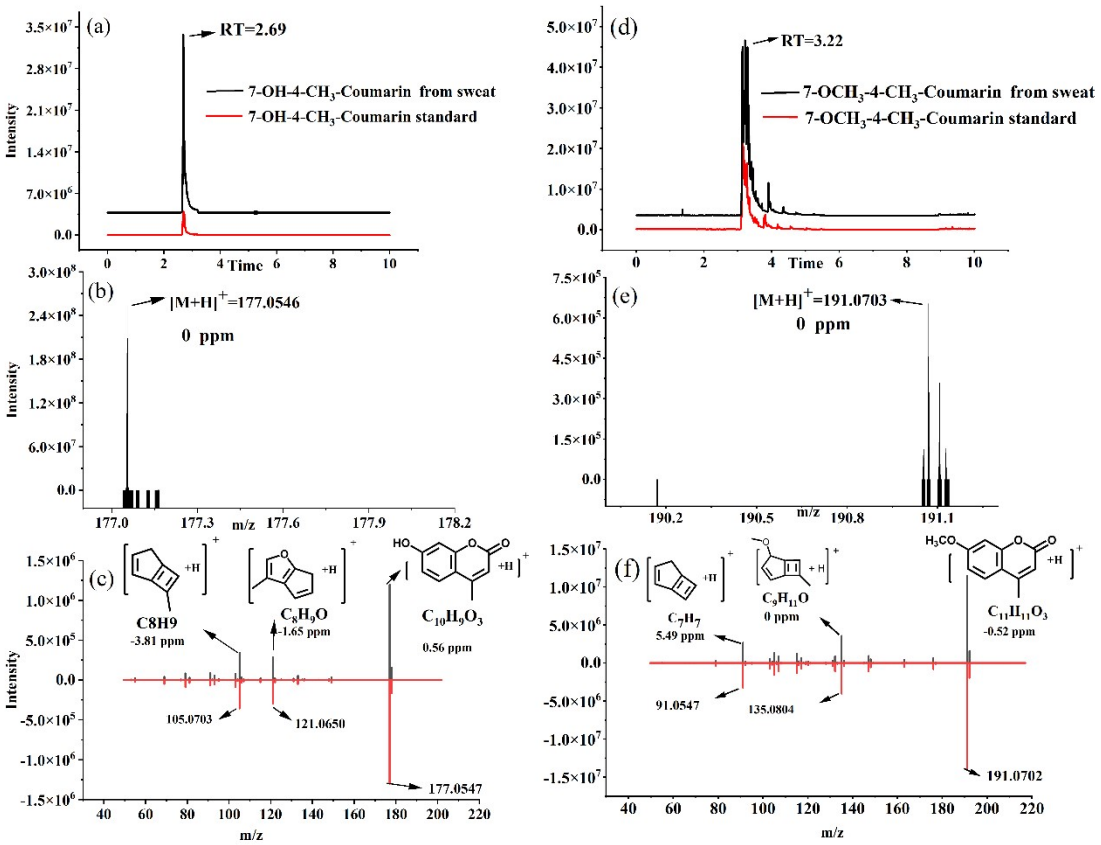


Figure S4. Mass spectra of commonly detected coumarin and 7-OH-4-CH_3-coumarin and 7-OCH_3-4-CH_3-coumarin in two different sources of sweat samples: (a) extracted ion chromatogram (EIC), (b) full mass scan, and (c) MS^2 spectra of 7-OH-4-CH_3-coumarin in sweat samples; (d) EIC, (e) full mass scan, and (f) MS^2 spectrum of 7-OCH_3-4-CH_3-coumarin in sweat samples.

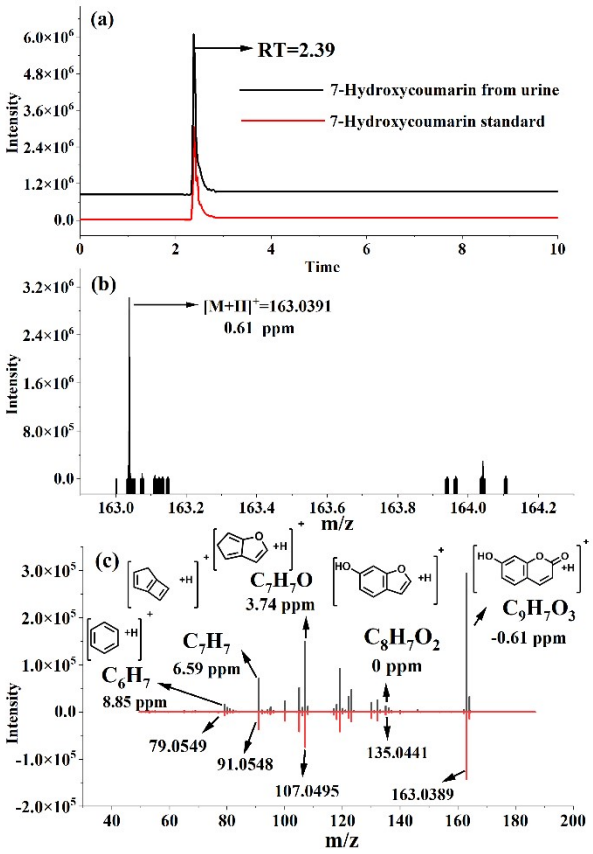
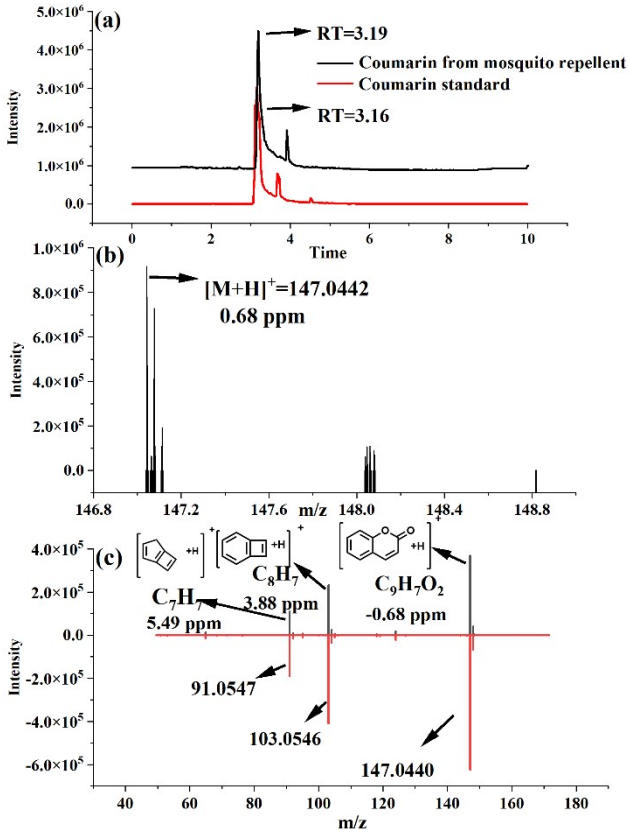


Figure S5. Mass spectra of commonly detected 7-OH-coumarin in three different sources of urine samples: (a) extracted ion chromatogram (EIC), (b) full mass scan, and (c) MS^2 spectrum of 7-OH-coumarin in urine samples.



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154 **Figure S6.** Mass spectra of coumarin in mosquito repellent: (a) extracted ion
155 chromatogram (EIC), (b) full mass scan, and (c) MS^2 spectrum of the detected
156 coumarin.

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160 **Figure S7.** Removal of coumarins by sand filtration.

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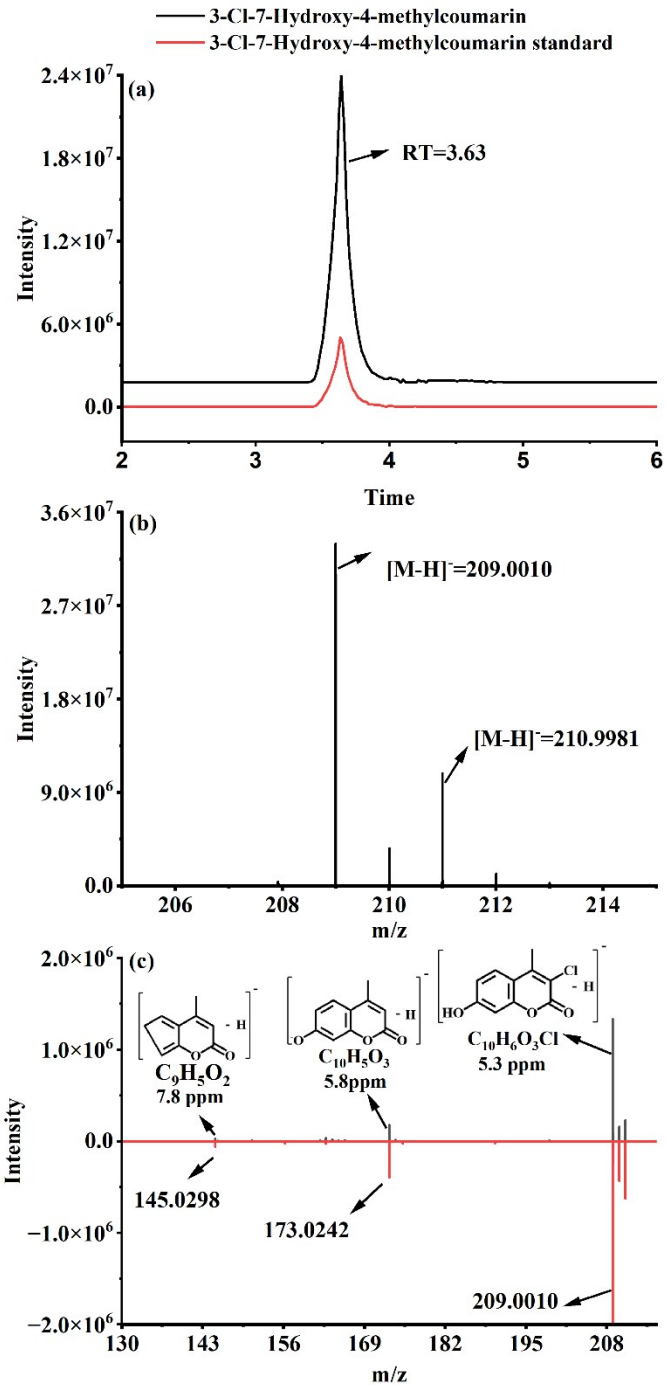

Figure S8. Mass spectra of 3-Cl-7-OH-4-CH_3-coumarin in chlorination (2 mg/L as free chlorine) of tap water spiked with 7-OH-4-CH_3-coumarin: (a) extracted ion chromatogram (EIC), (b) full mass scan, and (c) MS^2 spectrum of the detected 3-Cl-7-OH-4-CH_3-coumarin.

Table S1. pH of swimming pool (SP) water, residual free chlorine in SP water, and dissolved organic carbon (DOC) in tap water (near pool) and in SP water.

	pH of SP water	Residual free chlorine in SP water (mg/L)	DOC in tap water (near pool) (mg/L)	DOC in SP water (mg/L)
Swimming pool 1 in Nanjing	8.3	1.85	0.60	1.90
Swimming pool 2 in Changzhou	8.5	1.58	0.78	2.00

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177 **Table S2.** MRM parameters for LC-MS/MS analysis of coumarins.

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Name	Q1	Q3	EP(V)	CE(V)	CXP(V)
Coumarin	146.9	102.9	10	24	15
		90.9	10	32	15
		76.9	10	33	15
5,7-Di-OH-4-CH_3-coumarin	193.2	91.1	10	41	15
		119.1	10	17	15
		137.1	10	32	15
7-OH-4-CH_3-coumarin	177.0	121.0	10	29	15
		103.0	10	35	15
		77.0	10	50	15
7-OH-coumarin	163.1	107.1	10	29	15
		77.1	10	43	15
		91.1	10	47	15
6,7-Di-OH-coumarin	179.0.	123.1	10	30	15
		133.0	10	28	15
		77.1	10	44	15
3-NH_2-coumarin	162.1	106.1	10	24	15
		79.1	10	31	15
		134.1	10	21	15
7-OCH_3-4-CH_3-coumarin	191.0	91.0	10	57	15
		103.1	10	42	15
		148.2	10	40	15
7,8-Di-OH--6-CH_3-coumarin	209.0	194.0	10	32	15
		149.0	10	29	15
7-Cl-7-OH-4-CH_3-coumarin	209.0	173.0	-10	-24	-15
		35.0	-10	-33	-15

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Table S3. MRM parameters for GC-MS/MS analysis of HAAs, THMs and HALs.

Name	RT(min)	Mass	Product Mass	CE(eV)
MCAA	5.57	108.0	76	8
MBAA	8.38	120.9	93	8
MBAA	8.38	123.0	95	10
DCAA	8.70	85.0	48	30
TCAA	10.53	116.9	82	26
IAA	10.59	140.9	63	12
BCAA	10.65	128.9	127.1	38
DBAA	12.99	172.9	94	38
TBAA	17.77	250.8	171.9	36
TCM	2.89	82.9	47	24
BDCM	4.17	83.0	47	24
TCA	4.20	82.0	47	26
DBCM	5.87	128.9	48	34
TBM	8.72	170.8	91.8	38
TBA	11.84	172.9	91.1	28

Table S4. Recoveries, instrument limits of detection (LODs), instrument limits of quantification (LOQs), and method detection limits (MDLs) of HAAs, THMs, HALs and coumarins.

Compounds	LOD (μg/L)	LOQ (μg/L)	MDL (ng/L)	Recovery (%)
MCAA	0.500	1.00	62.8	91.5
DCAA	5.00	10.0	73.0	69.9
TCAA	1.00	5.00	630	65.9
IAA	1.00	5.00	1.16×10^3	56.2
TCM	6.00	10.0	4.23×10^3	64.9
BDCM	10.0	50.0	650	62.9
TCA	10.0	25.0	2.17×10^3	75.7
DBCM	1.00	5.00	9.33×10^3	82.2
TBM	0.500	1.00	420	71.5
TBA	5.00	10.0	1.23×10^3	76.4
Coumarin	0.0100	0.0500	0.120	80.0
6-OH-4-CH_3-coumarin	0.0500	0.100	0.0800	86.9
7-OCH_3-4-CH_3-coumarin	0.0500	0.100	0.0500	70.8
7-OH-4-CH_3-coumarin	0.0500	0.100	0.00200	83.0
7-OH-coumarin	0.0500	0.100	0.0100	89.3
6,7-Di-OH-coumarin	0.0500	0.100	0.5400	75.3
3-NH_2-coumarin	0.0500	0.100	0.00200	71.0
5,7-Di-OH-4-CH_3-coumarin	0.0500	0.100	0.0900	69.0
7-Cl-6-OH-4-CH_3-coumarin	0.0500	0.100	0.003	26.15