Electronic Supplementary Material (ESI) for Environmental Science: Water Research & Technology.

Electronic Supplementary Information for: Microbial degradation pathways of the herbicide bentazone in filter sand used for drinking water treatment

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S1 Introduction

Table S1 Chemical names, abbreviations and IUPAC definitions.

Name	Abbreviation	IUPAC definition
Bentazone	BTZ	3-Isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one-2,2-dioxide
6-OH-bentazone	6-OH-BTZ	6-Hydroxy-3-Isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one-2,2-dioxide
8-OH-bentazone	8-OH-BTZ	8-Hydroxy-3-Isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one-2,2-dioxide
AIBA	-	2-amino-N-propan-2-ylbenzamide
***N*-methyl-bentazone**	*N*-methyl-BTZ	3-isopropyl-1-methyl-1H-2,1,3-benzothiadiazin- 4(3H)-one 2,2-dioxide
Isopropyl-OH-bentazone	Isopropyl-OH-BTZ	3-(1-hydroxypropan-2-yl)-1*H*-benzo[c][2,1,3]thiadiazin-4(3*H*)-one-2,2-dioxide
Di-OH-bentazone	Di-OH-BTZ	-

S2 Materials and methods

S2.5.3 Bentazone and OH-bentazone measurements by HPLC-DAD

Chromatographic separation was achieved on a 150 mm x 4.6 mm x 5 µm BDS Hypersil C18 column (Thermo Scientific), held at 30 degrees, using 5mM H_2SO_4/acetonitrile (55/45) isocratic for 5 minutes at 1.25 ml min^{-1}. Detection was achieved at 230 nm with the absorbance spectrum collected between 190 and 400 nm.

S2.5.4 Identification of bentazone transformation products by high-resolution MS

The ion source parameters were: source temperature 550 °C; capillary voltage -4.5 kV; curtain gas 40 psi; ion source gas 1 35 psi, ion source gas 2 45 psi. Exact mass of parent compounds and main fragments were obtained using full scan TOF-MS and MS/MS mode with information dependent acquisition (IDA) experiments (MS^2). The resolution of measurements was 35,000 at m/z = 400 and the mass accuracy below 5 ppm. A scan range of m/z 100-500 was used.

Chromatographic separation was carried out on a 4 µm Synergi Hydro-RP column (150 x 3 mm i.d.) equipped with a SecurityGuard column (4 x 3 mm i.d.; Phenomenex, Aschaffenburg, Germany). Flow rate was set to 400 mL min^{-1} using 0.1% formic acid (A) and acetonitrile (B) as mobile phases. The percentage of (A) was changed linearly as follows: 0-10 min, 100%; 25 min, 10%; 28 min, 10%; 29 min, 100%; 35 min, 100%.

S2.5.5 LC/MS/MS analysis

A Hydro-RP column (150x3 mm, 4 µM; Phenomenex, Aschaffenburg, Germany) was used for chromatographic separation using 0.1% acetic acid (A) and methanol (B) as mobile phases. Separation of analytes was achieved applying the following gradient: 0-4 min, 100% A; 7 min, 30% A; 17 min, 10% A; 18 min, 100% A. The run time was 22 min, flow rate was 0.4 mL min^{-1}, and column oven temperature was set to 25 °C. Sample volume was set to 100 µL. Multi-reaction monitoring (MRM) was used for detection of all compounds (Table S2) using the following parameters: gas temp.: 350°C; gas flow: 9 L min^{-1}; nebulizer: 45 psi; sheath gas heater: 400°C; sheath gas flow: 9 L min^{-1}; capillary voltage: -3600 V.

Table S2 LC/MS/MS multi-reaction monitoring (MRM) parameters used for detection of TPs identified in biodegradation experiments with bentazone, 6-OH-bentazone and 8-OH-bentazone.

Name	Parent ion mass $[M-H]^-$	Fragment ion mass	Fragmentor voltage [V]	Collision energy [V]	Cell exit potential [V]
Bentazone	239	197	130	24	6
		175	130	22	4
		132	130	22	4
6-OH-bentazone	255	213	130	14	6
		191	130	12	4
		148	130	18	4
8-OH-bentazone	255	213	130	14	6
		191	130	12	4
		148	130	18	4
TP304	303	259	130	10	7
		215	130	10	7
		173	130	20	7
TP284	283	239	130	10	7
		197	130	20	7
		132	130	30	7
TP278	277	233	130	10	7
		189	130	10	7
		125	130	10	7
TP270	269	225	130	10	7
		197	130	20	7
		189	130	10	7
TP244	243	201	130	20	7
		121	130	20	7
		78	130	30	7
TP235	234	191	130	10	7
		173	130	20	7
		147	130	20	7
TP192	191	163	130	10	7
		122	130	20	7
		78	130	40	7

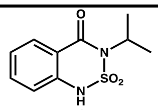
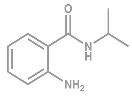
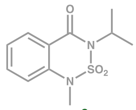
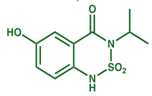
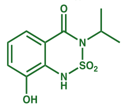
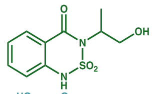
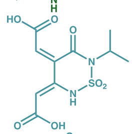
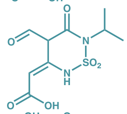
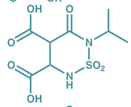
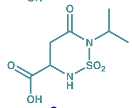
S3 Results and discussion

Table S3 Identified transformation products (TPs) from degradation of bentazone (5 mg L^{-1}) in contact with filter sand from a rapid sand filter in FTP1 (28 days) using high-resolution mass spectrometry in negative ionization mode. * Mark the TPs which presence were confirmed after 14 days in FTP2. # Mark the TPs which were detected during degradation of 6-OH- and/or 8-OH-bentazone. ¤ Mark the TPs which were detected during degradation of bentazone at low concentrations (10 µg L^{-1}).

	ESI$^-$			
	Exact mass	**Sum formula**	**Δppm**	
BTZ	**239.0494**	**$C_{10}H_{11}N_2O_3S$**	**1.5**	
	197.0026	$C_7H_5N_2O_3S$	2.7	$-C_3H_6$
	175.0877	$C_{10}H_{11}N_2O$	3.5	$-SO_2$
	147.0816	$C_{10}H_{11}O$	4.6	
	133.0409	$C_7H_5N_2O$	5.5	
	132.0331	$C_7H_4N_2O$	5.6	
TP304*#	303.0291	$C_{10}H_{11}N_2O_7S$	1.3	
	259.0374	$C_9H_{11}N_2O_5S$	5.3	$-CO_2$
	215.0477	$C_8H_{11}N_2O_3S$	5.9	$-CO_2$, $-CO_2$
	173.0019	$C_5H_5N_2O_3S$	1.0	$-CO_2$, $-CO_2$, $-C_3H_6$
	110.0244	$C_5H_4NO_2$	1.8	
	94.02964	C_5H_4NO	3.8	
	77.9641	NO_2S	9.9	
TP284	283.0395	$C_{11}H_{11}N_2O_5S$	2.2	
	240.9915	$C_8H_5N_2O_5S$	1.3	$-C_3H_6$
	239.0502	$C_{10}H_{11}N_2O_3S$	4.8	$-CO_2$
	197.0028	$C_7H_5N_2O_3S$	3.9	$-CO_2$, $-C_3H_6$
	175.0870	$C_{10}H_{11}N_2O$	0.3	$-CO_2$, $-SO_2$
	133.0397	$C_7H_5N_2O$	3.0	$-CO_2$, $-SO_2$, $-C_3H_6$
	132.0325	$C_7H_4N_2O$	1.1	
	117.0455	$C_7H_5N_2$	2.8	
TP278*#	277.0131	$C_8H_9N_2O_7S$	0.1	
	233.0228	$C_7H_9N_2O_5S$	1.5	$-CO_2$
	189.0334	$C_6H_9N_2O_3S$	0.1	$-CO_2$, $-CO_2$
	146.9861	$C_3H_3N_2O_3S$	1.6	$-CO_2$, $-CO_2$, $-C_3H_6$
	125.0714	$C_6H_9N_2O$	0.4	$-CO_2$, $-CO_2$, $-SO_2$
	68.01405	C_3H_2NO	6.0	
TP276*	275.0341	$C_9H_{11}N_2O_6S$	1.1	
	231.0431	$C_8H_{11}N_2O_4S$	3.3	$-CO_2$
	188.9985	$C_5H_5N_2O_4S$	7.9	$-CO_2$, $-C_3H_6$
	162.0210	$C_5H_8NO_3S$	9.0	
	145.9921	$C_4H_4NO_3S$	6.5	
	110.0242	$C_5H_4NO_2$	0.4	
TP270*¤	269.0238	$C_{10}H_9N_2O_5S$	2.1	
	225.0341	$C_9H_9N_2O_3S$	3.1	$-CO_2$
	197.0027	$C_7H_5N_2O_3S$	3.1	$-C_3H_4O_2$
	189.0665	$C_{10}H_9N_2O_2$	0.8	$-SO_3$
	161.0723	$C_9H_9N_2O$	5.4	
	145.0407	$C_8H_5N_2O$	3.6	
	143.0591	$C_6H_9NO_3$	6.2	
	133.0396	$C_7H_5N_2O$	3.9	$-C_3H_4O_2$, $-SO_2$

	132.0324	$C_7H_4N_2O$	0.4	
	117.0454	$C_7H_5N_2$	1.7	
	105.0333	C_7H_5O	6.4	
	92.05052	C_6H_6N	5.4	
	79.9579	SO_3	13.9	
TP259	258.0171	$C_5H_{10}N_2O_8S$	5.5	
	215.9692	$C_2H_4N_2O_8S$	1.7	$-C_3H_6$
	172.9662	$C_4HN_2O_4S$	3.1	
	136.0132	$C_2H_4N_2O_5$	2.7	$-C_3H_6$, $-SO_3$
	107.0587	$C_3H_9NO_3$	4.8	
	77.9651	NO_2S	2.0	
TP246	245.0234	$C_8H_9N_2O_5S$	0.7	
	202.9773	$C_5H_3N_2O_5S$	5.1	$-C_3H_6$
	123.0188	$C_5H_3N_2O_2$	5.0	$-C_3H_6$, $-SO_3$
	77.9653	NO_2S	4.4	
TP244[#]	243.0445	$C_9H_{11}N_2O_4S$	2.2	
	200.9960	$C_6H_5N_2O_4S$	4.8	$-C_3H_6$
	121.0408	$C_6H_5N_2O$	5.6	$-C_3H_6$, $-SO_3$
	77.96553	NO_2S	7.2	
TP234*	233.0241	$C_7H_9N_2O_5S$	3.7	
	190.9766	$C_4H_3N_2O_5S$	1.9	$-C_3H_6$
	189.0334	$C_6H_9N_2O_3S$	0.1	$-CO_2$
	172.9664	$C_4HN_2O_4S$	4.3	$-C_3H_6$, $-H_2O$
	146.9861	$C_3H_3N_2O_3S$	1.7	$-CO_2$, $-C_3H_6$
	125.0714	$C_6H_9N_2O$	0.6	$-CO_2$, $-SO_2$
	83.0251	$C_3H_3N_2O$	7.8	$-CO_2$, $-SO_2$, $-C_3H_6$
	79.9578	SO_3	12.4	
TP192[#]	191.0138	$C_5H_7N_2O_4S$	5.6	
	163.0174	$C_4H_7N_2O_3S$	1.4	-CO
	121.9550	CNO_4S	2.2	
	77.9651	NO_2S	2.4	

Table S4 Structure of substances in the hazard screening.

BTZ	
AIBA	
N-methyl-BTZ	
6OH-BTZ	
8OH-BTZ	
Isopropyl-OH-BTZ	
TP304	
TP276	
TP278	
TP234	
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