

Long-Term Toxicological Monitoring of a Multibarrier Advanced Wastewater Treatment Plant Comprising Ozonation and Granular Activated Carbon with In Vitro Bioassays

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Table S1. Frequency of sampling for each sampling point, sorted by specific ozone dose. Bed volumes (BV) are only given for routine monitoring campaigns.

	D _{spec} [g O ₃ /g DOC]	BV [-]	Routine Monitoring	O ₃ Dose- Campaign	CAS-OUT	O ₃ - OUT	GAC-OUT
18.09.2018	0.18			x	x	x	
19.03.2019	0.27			x	x	x	
18.09.2018	0.31	6,764		x	x	x	
22.05.2019	0.43	33,098	x		x	x	x
09.04.2019	0.47	28,854	x		x	x	x
18.09.2018	0.55			x	x	x	
19.03.2019	0.57	26,776		x	x	x	x
14.05.2018	0.62	978	x		x	x	x
12.11.2018	0.62	16,212	x		x	x	x
16.10.2018	0.66	14,991	x		x	x	x
19.02.2019	0.71	n.a.	x		x	x	x
18.09.2018	0.75			x	x	x	
19.03.2019	0.78			x	x	x	
13.06.2018	0.83	2,591	x		x	x	x
03.07.2018	0.89	3,667	x		x	x	x
19.03.2019	0.92			x	x	x	

n.a. not available.

Table S2. Frequency of analysis for the applied bioassays at the sampling points including method references.

Calux Bioassay Panel	Frequency of Analysis			Method Reference
	CAS-OUT	O3-OUT	GAC-OUT	
Cytotox	16	16	7	[1]
ER α	16	16	7	[2]
anti-ER α	2	2	2	[3]
anti-AR	16	16	7	[4]
Nrf2	13	13	5	[1]
p53 +S9	5	5	5	[1]
p53 -S9	3	3	3	[1]
PAH	8	8	4	[5]
PXR	3	3	3	[6]

Table S3. BEQs for cytotoxicity, estrogenicity and toxic PAH-like activities along the multibarrier system for each sampling campaign.

Date	D _{spec} g O ₃ / g DOC	Cytotox (μ g TBT-EQ/L)			ER (ng EEQ/L)			PAH (ng B[a]P-EQ/L)		
		CAS-OUT	O3-OUT	GAC-OUT	CAS-OUT	O3-OUT	GAC-OUT	CAS-OUT	O3-OUT	GAC-OUT
18.09.2018	0.18	0.24	0.23		0.15	0.05				
19.03.2019	0.27	2.80	0.25		0.27	0.09		120	62	
18.09.2018	0.31	0.25	0.24		0.44	0.01				
22.05.2019	0.43	0.59	0.30	0.295	1	0.04	0.16	200	52	28
09.04.2019	0.47	2.00	0.28	0.28	0.57	0.03	0.028			
18.09.2018	0.55	0.55	0.22		0.31	0.04				
19.03.2019	0.57	1.15	0.23		0.09	0.02		140	25	
14.05.2018	0.62	0.35	0.28	0.31	0.59	0.03	0.055	140	99	39
12.11.2018	0.62	0.23	0.23	0.205	0.56	0.02	0.048			
16.10.2018	0.66	1.20	0.26	0.23	1.2	0.04	0.015	270	170	210
19.02.2019	0.71	1.50	0.26		0.53	0.12				
18.09.2018	0.75	0.68	0.23		0.39	0.04				
19.03.2019	0.78	3.30	0.26		0.24			100	50	
13.06.2018	0.83	0.24	0.34	0.295	0.37	0.03	0.056	260	100	130
03.07.2018	0.89	0.19	0.19	0.19	0.5	0.08	0.061			
19.03.2019	0.92	2.70	0.26		0.23			150	45	
EBT		not available				0.1			6.2	

Numbers in bold: data below the limit of quantification (LOQ) was taken as $\frac{1}{2}$ LOQ

Table S4. BEQs for anti-androgenicity (Anti-AR), xenobiotic sensing (PXR) and oxidative stress (Nrf2) response along the multibarrier system for each sampling campaign.

Date	D _{spec} g O ₃ / g DOC	Anti-AR (µg Flu-EQ/L)			Nrf2 (ng Cur-EQ/L)			PXR (ng Nic-EQ/L)		
		CAS-OUT	O3-OUT	GAC-OUT	CAS-OUT	O3-OUT	GAC-OUT	CAS-OUT	O3-OUT	GAC-OUT
18.09.2018	0.18	6.20	2.20		34	12.5				
19.03.2019	0.27	0.95	0.95		86	16				
18.09.2018	0.31	18.00	2.45		73	73				
22.05.2019	0.43	0.28	0.95	0.95	130	97	97	40	21	15
09.04.2019	0.47	0.32	0.60	0.60						
18.09.2018	0.55	4.10	2.60		70	54				
19.03.2019	0.57	1.05	1.05		160	79				
14.05.2018	0.62	3.05	2.70	3.45	160	190	130	53	18	65
12.11.2018	0.62	9.90	2.65	2.20	110	37	88			
16.10.2018	0.66	15.00	2.60	2.45	36.5	54	12.5	4.15	1.3	1.2
19.02.2019	0.71	1.05	0.95							
18.09.2018	0.75	1.20	2.65		70	63				
19.03.2019	0.78	0.90	0.90		70	67				
13.06.2018	0.83	2.25	3.05	2.65						
03.07.2018	0.89	7.30	2.15	2.20	120	130	63			
19.03.2019	0.92	0.90	0.90		160	100				
EBT			14			10			3	

Numbers in bold: data below the limit of quantification (LOQ) was taken as ½ LOQ

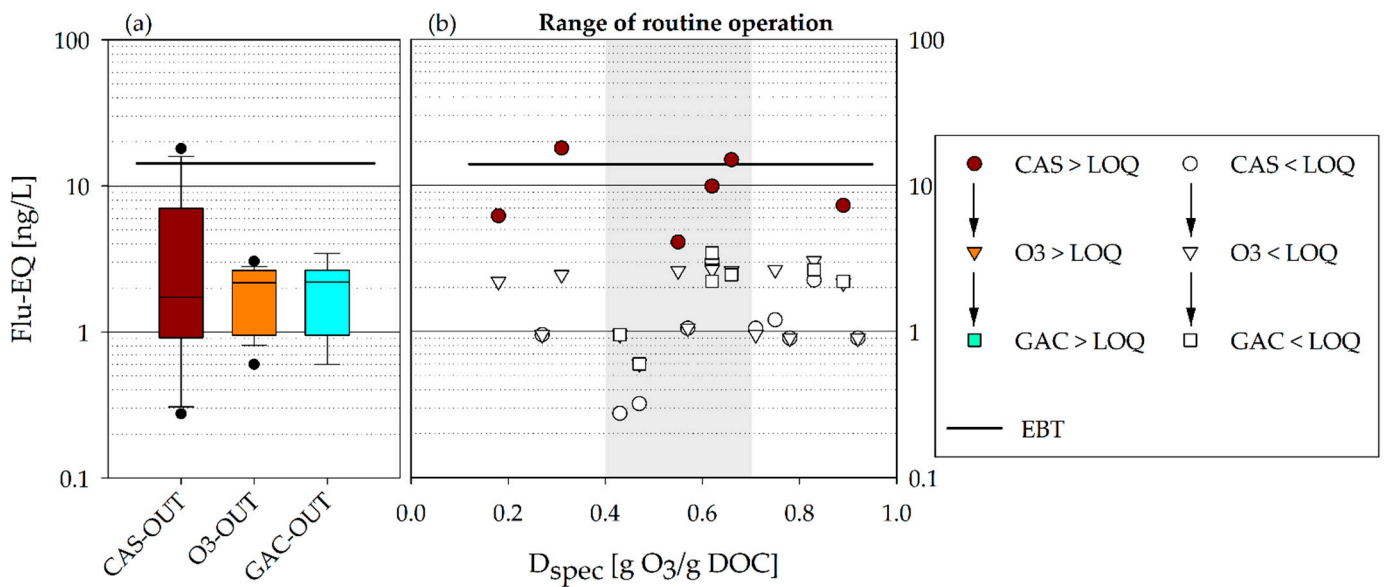


Figure S1. (a) Boxplots showing the range of flutamide equivalents over all campaigns along the multibarrier system; (b) Flu-EQ along the multibarrier system for each sampling campaign.

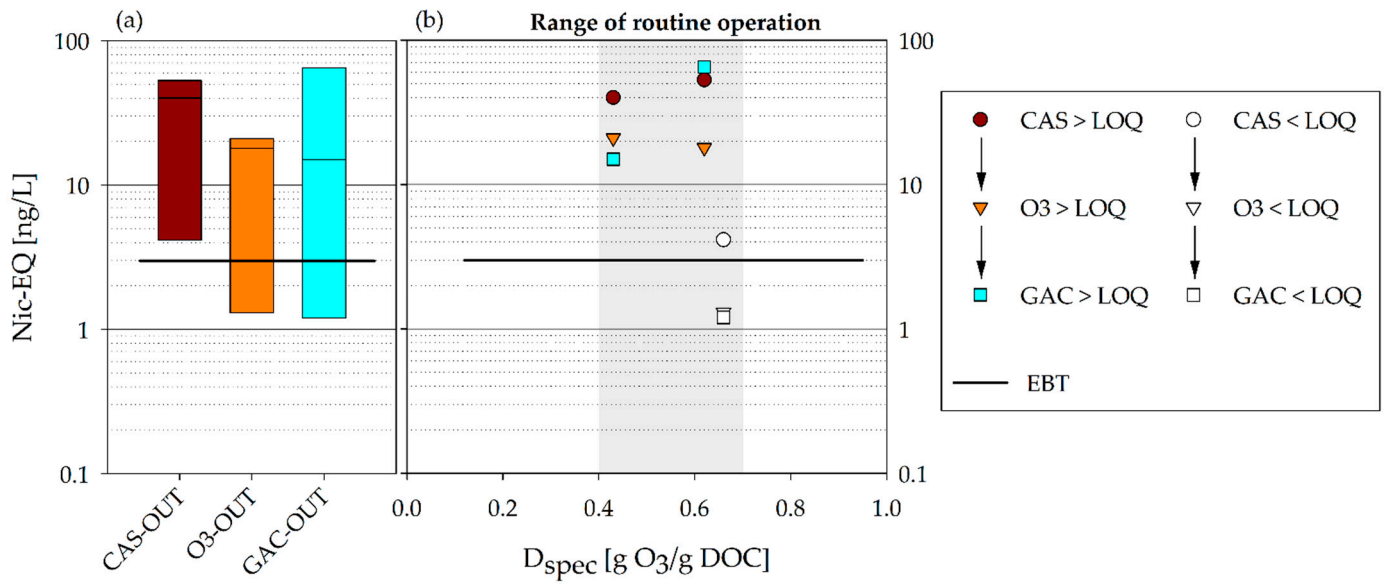


Figure S2. (a) Boxplots showing the range of nicardipine equivalents over all campaigns along the multibarrier system; (b) Nic-EQ along the multibarrier system for each sampling campaign.

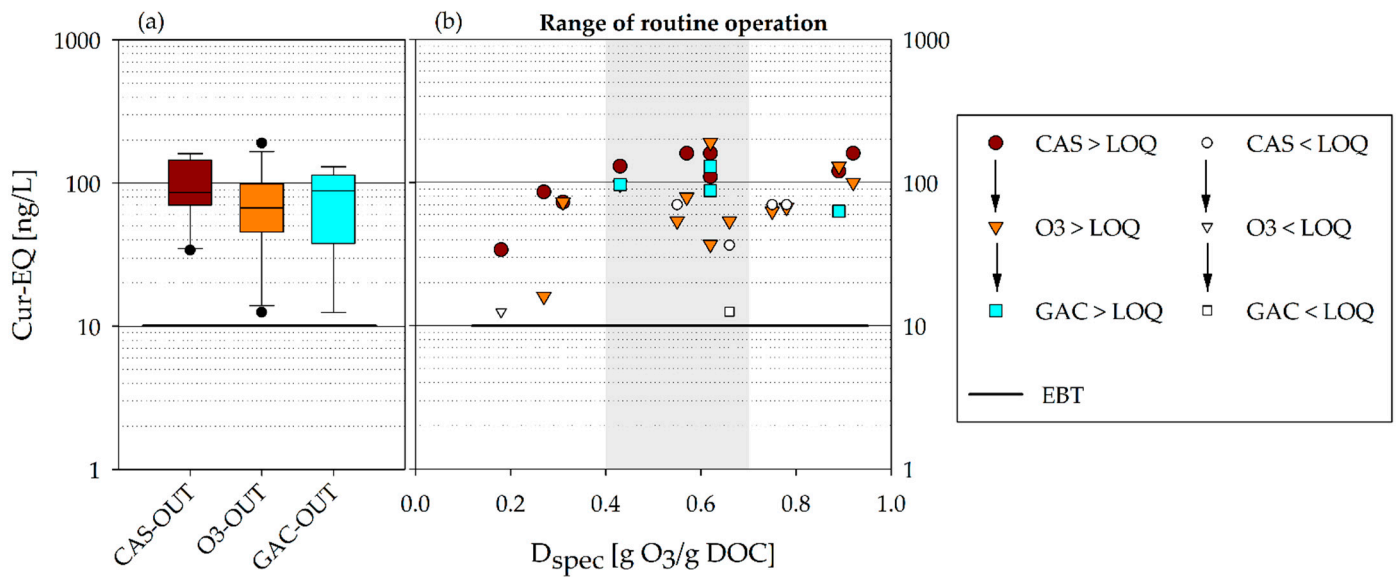


Figure S3. (a) Boxplots showing the range of curcumin equivalents over all campaigns along the multibarrier system; (b) Cur-EQ along the multibarrier system for each sampling campaign.

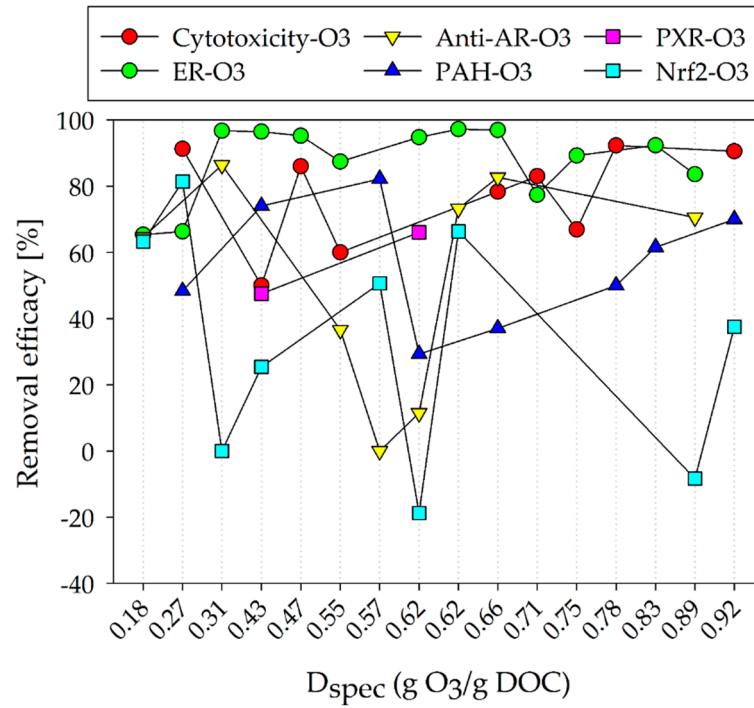


Figure S4. The range of removal for the investigated MOA after ozonation over the one-year monitoring.

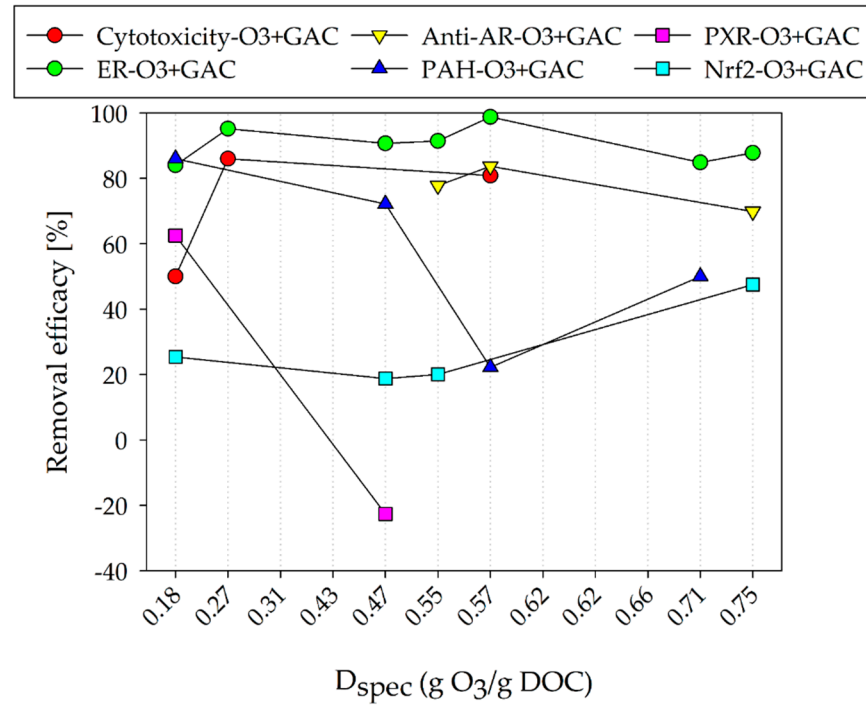


Figure S5. The range of removal for the investigated MOA after the multibarrier system (ozonation and GAC) over the one-year monitoring.

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