Supporting Information

Formation of transformation products during ozonation of secondary wastewater effluent and their fate in post-treatment: From laboratory- to full-scale

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Additionally to this SI pdf, an excel file with tree tables and a second pdf file are attached, which include the following information:

Excel Table S1: Information on the studied 87 parent micropollutants

Excel Table S2: OTP signals (777 in positive and 972 in negative ionization modes) of *O3bMix* experiments for 70 parent micropollutants (found in Approach 1)

Excel Table S3: OTP signals (totally 84) found in wastewater samples of four WWTPs which were matching signals in the *O3bAll* samples (Approach 2)

Figure S19: Figures with all 84 OTPs found in batch experiments and in wastewater treatment.

Table of content

Text S1. Micropollutant selection and separation into mixtures

Details on all parent micropollutants (MPs), including chemical structures, are provided in Table S1 in the supporting Excel file.

The MPs were spiked into 19 different mixtures, whereby each MP was present in two mixtures, while no other MP occurred twice in these two mixtures. The separation is visualized in Figure S1 and Figure S2. Figures S1 and S2 represent a 9 to 10 matrix, where each position is taken up by a MP. Each row was mixed together and these 9 mixtures were called MR0 – MR8. Accordingly, each column was mixed together and these 10 mixtures were called MC0 – MC9.

	MC0	MC1	MC2	MC3	MC4	MC5	MC6	MC7	MC8	MC9
MR0	Venlafaxine	Lidocaine	Flecainide	Atenolol-desisopropyl	Eprosartan	Lamotrigine	Sulfamethazine	Ketoprofen	Levamisole	Sucralose
MR1	Diclofenac	Mecoprop	Citalopram	Cetirizine	Propranolol	Sitagliptin	-	Prometon	Sulfapyridin	Metformin
MR2	Emtricitabine	Caffeine	Thiacloprid	Fenpropidin	Diphenhydramine	Norlidocaine	Mycophenolic acid	Sulfamethoxazole	2-Naphthalic sulfonic acid	Flufenamic acid
MR3	5-Methyl-benzotriazole	Cyprodinil	Atazanavir	Diuron	Norfenfluramine	Clindamycin	Atenolol acid	Amitriptyline	Rosuvastatin	2-7-naphthalic disulfonic acid
MR4	Etodolac	Benzotriazole	Trimethoprim	Methoxyfenozide	Ibuprofen	Tramadol	Clozapine	Atenolol	Oseltamivir	Hydrochlorothiazide
MR5	Acesulfame	Carbamazepine	Candesartan	Benzisothiazolone	Benzophenon 3	Bezafibrate	N-Bisdesmet. Tramadol	Sulpiride	Metoprolol	Pravastatin
MR6	Aliskiren	Cephalexin	Resveratrol	Valsartan acid	-	2,4-D	Phenazone	Clarithromycin	Amisulpride	N-Desmethyl-tramadol
MR7	Efavirenz	-	Progesterone	Fenfluramine	Valsartan	Naproxen	Atrazine	Oxcarbazepine	Diltiazem	Triclosan
MR8	Levetiracetam	Propyzamide	Gabapentin	Methyl-prednisolone	Codeine	Irbesartan	Napropamide	Oxazepam	Mefenamic acid	Ranitidine

3° amines	2° amines	1° amines	olefins	ethynes	sulfonamide	aromatic compounds	Similar to benzotriazole	napthalenes	N-hetero aromatics

Figure S1: *Visualization of the micropollutant (MP) mixtures for the ozonation batch experiments (O3bMix). All MPs of a row were mixed together (called MR0 – MR8) and all MPs of a column were mixed together (called MC0 - MC9). The background colors indicate the ozone reactive functional groups of the micropollutants.*

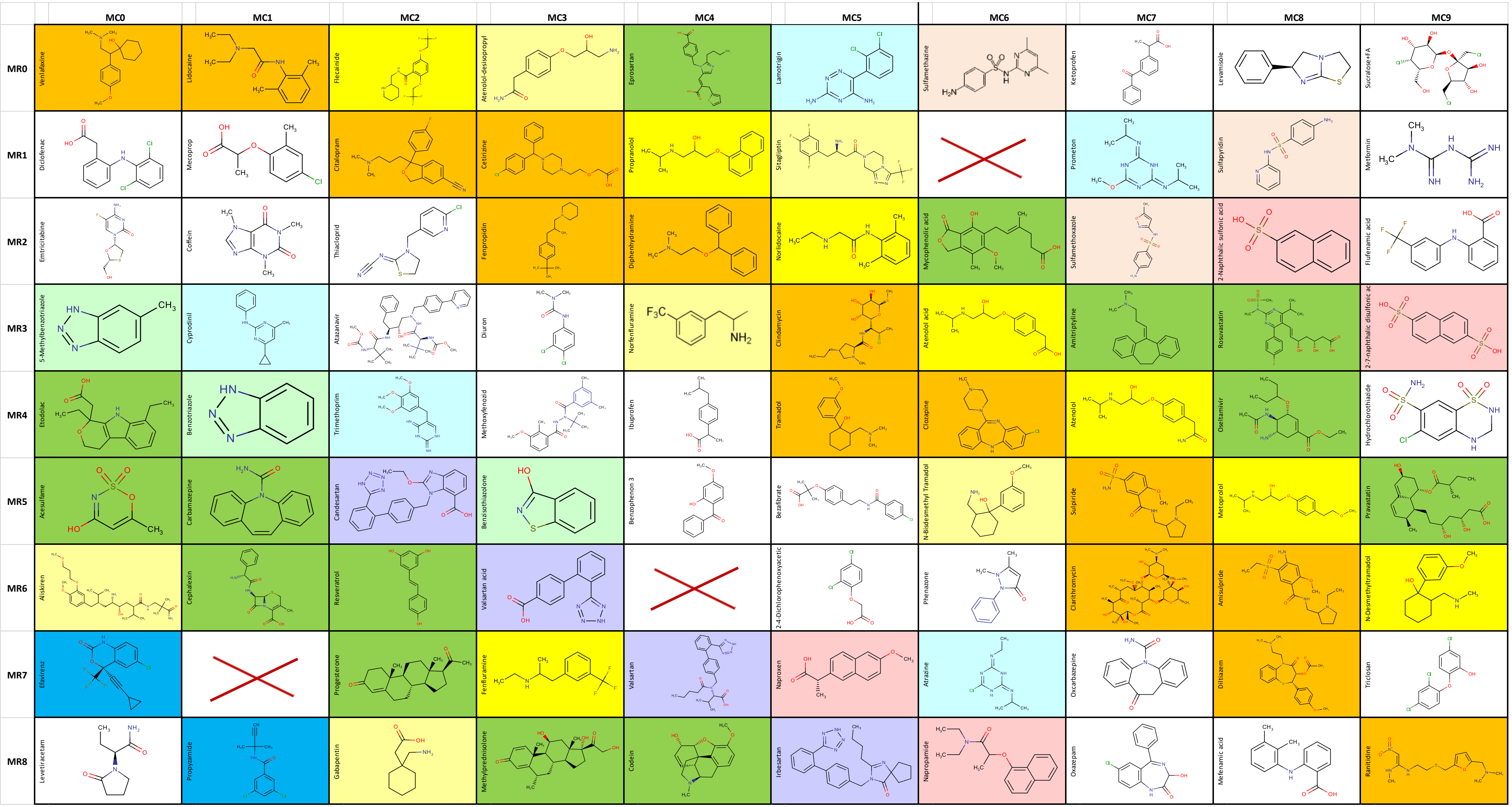

Figure S2: *Visualization of the micropollutant (MP) mixtures for the ozonation batch experiments (O3bMix). All MPs of a row were mixed together (called MR0 – MR8) and all MPs of a column were mixed together (called MC0 - MC9). The background colors indicate the ozone reactive functional groups of the micropollutants. Color coding as in Figure S1: 3° amines (orange), 2° amines (yellow), 1° amines (light yellow), olefins (green), ethynes (blue), sulfonamide (light pink), aromatic compounds (white), similar to benzotriazole (light green), napthalenes (pink), N-hetero aromatics - triazines (light blue), sartanes (purple).*

Text S2. Laboratory ozonation batch experiments

Text S2.1 Simulation of organic matter containing water matrices and ozonation conditions

Before conducting the ozonation batch experiments with micropollutants, two water matrices with a DOC content of 1.3 and 5 were simulated by mimicking the organic matter with methanol and acetate. Methanol acts as promoter for the radical chain reaction with hydroxyl radicals (•OH), which enhances the rate of ozone decomposition, while acetate is an inhibitor, which stops the radical chain reaction. Therefore, methanol and acetate represent sites in the dissolved organic matter (DOM), which control the ozone stability (Buffle et al. 2006, Elovitz and von Gunten 1999, Hoigné and Bader 1994, Staehelin and Hoigne 1985). The aim was to yield similar ozonation conditions as in real waters, i.e. ozone- and •OH exposure (and the concentration ratio of •OH/O_3 known as R_{ct}), but with less interferences than DOM in the LC-HR-MS/MS screening for OTP signals. Details on the composition of the simulated water matrices as well as on the resulting ozonation conditions in the pre-experiments are provided in Table S4. Experiments were performed in a phosphate buffer at pH 7.5 (1-5 mM depending on the experiment), which does not affect the oxidation by ozone as long as the pH remains constant. Figure S3 shows the evolution of the ozone concentration and the R_{ct} values for various conditions. The specific ozone dose was chosen depending on the simulated DOC content to be 1 gO_3/gDOC and therefore the absolute ozone doses differed among the two water matrices. As can be seen from Table S4 and Figure S3, also the duration of the experiments differed, whereas the ozone exposure (0.020 - 0.035 Ms) at full ozone depletion and the R_{ct} values (1.3×10^{-8} - 2.4×10^{-8}) were in a similar range. By taking samples at different time points and quenching the ozone with indigo or sulfite, different ozonation conditions can be selected to best mimic real water conditions. The ozone exposures of 9 different municipal wastewaters were determined to be in the range of 0.0002-0.0028 Ms for a specific ozone dose of 0.5 gO_3/gDOC, and 0.004-0.013 Ms for a specific ozone dose of 1 gO_3/gDOC (Lee et al. 2013). For 5 lakes and 2 river waters, Elovitz et al. (2000) determined ozone exposures in the range of 0.0015-0.024 Ms for specific ozone doses of 0.67-1.0 gO_3/gDOC. The R_{ct} values for the seven surface waters were in the range of 0.95×10^{-8} -5.8×10^{-8} and for the nine wastewaters in the range of 5.0×10^{-8} -19×10^{-8} (for 0.5 gO_3/gDOC) and of 2.2×10^{-8} -6.3×10^{-8} (for 1.0 gO_3/gDOC). By time-resolved experiments any water matrix can be simulated in terms of ozone and hydroxyl radical exposure and the R_{ct} values. This enables to carry out product studies under realistic ozonation conditions in absence of a cumbersome matrix, which may cause analytical problems.

Table S4 shows the water matrices simulating DOC concentrations of 1.3 mg/L and 5 mg/L which were used for the ozonation batch experiments with micropollutants (see Text S2.2 and S2.3).

The simulated DOC concentrations refer to a lake water and a wastewater effluent in Switzerland, respectively. The scavenging rates for the different water constituents are also shown in Table S4. They were calculated by the product of the second-order rate constants of the reactions with hydroxyl radicals and the concentration of the scavengers. For the simulation of the 1.3 mg/L DOC matrix, the relative scavenging rates of acetate and methanol are 30% and 70%, respectively. For 5 mg/L DOC matrix, the relative scavenging rates of acetate and methanol are 5% and 95%, respectively.

Table S4: *Details on the simulated water matrices without addition of micropollutants.*

Simulated DOC concentration in mg/L (scavenging rate) $k_{DOC,OH}$ = 3x10^4 L/mg/s [a]	Acetate in mM (scavenging rate) $k_{ac,OH}$ = 7.9x10^7 $M^{-1}s^{-1}$ [b]	MeOH in mM (scavenging rate) $K_{MeOH,OH}$ = 9.7x10^8 $M^{-1}s^{-1}$ [b]	pH (Phosphate buffer 1-5 mM)	Targeted Ozone dose in mg/L	Ozone exposure in M·s (full-depletion)	R_{ct}
1.3 (3.9x10^4 s^{-1})	0.15 (1.19x10^4 s^{-1})	0.029 (2.8x10^4 s^{-1})	7.5	1.3	0.035	1.3×10^{-8}
5 (1.5x10^5 s^{-1})	0.10 (7.9x10^3 s^{-1})	0.16 (1.55x10^5 s^{-1})	7.5	5	0.020	2.4×10^{-8}

[a] (von Sonntag and von Gunten 2012), [b] (Elovitz and von Gunten 1999)

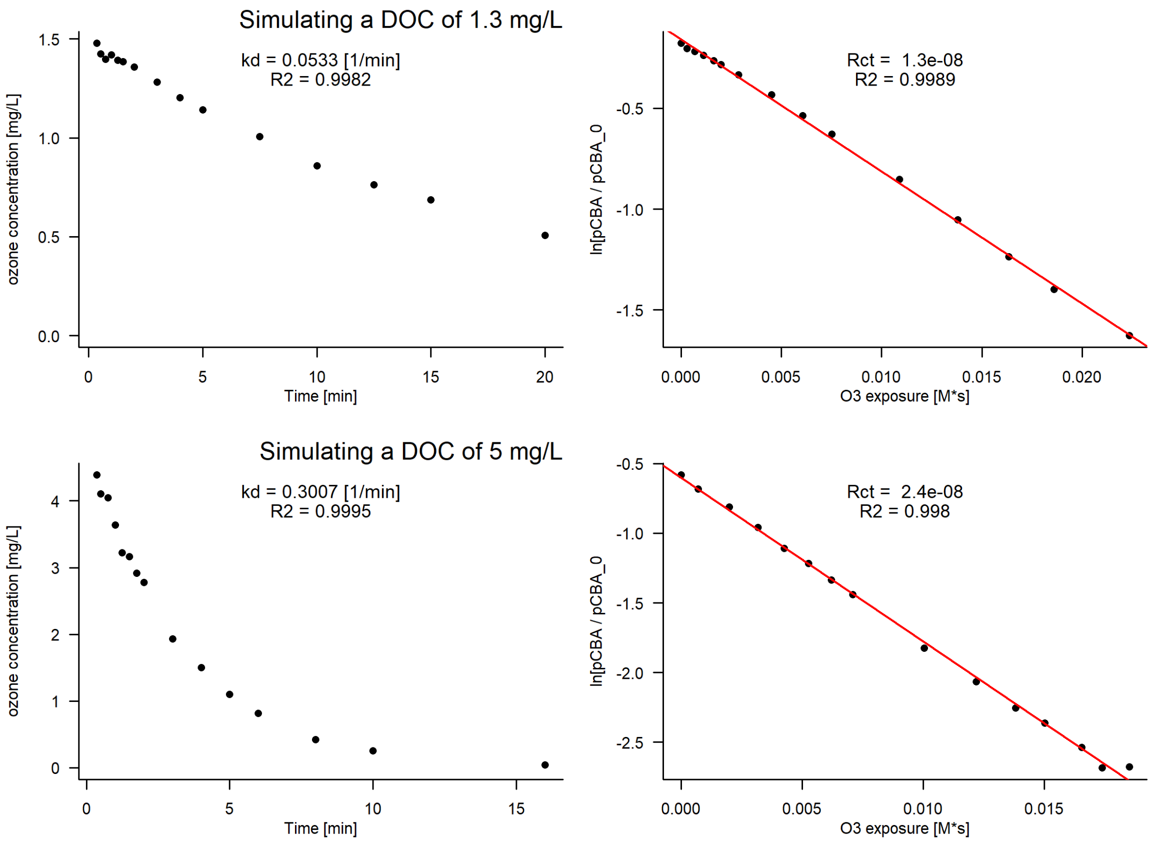

Figure S3: *Ozonation conditions for the two different simulated water matrices without addition of micropollutants. Left: plots of ozone concentrations as a function of the reaction time. Right: plots of ln(pCBA/pCBA_0) as a function of the ozone exposure, the* R_{ct} *can be calculated from the slopes and is provided in the graphs.*

Text S2.2 Ozonation conditions in the O3bMix experiments

In Figure S4 - Figure S8 the ozonation conditions of the 19 *O3bMix* experiments (spiked with 19 different MP mixtures as described in Section 2.1, main manuscript) are illustrated. These experiments were performed with 0.15 mM acetate and 0.029 mM methanol, simulating a DOC of 1.3 mg/L. The resulting average ozone exposure for complete ozone depletion was 0.011±0.003 Ms and the estimated R_{ct} from the two-point fit of the 19 experiments (note that ozone quenching with sulfite did not work in the *O3bMix* samples) was $(2.3\pm0.7) \times 10^{-8}$. These experiments had a lower ozone exposure than expected from the experiments defining the simulation conditions (0.035 Ms) described above. The reason might be the different handling due to the addition of MPs. Nevertheless, the ozone exposure of the *O3bMix* experiments at full depletion of 0.011±0.003 Ms fitted better to conditions with real water matrices (wastewaters and surface waters, see previous section) compared to the conditions of the preliminary experiments (Table S4).

These experiments were performed also with another matrix (0.10 mM acetate and 0.16 mM methanol, simulating a DOC of 5 mg/L, average ozone exposure at full ozone consumption was 0.016±0.002 Ms (data not shown). However, the ozone exposure of this matrix was higher than the values for real wastewater effluents and is therefore less representative for wastewater than the matrix simulating a DOC of 1.3 mg/L. Therefore, it is reasonable that only few additional OTP signals could be identified in the examined WWTP samples from the evaluation of this matrix. Based on these observations, we decided to focus our evaluation and presentation of the results only on the 1.3 mg/L DOC matrix. However, due to failure of one reactor in the first matrix (MC8), the MPs 2NS, ASP, MTO, OSE, SPD were evaluated in the other matrix (simulated DOC 5.0 mg/L).

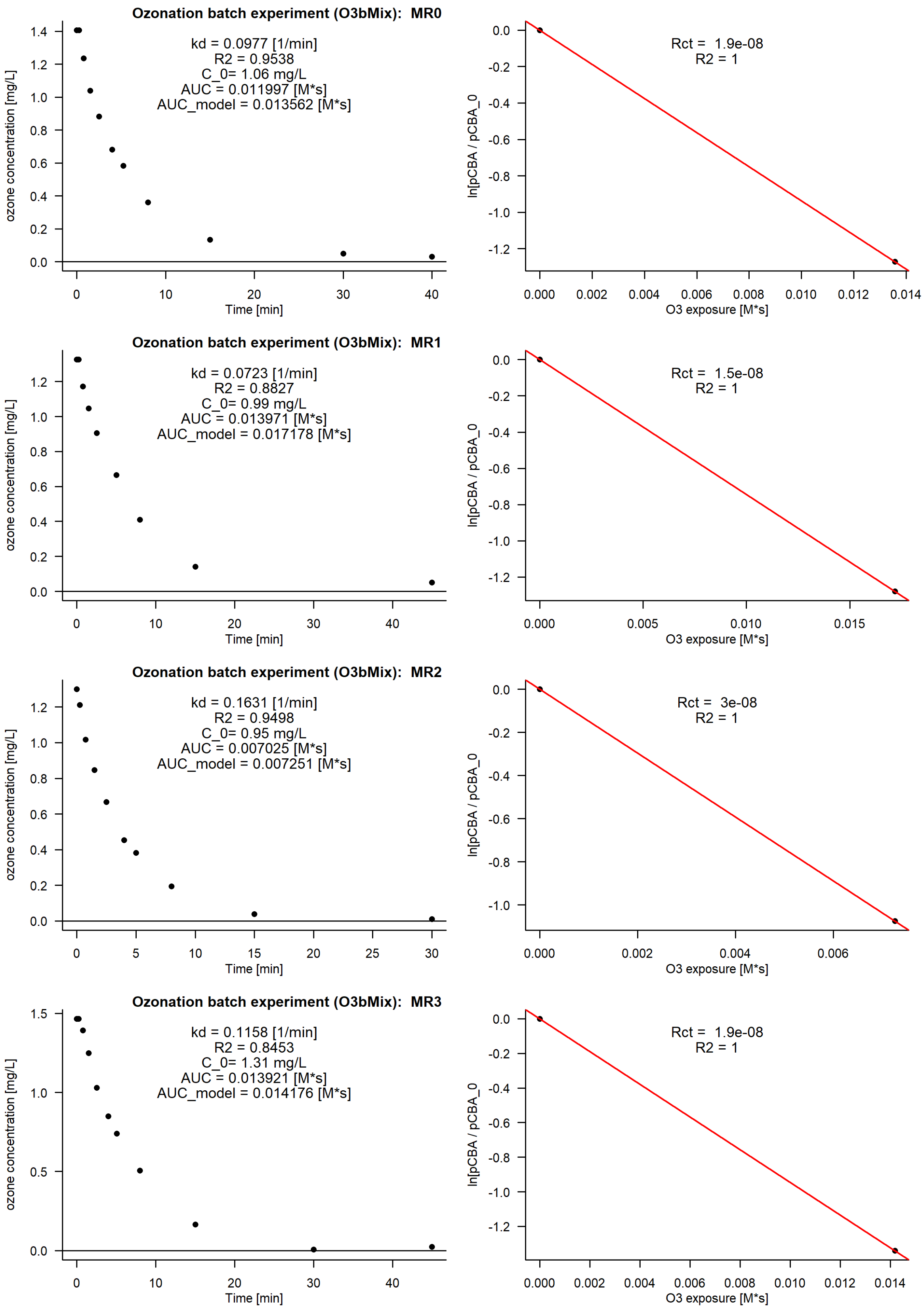

Figure S4: *Ozonation conditions of O3bMix experiments with mixtures MR0-MR3 (1.3 mg/L DOC matrix). Left: Evolution of the ozone concentration, right: Calculation of* R_{ct}.

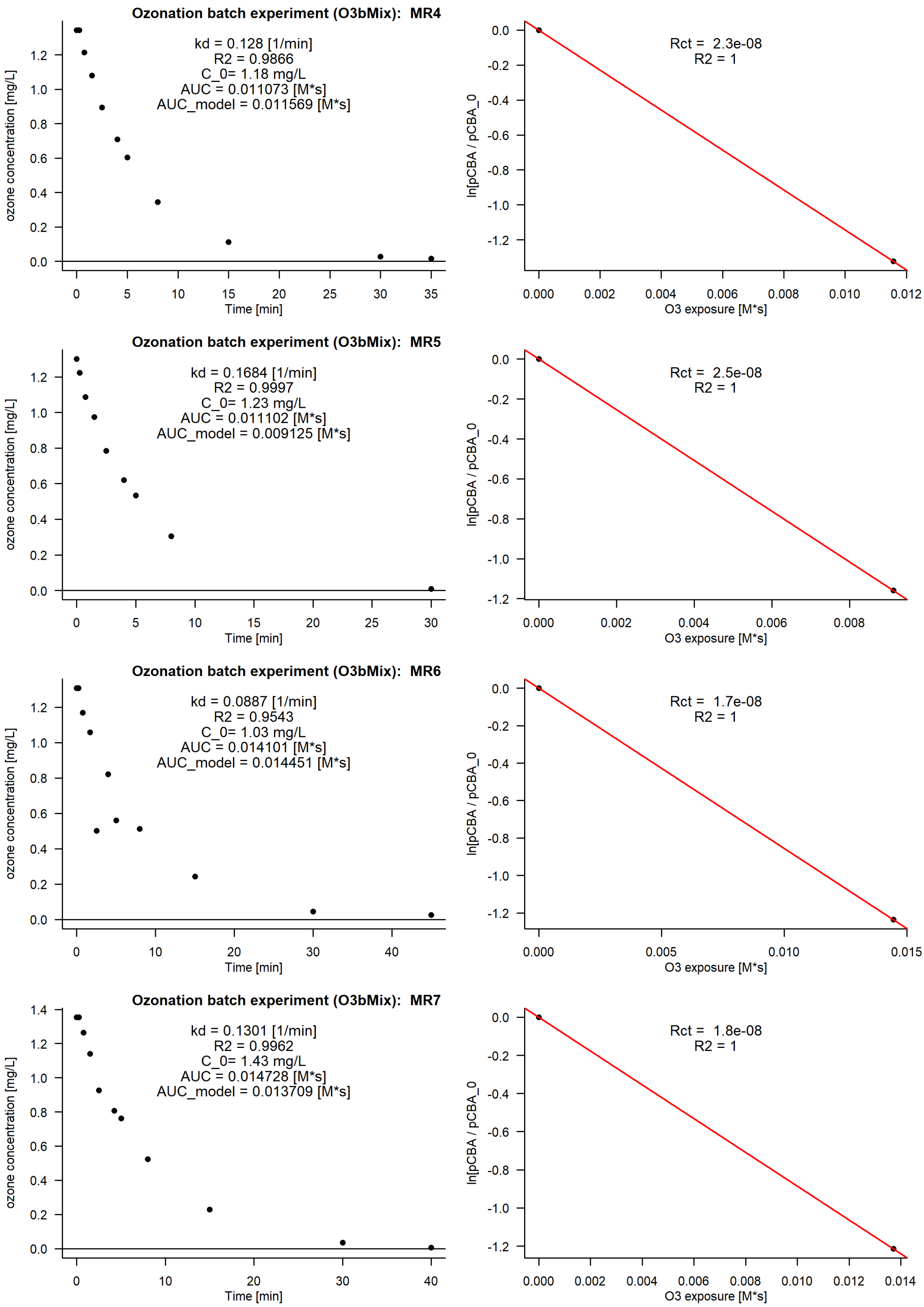

Figure S5: *Ozonation conditions of O3bMix experiments with mixtures MR4-MR7 (1.3 mg/L DOC matrix). Left: Evolution of the ozone concentration, right: Calculation of* R_{ct}.

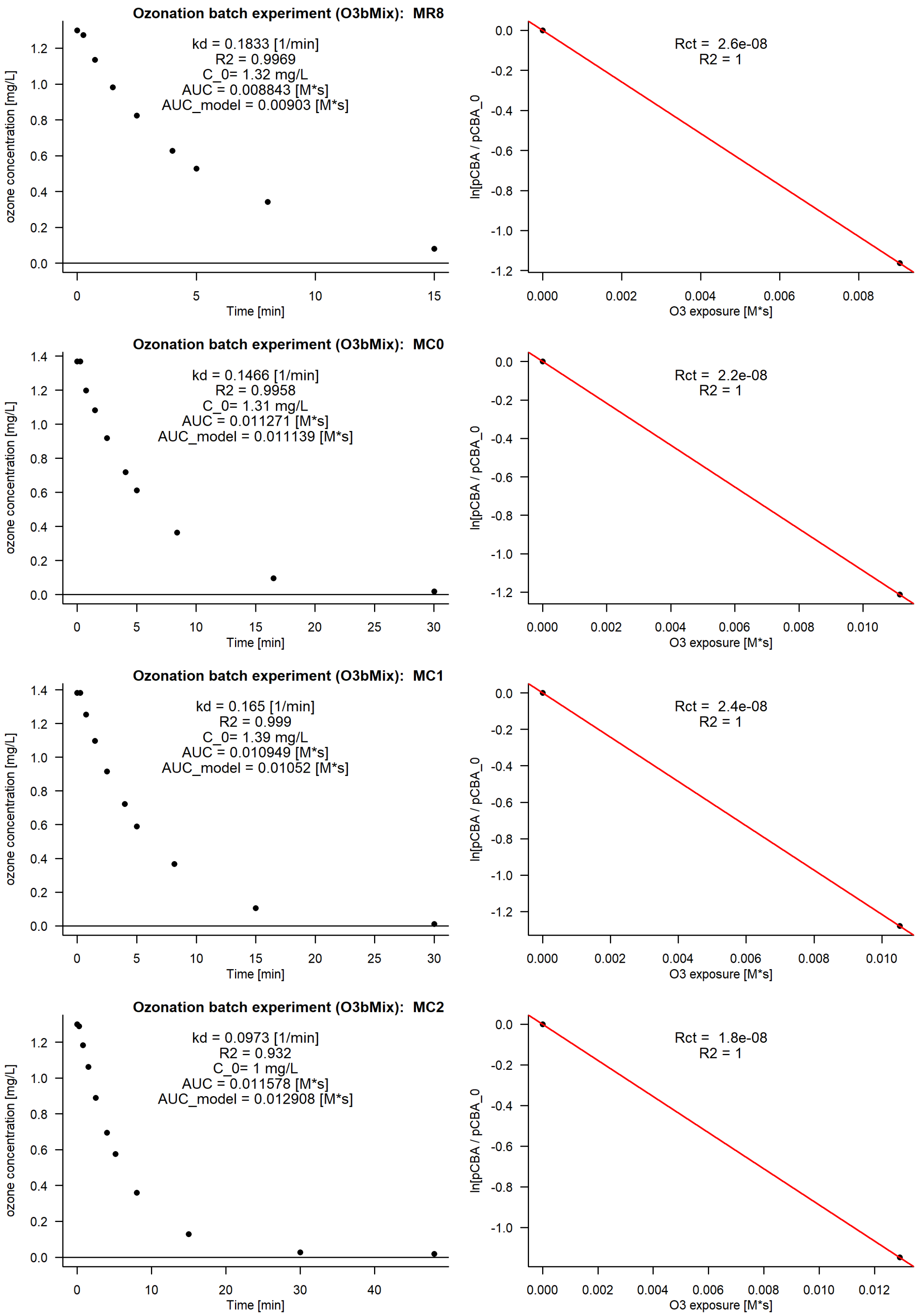

Figure S6: *Ozonation conditions of O3bMix experiments with mixtures MR8 and MC0-MR2 (1.3 mg/L DOC matrix). Left: Evolution of the ozone concentration, right: Calculation of* R_{ct}.

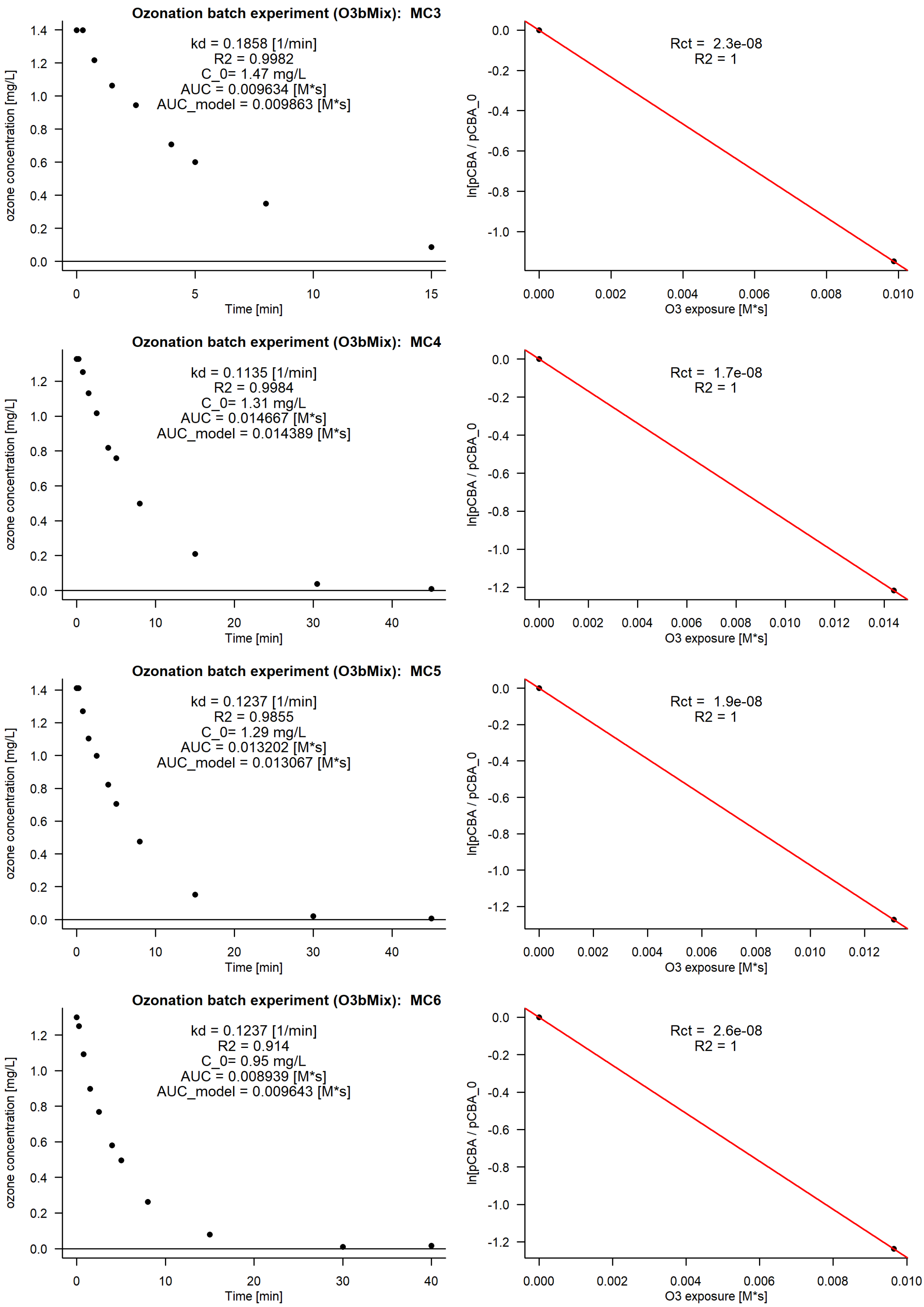

Figure S7: *Ozonation conditions of O3bMix experiments with mixtures MC3-MC6 (1.3 mg/L DOC matrix). Left: Evolution of the ozone concentration, right: Calculation of* R_{ct}.

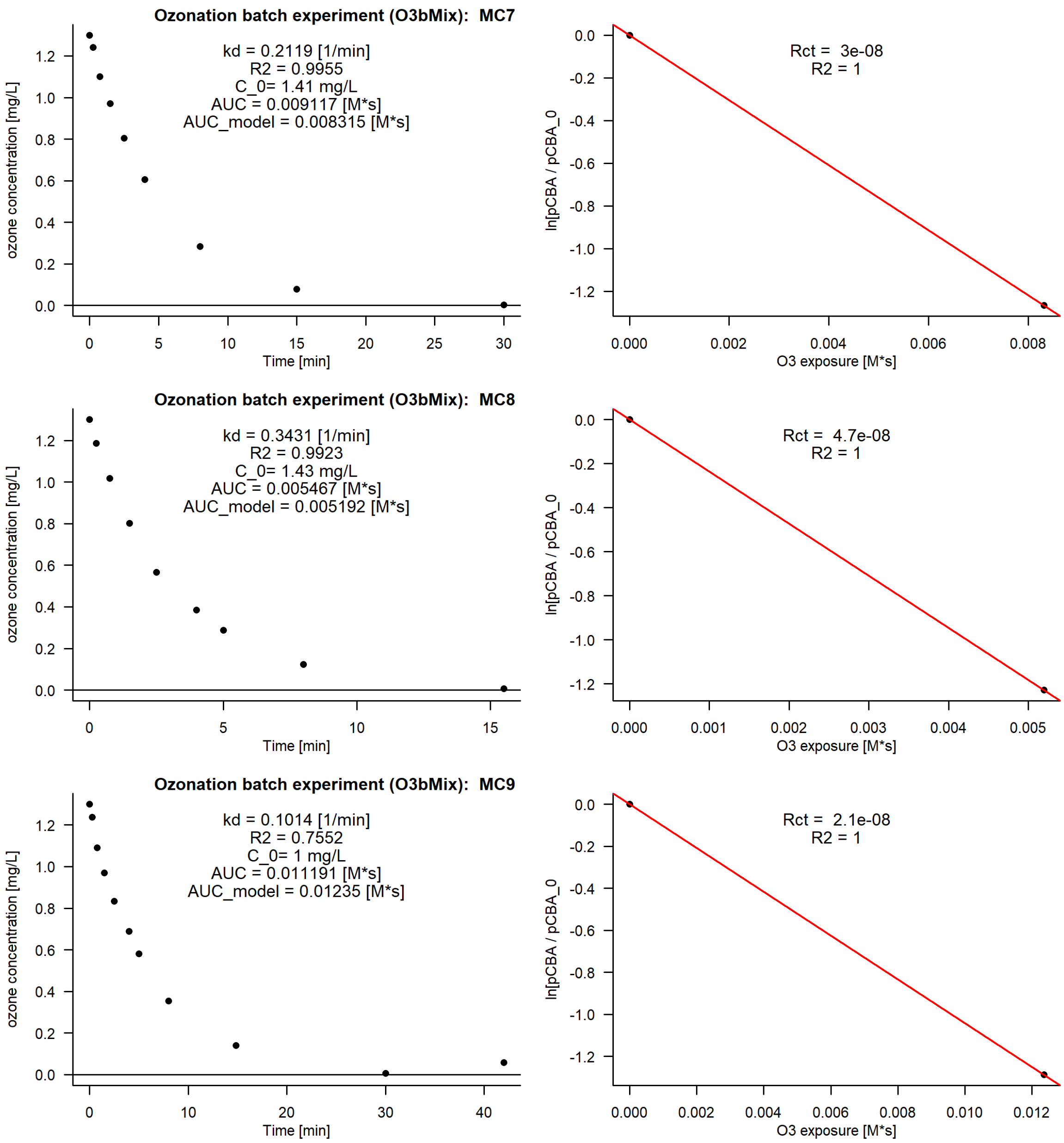

Figure S8: *Ozonation conditions of O3bMix experiments with mixtures MR7-MR9 (1.3 mg/L DOC matrix). Left: Evolution of the ozone concentration, right: Calculation of* R_{ct}*.*

Text S2.3 Ozonation conditions in the O3bAll batch experiment

Figure S9 shows the results of ozonation conditions of the *O3bAll* experiment. This experiment was performed with 0.15 mM acetate and 0.029 mM methanol, simulating a DOC of 1.3 mg/L.

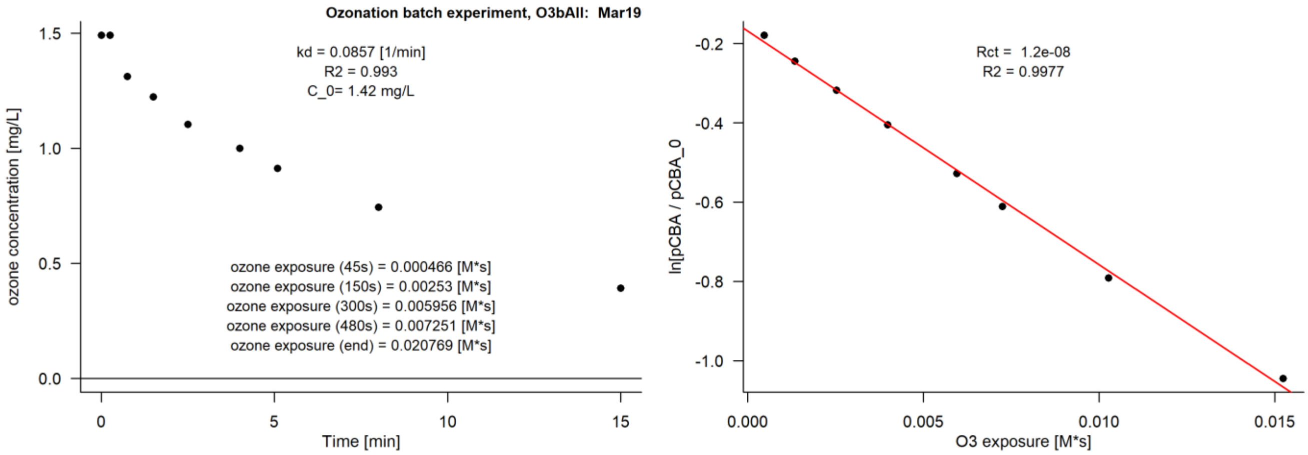

Figure S9: *Ozonation conditions of the O3bAll experiment. Left: Evolution of the ozone concentration, right: Calculation of* R_{ct}.

Text S3. Chemical analyses

Text S3.1 Method for the O3bMix samples

The LC gradient at a flow rate of 300 µL/min and a column temperature of 30°C was:

Time in min	% (Nanopure water +0.1% formic acid)	% (Methanol + 0.1% formic acid)
0	100	0
4	100	0
19	5	95
25	5	95
25.1	100	0
30	100	0

The Electrospray ionization was triggered at a capillary temperature of 320°C and a spray voltage of 4 kV and 3kV for positive and negative ionization, respectively. The sheath gas flow rate was adjusted to 40, the auxiliary gas flow rate to 10, and the sweep gas flow rate to 0. Mass calibration and mass accuracy checks were carried out prior to the measurement with an in-house amino acid solution, which enhanced calibration for small masses. Full-scan acquisition was conducted at a resolution of 140’000 at m/z 200 and a scan range of m/z 50-750 with an injection time of 50 ms and an AGC target value of 5×10^5. Ten data-dependent MS/MS scans were conducted after each full-scan with a resolution of 17’500 at m/z 200, an injection time of 100 ms, an AGC target value of 1×10^5, and an isolation width of 1.0 Da. The dynamic exclusion was set to 3 sec. The data-dependent MS/MS were triggered after each full-scan on the [M+H]+ and if negative ionization mode acquisition was conducted on the [M-H]- on masses predicted by O3-PPD (Lee et al. 2017, Schollée et al. 2021). The collision energy with which the MS/MS scans were triggered were calculated as (-0.41) × exact mass+160 for masses below m/z 350 and were set to 15 for masses above 350 m/z. Quantification of the parent micropollutants was done with the Tracefinder 4.1 software (Thermo Scientific). The smallest standard of the calibration row with a reasonable peak was treated as limit of quantification (LOQ) as given in Table S1.

Text S3.2 Method for the O3bAll samples

The LC gradient for the separation of the sample on the chromatographic column at a flow rate of 300 µL/min and a column temperature of 30°C was:

Time in min	Nanopure water +0.1% FA in µL/min	Methanol + 0.1% FA in µL/min
0	260	40
5	260	40
20	15	285
29	15	285
29.5	260	40
35	260	40

The Electrospray ionization was triggered at a capillary temperature of 350°C and a spray voltage of 4 kV and 3 kV for positive and negative ionization, respectively. The sheath gas flow rate was adjusted to 40, the auxiliary gas flow rate to 10, and the sweep gas flow rate to 0. Mass calibration and mass accuracy checks were carried out prior to the measurement with an in-house amino acid solution, which enhanced calibration for small masses. Full-scan acquisition was conducted at a resolution of

140’000 at m/z 200 and a scan range of m/z 60-900 with an injection time of 100 ms and an AGC target value of 5×10^5. Seven data-dependent MS/MS scans were conducted after each full-scan with a resolution of 17’500 at m/z 200, an injection time of 65 ms, an AGC target value of 1×10^5, and an isolation width of 1.0 Da. The dynamic exclusion was set to 5 sec. The data-dependent MS/MS were triggered after each full-scan on the [M+H]+ and if negative ionization mode acquisition was conducted on the [M-H]- on masses that were identified from the O3bMix experiments. The collision energy with which the MS/MS scans were triggered were calculated as (-0.41)*exact mass+160 for masses below m/z 350 and were set to 15 for masses above 350 m/z. Quantification of the parent micropollutants was done with the Tracefinder 4.1 software (Thermo Scientific). The smallest standard of the calibration row with a reasonable peak was treated as limit of quantification (LOQ) as given in Table S1.

Text S4. Identification of ozonation transformation products

Please note for 5 parent MPs (2-napthalic-sulfonic-acid 2NS, amisulpride ASP, metoprolol MTO, oseltamivir OSE, and sulfapyridin SPD), for which an *O3bMix* reactor (MC8) failed, no OTP signals could be identified within the matrix simulating a DOC of 1.3 mg/L. However, the experiments were repeated within another matrix simulating a DOC of 5 mg/L (see Text S2.2). The results of these experiments are not shown. However, for these 5 MPs the result of the additional experiments were adopted.

Text S4.1 Generation of a list of suspect OTPs using laboratory ozonation batch experiments (O3bMix) with Compound Discoverer 2.1 (Approach 1)

For each parent MP, a separate Compound Discoverer 2.1 run in positive and negative mode was conducted to screen for corresponding OTP signals. The following details were chosen for the Compound Discoverer 2.1 run.

Two study factors were defined

- *Mix* with the levels: column mix, row mix, control mix
- *Time Points* with the levels: 0h (time zero sample, before ozone addition), S2 (2nd sample at 53s), S4 (4th sample at 158 s), S6 (6th sample at 308 s), S7 (7th sample at 488 s), Sc (sample of the control reactor)

For the uploaded measurement files, the study factors were selected. For the files of the control reactor, the *Time Points* Sc were selected instead of assigning different time point levels. In this way, the automatically calculated mean value of this group could be used. Figure S10 illustrates the selection.

At the Sample Groups and Ratios step, *Time points: 0h* was selected to generate Ratios as can be seen in Figure S11.

The applied Workflow is illustrated in Figure S12, the node settings are given in Table S5.

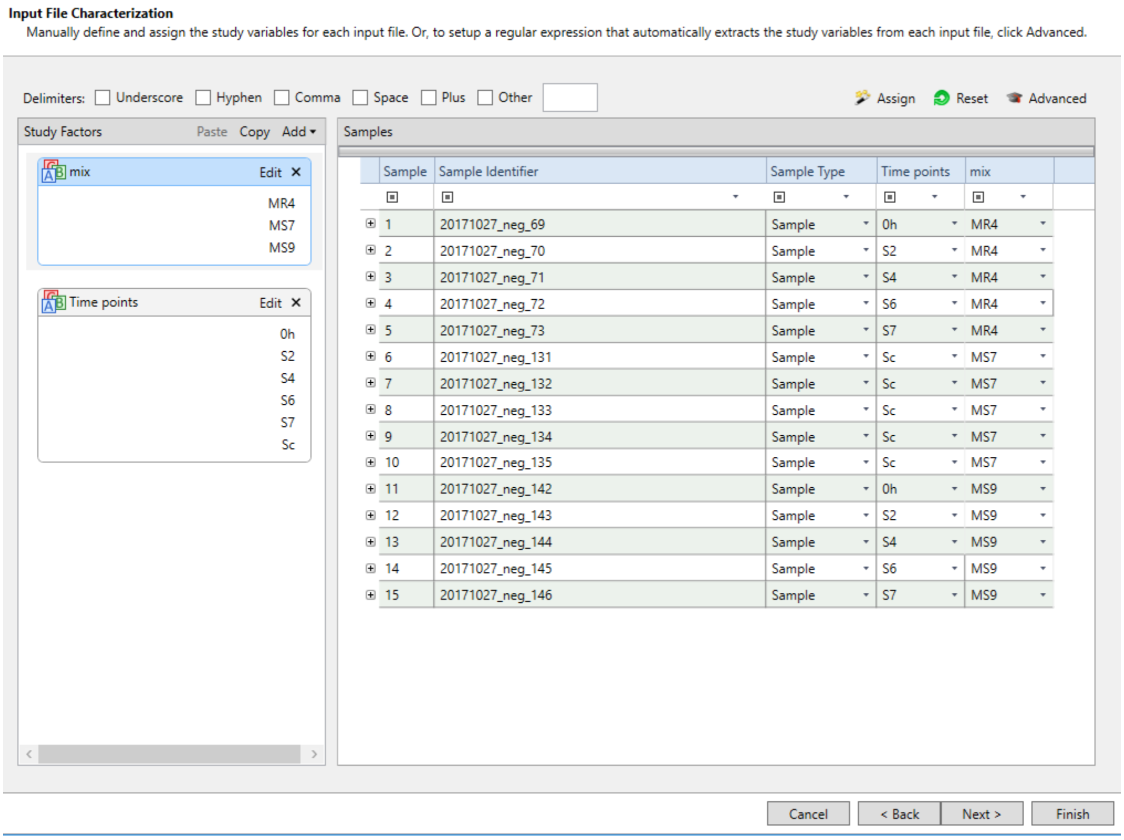

Figure S10: *Input file assignment for Compound Discoverer 2.1 (Approach 1).*

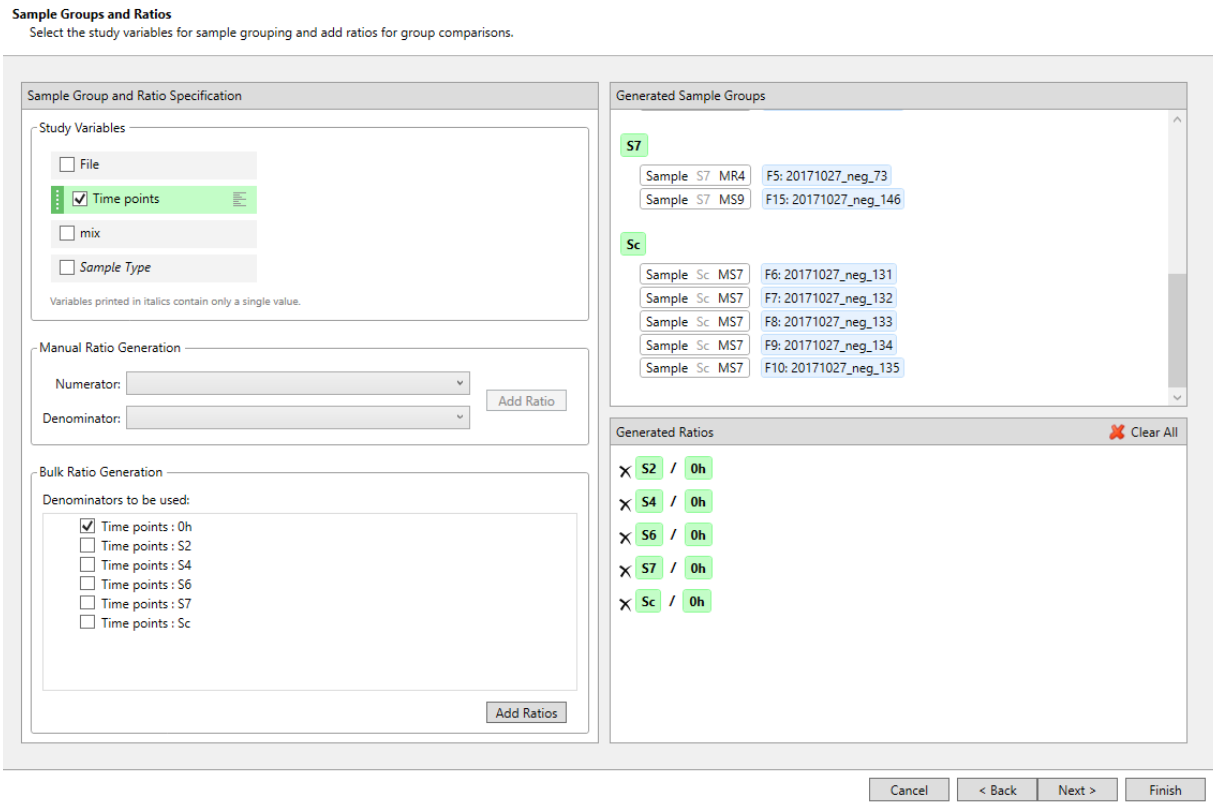

Figure S11: *Sample Groups and Ratios for Compound Discoverer 2.1 (Approach 1).*

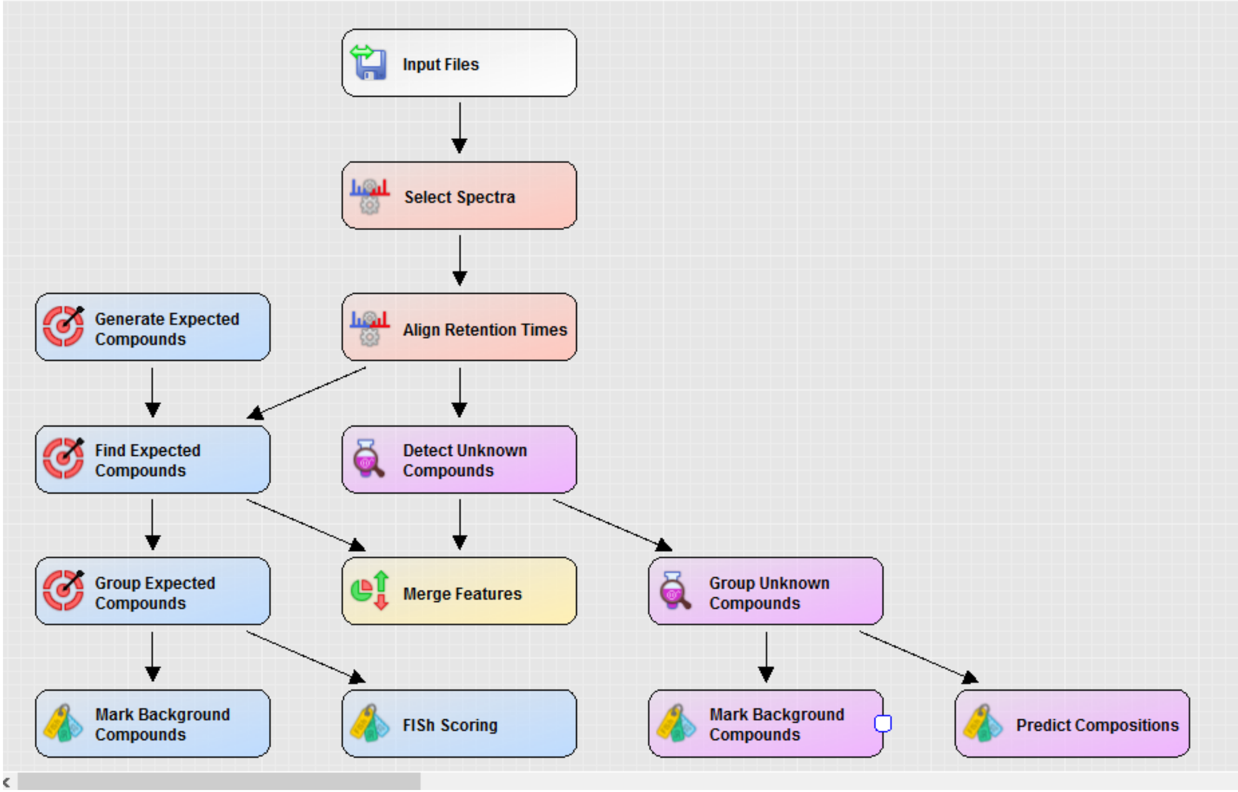

Figure S12: *Applied workflow in Compound Discoverer 2.1 (Approach 1).*

Table S5: *Compound Discoverer 2.1 settings for the evaluation of the O3bMix experiments (Approach 1). In blue are highlighted settings that were different for the evaluation of samples measured in positive and negative mode. The first settings refer to the evaluation in positive mode and the second to the evaluation in negative mode. In the Generate Expected Compounds note the respective parent MP was selected as highlighted in green.*

<table>
<tr>
<td rowspan="3">Select Spectra

1. General Settings:
- Precursor Selection: Use MS(n - 1) Precursor

2. Spectrum Properties Filter:
- Lower RT Limit: 0
- Upper RT Limit: 0
- First Scan: 0
- Last Scan: 0
- Ignore Specified Scan:
- Lowest Charge State: 0
- Highest Charge State: 0
- Min. Precursor Mass: 100 Da
- Max. Precursor Mass: 5000 Da
- Total Intensity Threshold: 0
- Minimum Peak Count: 1

3. Scan Event Filters:
- Mass Analyzer: (not specified)
- MS Order: Any
- Activation Type: (not specified)
- Min. Collision Energy: 0
- Max. Collision Energy: 1000
- Scan Type: Any
- Polarity Mode: Is + / Is-

4. Peak Filters:
- S/N Threshold (FT-only): 1.5</td>
<td>Detect Unknown Compounds

1. General Settings:
- Mass Tolerance [ppm]: 5 ppm
- Intensity Tolerance [%]: 30
- S/N Threshold: 3
- Min. Peak Intensity: 10000/1000
- Ions: [M+H]+H / [M-H]-1
- Min. Element Counts: C H
- Max. Element Counts: C90 H190 Br3 Cl4 K2 N10 Na2 O18 P3 S5</td>
<td rowspan="2">Generate Expected Compounds

1.Compound Selection
- Compound: respective parent Micropollutant

2. Dealkylation
- Apply Dealkylation: True
- Apply Dearylation: True
- Max. #Steps: 1
- Min. Mass [Da]: 200

3. Transformations
- Phase |: some meaningful reactions
- Phase ||: 0
-Others: manually entered reactions according to (Schollée et al. 2018)
- Max. # Phase ||: 0
- Max.# All Steps: 3

4.Ionization
-Ions: [M+H]+ / [M-H]-</td>
</tr>
<tr>
<td>Group Unknown Compounds

1. Compound Consolidation
- Mass Tolerance: 5 ppm
- RT Tolerance [min]: 0.5

2. Fragment Data Selection:
- Preferred Ions: [M+H]+ / [M-H]-</td>
</tr>
<tr>
<td>Mark Background Compounds

1. General Settings:
- Max. Sample/Blank: 5
- Max. Blank/Sample: 0
- Hide Background: True</td>
<td>Find Expected Compounds

1.General Settings
- Mass Tolerance: 5ppm
- Intensity Tolerance [%]: 30
- Intensity Threshold [%]: 0.1
- SN Threshold: 3</td>
</tr>
</table>

5. Replacements for Unrecognized Properties: - Unrecognized Charge Replacements: 1 - Unrecognized Mass Analyzer Replacements: ITMS - Unrecognized MS Order Replacements: MS2 - Unrecognized Activation Type Replacements: CID		- Min. # Isotopes: 2 - Min. Peak. Intensity: 1000 - Average Peak Width [min]: 0
	Merge Features 1. Peak Consolidation - Mass Tolerance: 5 ppm - RT Tolerance [min]: 0.5	**Group Expected Compounds** 1.Compound Consolidation - RT Tolerance [min] : 0.5 2. Fragment Data Selection - Preferred Ions : [M+H]+ / [M-H]-
	Predict Compositions 1.Prediction Settings - Mass Tolerance: 5 ppm - Min. Element Counts: CH - Max. Element Counts: : C90 H190 Br3 Cl4 K2 N10 Na2 O18 P3 S5 - Min. RDBE: 0 - Max. RDBE: 40 - Min. H/C: 0.1 - Max. H/C: 3.5 - Max. # Candidates: 10 2. Pattern Matching - Intensity Tolerance [%]: 30 - Intensity Threshold [%]: 0.1 - S/N Threshold: 3 - Use Dynamic Recalibration: True 3.Fragments Matching - Use Fragments Matching: True - Mass Tolerance: 5ppm - S/N Threshold: 3	**Mark Background Compounds** 1. General Settings: - Max. Sample/Blank: 5 - Max. Blank/Sample: 0 - Hide Background: True
Align Retention Times 1. General Settings: - Alignment Model: Adaptive curve - Maximum Shift [min]: 2 - Mass Tolerance: 5 ppm		**FISh Scoring** 1.General Settings - Annotate Full Tree: True - Match Transformations: True - High Acc. Mass Tolerance: 2.5mmu - Low Acc. Mass Tolerance: 0.5 Da 2. Fragmented Prediction Settings - Use General Rules: True - Use Libraries: True - Max. Depth: 5 - Aromatic Cleavage: True - Min. Fragment m/z: 50

After the processing of Compound Discoverer 2.1, the following steps were conducted:

- Checking the presence of 3-5 internal standards and 1-3 parent compounds.
- Reducing the hits within the Merged Features tab by an automatic filtering with the following criteria as illustrated in Figure S13:
    1. the peak had to increase at least 5 times from the samples before to the samples after ozone addition
    2. presence in both mixtures containing the respective parent MP
    3. absence in the control mixture

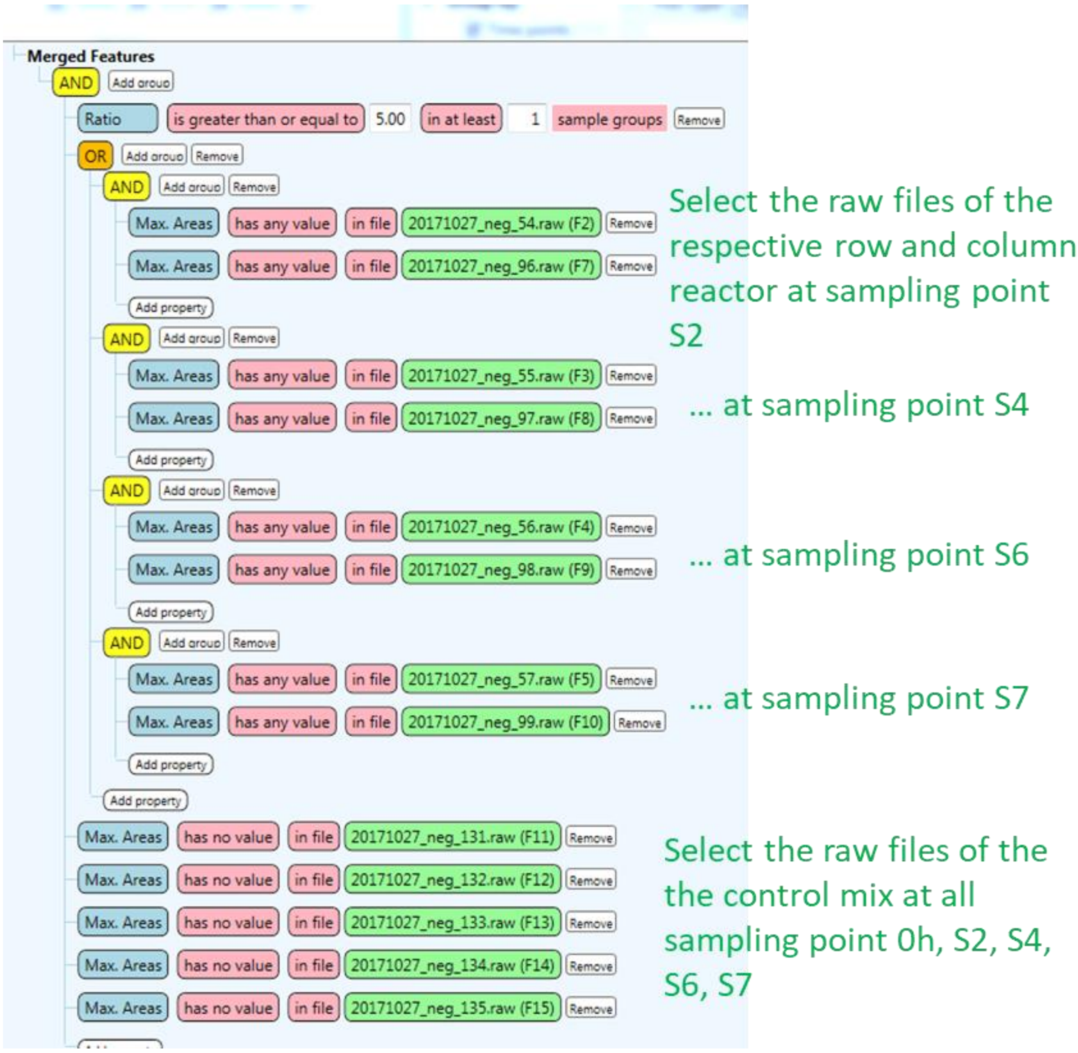

Figure S13: *Filter criteria applied for Merged Features in Compound Discoverer 2.1 (Approach 1).*

Afterwards, the profiles were manually investigated and selected based on the following criteria:

- reasonable peak shape by visual inspection
- meaningful presence / absence in the treated / control samples
- higher chance to be selected if questionable if present in the *Expected Compounds* tab

Text S4.2 Identification of OTP signals that are simultaneously forming in O3bAll and WWTP samples with Compound Discoverer 2.1 (Approach 2)

To identify OTP signals that are formed in our reference laboratory ozonation batch experiment (*O3bAll*) and in the WWTPs, we used Compound Discoverer 2.1 (Thermo Scientific) for a separate evaluation in positive and negative mode. We uploaded the samples from the *O3bAll* experiments and from the four different WWTPs. Since the *O3bMix* experiments were already performed and measured within Approach 1 (A-C), we uploaded these samples as well. By doing so, we performed Approach 2C and 2F at the same time.

Three study factors (*Mix, Sample points, type*) with different levels were applied (see Figure S14). Most of them were only important for a neat arrangement in the resulting table. Relevant for the filtering were only the following three levels of the study factor *type*:

- *13_70_all* (assigned to all samples of the *O3bAll* experiment after ozone addition)
- *all_S0* (assigned to all samples of the *O3bAll* experiment before ozone addition)
- *Blind* (assigned to blind samples)

The applied Workflow is illustrated in Figure S15, the node settings are given in Table S6.

Study Definition | Input Files | Samples | Analysis Results

Sample	Sample Identifier	Sample Type	mix	Sample points	Type
S1	20181203_online_neg_017	Sample	3_NG	3_EFF	3_EFF
S2	20181203_online_neg_018	Sample	3_NG	3_EFF	3_EFF
S3	20181203_online_neg_019	Sample	2_GL	3_EFF	3_EFF
S4	20181203_online_neg_020	Sample	2_GL	3_EFF	3_EFF
S5	20181203_online_neg_021	Sample	2_GL	3_EFF	3_EFF
S6	20181203_online_neg_022	Sample	1_AR	3_EFF	3_EFF
S7	20181203_online_neg_023	Sample	4_PR	3_EFF	3_EFF
S8	20181203_online_neg_025	Sample	3_NG	2_OZO	2_OZO
S9	20181203_online_neg_026	Sample	3_NG	2_OZO	2_OZO
S10	20181203_online_neg_027	Sample	2_GL	2_OZO	2_OZO
S11	20181203_online_neg_028	Sample	2_GL	2_OZO	2_OZO
S12	20181203_online_neg_029	Sample	2_GL	2_OZO	2_OZO
S13	20181203_online_neg_030	Sample	1_AR	2_OZO	2_OZO
S14	20181203_online_neg_031	Sample	4_PR	2_OZO	2_OZO
S15	20181203_online_neg_033	Sample	3_NG	1_BIO	1_BIO
S16	20181203_online_neg_034	Sample	3_NG	1_BIO	1_BIO
S17	20181203_online_neg_035	Sample	2_GL	1_BIO	1_BIO
S18	20181203_online_neg_036	Sample	2_GL	1_BIO	1_BIO
S19	20181203_online_neg_037	Sample	2_GL	1_BIO	1_BIO
S20	20181203_online_neg_038	Sample	1_AR	1_BIO	1_BIO
S21	20181203_online_neg_039	Sample	4_PR	1_BIO	1_BIO
S22	20181203_online_neg_042	Sample	control	Blind	Blind
S23	20181203_online_neg_071	Sample	control	Blind	Blind
S24	20181203_online_neg_086	Sample	control	Blind	Blind
S25	20181203_online_neg_112	Sample	control	Blind	Blind
S26	20181203_online_neg_113	Sample	MRall	S0	all_S0
S27	20181203_online_neg_114	Sample	MRall	S2	_13_70all
S28	20181203_online_neg_115	Sample	MRall	S4	_13_70all
S29	20181203_online_neg_116	Sample	MRall	S6	_13_70all
S30	20181203_online_neg_117	Sample	MRall	S7	_13_70all
S31	20181203_online_neg_119	Sample	MRall	S0	all_S0
S36	20181203_online_neg_124	Sample	control	Blind	Blind
S37	20181203_online_neg_125	Sample	MR0	S2	_13_70_S2
S38	20181203_online_neg_126	Sample	MR0	S6	_13_70_S6
S39	20181203_online_neg_127	Sample	MR1	S2	_13_70_S2
S40	20181203_online_neg_128	Sample	MR1	S6	_13_70_S6
S41	20181203_online_neg_129	Sample	MR2	S2	_13_70_S2
S42	20181203_online_neg_130	Sample	MR2	S6	_13_70_S6
S43	20181203_online_neg_131	Sample	MR3	S2	_13_70_S2
S44	20181203_online_neg_132	Sample	MR3	S6	_13_70_S6
S45	20181203_online_neg_133	Sample	MR4	S2	_13_70_S2
S46	20181203_online_neg_134	Sample	MR4	S6	_13_70_S6
S47	20181203_online_neg_136	Sample	MR5	S2	_13_70_S2
S48	20181203_online_neg_137	Sample	MR5	S6	_13_70_S6
S49	20181203_online_neg_138	Sample	MR6	S2	_13_70_S2
S50	20181203_online_neg_139	Sample	MR6	S6	_13_70_S6
S51	20181203_online_neg_140	Sample	MR7	S2	_13_70_S2
S52	20181203_online_neg_141	Sample	MR7	S6	_13_70_S6
S53	20181203_online_neg_142	Sample	MR8	S2	_13_70_S2
S54	20181203_online_neg_143	Sample	MR8	S6	_13_70_S6

Figure S14: *Input file assignment for Compound Discoverer 2.1 (Approach 2).*

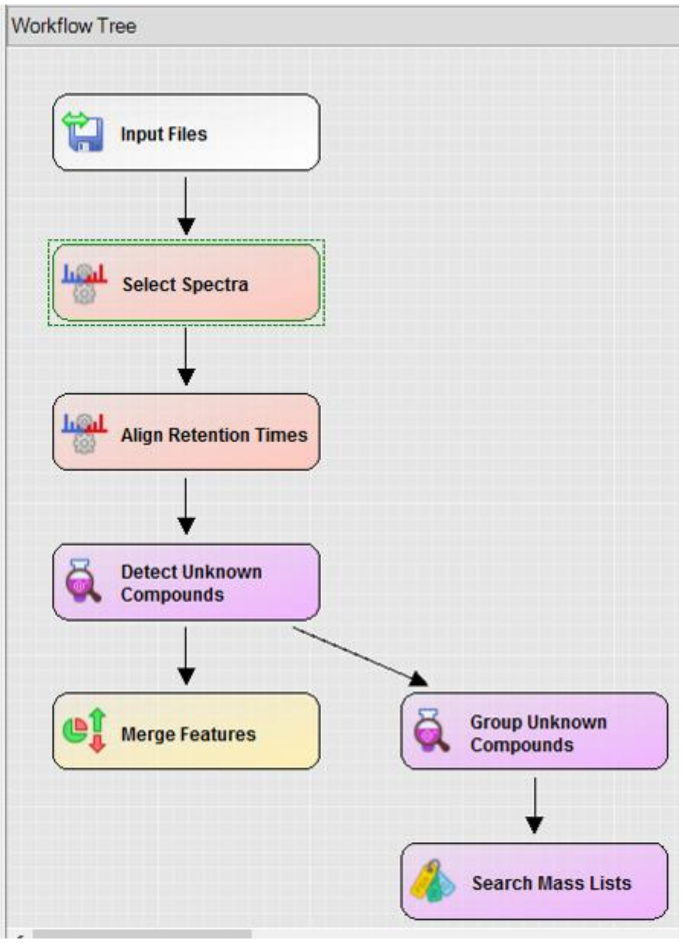

Figure S15: *Applied workflow in Compound Discoverer 2.1 (Approach 2).*

Table S6: *Compound Discoverer 2.1 settings for the evaluation of the O3bAll experiments and WWTP samples (Approach 2). In blue are highlighted settings that were different for the evaluation of samples measured in positive and negative mode. The first settings refer to the evaluation in positive mode and the second to the evaluation in negative mode. In green are the two lists of suspected neutral OTP masses that were uploaded. The first list was generated through Approach 1 and the second list is a compilation of OTP masses known from literature.*

<table>
<tr>
<td rowspan="3">Select Spectra

1. General Settings:
- Precursor Selection: Use MS(n - 1) Precursor

2. Spectrum Properties Filter:
- Lower RT Limit: 0
- Upper RT Limit: 0
- First Scan: 0
- Last Scan: 0
- Ignore Specified Scan:
- Lowest Charge State: 0
- Highest Charge State: 0
- Min. Precursor Mass: 100 Da
- Max. Precursor Mass: 5000 Da
- Total Intensity Threshold: 0
- Minimum Peak Count: 1

3. Scan Event Filters:
- Mass Analyzer: (not specified)
- MS Order: Any
- Activation Type: (not specified)
- Min. Collision Energy: 0
- Max. Collision Energy: 1000</td>
<td>Align Retention Times

1. General Settings:
- Alignment Model: Adaptive curve
- Maximum Shift [min]: 2
- Mass Tolerance: 5 ppm</td>
<td>Group Unknown Compounds

1. Compound Consolidation
- Mass Tolerance: 5 ppm
- RT Tolerance [min]: 0.5

2. Fragment Data Selection:
- Preferred Ions: [M+H]+ / [M-H]-</td>
</tr>
<tr>
<td>Detect Unknown Compounds

1. General Settings:
- Mass Tolerance [ppm]: 5 ppm
- Intensity Tolerance [%]: 30
- S/N Threshold: 3
- Min. Peak Intensity: 10000/1000
- Ions: [M+H]+H / [M-H]-1
- Min. Element Counts: C H
- Max. Element Counts: C90 H190 Br3 Cl4 K2 N10 Na2 O18 P3 S5</td>
<td rowspan="2">Search mass list

Input file(s):
CDin_allMPs_CDRPneg_neutral.csv, CDin_allMPs_litneg_neutral.csv
- Mass Tolerance: 15 ppm
- RT Tolerance: 1
Consider Retention Time: False</td>
</tr>
<tr>
<td>Merge Features</td>
</tr>
</table>

- Scan Type: Any - Polarity Mode: Is + / Is- 4. Peak Filters: - S/N Threshold (FT-only): 1.5 5. Replacements for Unrecognized Properties: - Unrecognized Charge Replacements: 1 - Unrecognized Mass Analyzer Replacements: ITMS - Unrecognized MS Order Replacements: MS2 - Unrecognized Activation Type Replacements: CID	1. Peak Consolidation - Mass Tolerance: 5 ppm - RT Tolerance [min]: 0.5	

To identify signals in the WWTPs that were also formed in our *O3bAll* experiments, we used an automatic filter and a manual selection. The automatic filter had the following criteria (see Figure S16):

Signals had to

- be in at least one *O3bAll* sample after ozone addition
- increase at least 2 times in the *O3bAll* experiment from the sample before ozone addition to any sample at a later time point
- be 5 times higher in the *O3bAll* samples than in the blank samples (20 mL nanopure water augmented with 16 µL ISTD stock solution)
- have any value in WWTP samples after ozonation or post-treatment
- have a retention time between 4 and 28 min
- have a maximal area of at least 10000 / 1000 in positive / negative mode

Signals were manually selected, based on the pattern in the ozonation batch experiments over time and the occurrence in the WWTP samples. Since we had knowledge of suspect OTPs of 70 MPs from Approach 1, we used this information by uploading the list of all exact masses of identified OTP signals. That way, an easier selection of OTP signals was possible as MS^2 spectra were available. However, Approach 2 can be applied without this prior knowledge.

Figure S16: *Filter criteria applied for Merged Features in Compound Discoverer 2.1 (Approach 2).*

Text S5. Measured and calculated abatement of micropollutants in *O3bAll*

Figure S17 illustrates the observed abatement of the four parent MPs atrazine, bezafibrate, ibuprofen, and ketoprofen in the ozonation batch experiments (*O3bAll*) and the expected abatement based on second-order rate constants for their reactions with ozone and •OH (data derived from literature). The good match between experimental data and calculations shows that the ozonation batch experiments are well controlled in terms of ozone and hydroxyl radical exposures and the R_{ct}.

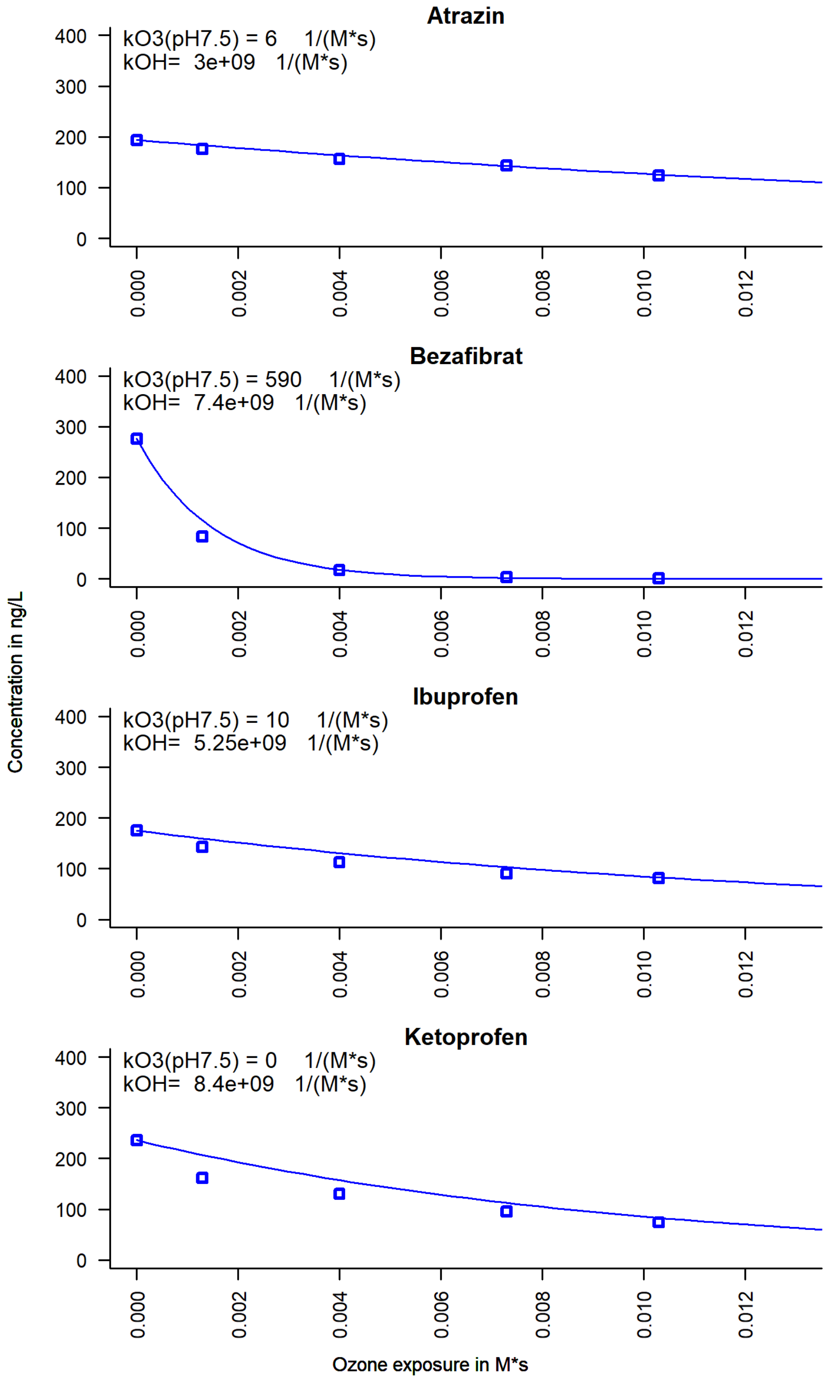

Figure S17: *Abatement of the MPs atrazine, bezafibrate, ibuprofen, and ketoprofen (symbols) as a function of the ozone exposure in the O3bAll experiment. The line illustrates the calculated abatement based on the second order rate constants for the reaction of the MPs with ozone and hydroxyl radical from the literature (Acero et al. 2000, Huber et al. 2003, Real et al. 2009).*

Text S6. Results of OTPs found in batch experiments and wastewater treatment

Overall, 84 OTPs could be detected, which originated from 40 of the 87 investigated MPs. For 47 MPs, no OTPs were assigned. Between 1 and 7 of the detected 84 OTPs were assigned to the same parent MPs (Figure S18). For most parents (22), only 1 OTP was found, for 17 MPs 2-4 OTPs, while for carbamazepine 6 OTPs and for sitagliptin 7 OTPs were observed.

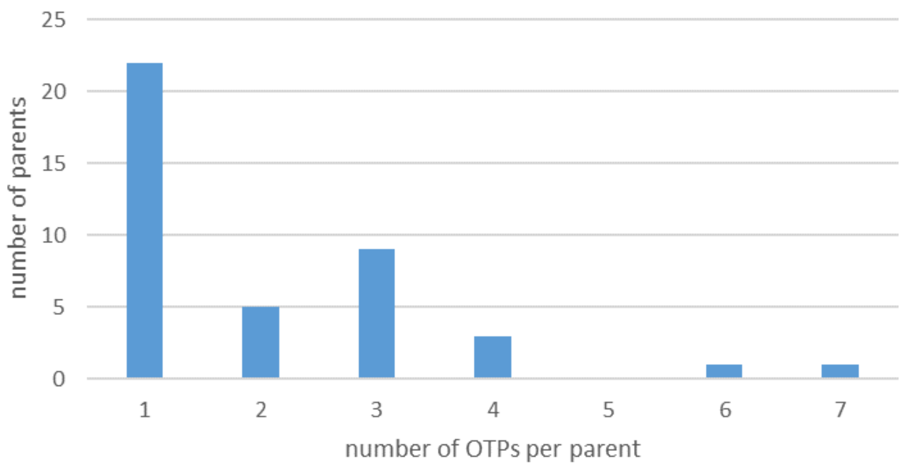

Figure S18: *Amount of OTPs (from all 84 detected OTPs) assigned to a parent.*

In the attached Excel Table S3, details on all the detected 84 OTPs are listed.

Figure S19:

In the attached pdf file, all the results for the 84 OTPs found in wastewater treatment are visually shown. The following information is given for each OTP:

- Top left: OTP formation in ozonation batch experiments
- Top right: Fate of parent MP in wastewater treatment
- Middle: (left) Fate of OTP in post-treatment (% formed or abated) and (right) OTP peak area in all wastewater samples in the four WWTPs. The score and evaluation of MS^2 spectra match of WWTP OZO samples to batch samples is given below.
- Bottom: Information on OTP identification (exact mass, formula, atomic modification, proposed structure, confidence level, Massbank identifier, MS^2 spectra and interpretation of it)

References

Abellan, M.N., Gebhardt, W. and Schroder, H.F. (2008) Detection and identification of degradation products of sulfamethoxazole by means of LC/MS and -MSn after ozone treatment. Water Sci. Technol. 58(9), 1803-1812.

Acero, J.L., Stemmler, K. and von Gunten, U. (2000) Degradation Kinetics of Atrazine and Its Degradation Products with Ozone and OH Radicals: A Predictive Tool for Drinking Water Treatment. Environ. Sci. Technol. 34(4), 591-597.

Azais, A., Mendret, J., Cazals, G., Petit, E. and Brosillon, S. (2017) Ozonation as a pretreatment process for nanofiltration brines: Monitoring of transformation products and toxicity evaluation. J. Hazard. Mater. 338, 381-393.

Bailey, P.S. (1982) Ozonation in Organic Chemistry, Volume II: Nonolefinic Compounds Academic Press

Barletta, B., Bolzacchini, E., Meinardi, S., Orlandi, M. and Rindone, B. (2003) The kinetics and the mechanism of the reaction of 2-chloro-4,6-dialkylamino-1,3,5-triazines with ozone. Ozone Sci. Eng. 25, 81-94.

Beltrán, F.J., García-Araya, J.F., Álvarez, P.M. and Rivas, J. (1998) Aqueous degradation of atrazine and some of its main by-products with ozone-hydrogen peroxide. J. Chem. Technol. Biotechnol. 71, 345-355.

Bollmann, A.F., Seitz, W., Prasse, C., Lucke, T., Schulz, W. and Ternes, T. (2016) Occurrence and fate of amisulpride, sulpiride, and lamotrigine in municipal wastewater treatment plants with biological treatment and ozonation. J. Hazard. Mater. 320, 204-215.

Borowska, E., Bourgin, M., Hollender, J., Kienle, C., McArdell, C.S. and von Gunten, U. (2016) Oxidation of cetirizine, fexofenadine and hydrochlorothiazide during ozonation: Kinetics and formation of transformation products. Water Res. 94, 350-362.

Buffle, M.O., Schumacher, J., Meylan, S., Jekel, M. and Von Gunten, U. (2006) Ozonation and advanced oxidation of wastewater: Effect of O3 dose, pH, DOM and HO.-scavengers on ozone decomposition and HO. generation. Ozone Sci. Eng. 28, 247-259.

Coelho, A.D., Sans, C., Aguera, A., Gomez, M.J., Esplugas, S. and Dezotti, M. (2009) Effects of ozone pre-treatment on diclofenac: intermediates, biodegradability and toxicity assessment. Sci. Total Environ. 407(11), 3572-3578.

Diehle, M., Gebhardt, W., Pinnekamp, J., Schäffer, A. and Linnemann, V. (2019) Ozonation of valsartan: Structural elucidation and environmental properties of transformation products. Chemosphere 216, 437-448.

Elovitz, M.S. and von Gunten, U. (1999) Hydroxyl radical/ozone ratios during ozonation processes. I. The R(ct) concept. Ozone Sci. Eng. 21, 239-260.

Favier, M., Dewil, R., Van Eyck, K., Van Schepdael, A. and Cabooter, D. (2015) High-resolution MS and MS(n) investigation of ozone oxidation products from phenazone-type pharmaceuticals and metabolites. Chemosphere 136, 32-41.

Feng, J., Zheng, Z., Luan, J., Zhang, J. and Wang, L. (2008) Degradation of diuron in aqueous solution by ozonation. J. Environ. Sci. Health B 43(7), 576-587.

Gao, S., Zhao, Z., Xu, Y., Tian, J., Qi, H., Lin, W. and Cui, F. (2014) Oxidation of sulfamethoxazole (SMX) by chlorine, ozone and permanganate--a comparative study. J. Hazard. Mater. 274, 258-269.

Gomez-Ramos, M.D.M., Mezcua, M., Aguera, A., Fernandez-Alba, A.R., Gonzalo, S., Rodriguez, A. and Rosal, R. (2011) Chemical and toxicological evolution of the antibiotic sulfamethoxazole under ozone treatment in water solution. J. Hazard. Mater. 192(1), 18-25.

Hermes, N., Jewell, K.S., Falås, P., Lutze, H.V., Wick, A. and Ternes, T.A. (2020) Ozonation of Sitagliptin: Removal Kinetics and Elucidation of Oxidative Transformation Products. Environ. Sci. Technol. 54, 10588-10598.

Hoigné, J. and Bader, H. (1994) Characterization of water quality criteria for ozonation processes. Part II: lifetime of added ozone. Ozone Sci. Eng. 16(2), 121-134.

Hörsing, M., Kosjek, T., Andersen, H.R., Heath, E. and Ledin, A. (2012) Fate of citalopram during water treatment with O3, ClO2, UV and Fenton oxidation. Chemosphere 89(2), 129-135.

Hu, R., Zhang, L. and Hu, J. (2017) Investigation of ozonation kinetics and transformation products of sucralose. Sci. Total Environ. 603-604, 8-17.

Huber, M.M., Canonica, S., Park, G.-Y. and von Gunten, U. (2003) Oxidation of Pharmaceuticals during Ozonation and Advanced Oxidation Processes. Environ. Sci. Technol. 37(5), 1016-1024.

Hübner, U., Seiwert, B., Reemtsma, T. and Jekel, M. (2014) Ozonation products of carbamazepine and their removal from secondary effluents by soil aquifer treatment - Indications from column experiments. Water Res. 49, 34-43.

Keen, O.S., Ferrer, I., Michael Thurman, E. and Linden, K.G. (2014) Degradation pathways of lamotrigine under advanced treatment by direct UV photolysis, hydroxyl radicals, and ozone. Chemosphere 117, 316-323.

Lajeunesse, A., Blais, M., Barbeau, B., Sauvé, S. and Gagnon, C. (2013) Ozone oxidation of antidepressants in wastewater– Treatment evaluation and characterization of new by-products by LC-QToFMS. Chem. Cent. J. 7(15).

Lee, M., Blum, L.C., Schmid, E., Fenner, K. and von Gunten, U. (2017) A computer-based prediction platform for the reaction of ozone with organic compounds in aqueous solution: kinetics and mechanisms. Environ. Sci. Process. Impacts 19, 465-476.

Lester, Y., Mamane, H., Zucker, I. and Avisar, D. (2013) Treating wastewater from a pharmaceutical formulation facility by biological process and ozone. Water Res. 47(13), 4349-4356.

Lim, S., McArdell, C.S. and von Gunten, U. (2019) Reactions of aliphatic amines with ozone: Kinetics and mechanisms. Water Res. 157, 514-528.

Martín de Vidales, M.J., Robles-Molina, J., Domínguez-Romero, J.C., Cañizares, P., Sáez, C., Molina-Díaz, A. and Rodrigo, M.A. (2012) Removal of sulfamethoxazole from waters and wastewaters by conductive-diamond electrochemical oxidation. J. Chem. Technol. Biotechnol. 87(10), 1441-1449.

McDowell, D.C., Huber, M.M., Wagner, M., von Gunten, U. and Ternes, T.A. (2005) Ozonation of Carbamazepine in Drinking Water: Identification and Kinetic Study of Major Oxidation Products. Environ. Sci. Technol. 39(20), 8014-8022.

Merel, S., Lege, S., Yanez Heras, J.E. and Zwiener, C. (2017) Assessment of N-Oxide Formation during Wastewater Ozonation. Environ. Sci. Technol. 51(1), 410-417.

Miao, H.F., Cao, M., Xu, D.Y., Ren, H.Y., Zhao, M.X., Huang, Z.X. and Ruan, W.Q. (2015) Degradation of phenazone in aqueous solution with ozone: influencing factors and degradation pathways. Chemosphere 119, 326-333.

Müller, A., Weiss, S.C., Beisswenger, J., Leukhardt, H.G., Schulz, W., Seitz, W., Ruck, W.K. and Weber, W.H. (2012) Identification of ozonation by-products of 4- and 5-methyl-1H-benzotriazole during the treatment of surface water to drinking water. Water Res 46(3), 679-690.

Quaresma, A.V., Sousa, B.A., Silva, K.T.S., Silva, S.Q., Werle, A.A. and Afonso, R. (2019) Oxidative treatments for atenolol removal in water: Elucidation by mass spectrometry and toxicity evaluation of degradation products. Rapid Commun. Mass Spectrom. 33(3), 303-313.

Ramseier, M.K. and von Gunten, U. (2009) Mechanisms of Phenol Ozonation-Kinetics of Formation of Primary and Secondary Reaction Products. Ozone Sci. Eng. 31(3), 201-215.

Real, F.J., Benitez, F.J., Acero, J.L., Sagasti, J.J.P. and Casas, F. (2009) Kinetics of the Chemical Oxidation of the Pharmaceuticals Primidone, Ketoprofen, and Diatrizoate in Ultrapure and Natural Waters. Ind. Eng. Chem. Res. 48(7), 3380-3388.

Rodayan, A., Roy, R. and Yargeau, V. (2010) Oxidation products of sulfamethoxazole in ozonated secondary effluent. J. Hazard. Mater. 177(1-3), 237-243.

Rosal, R., Rodriguez, A., Perdigon-Melon, J.A., Petre, A., Garcia-Calvo, E., Gomez, M.J., Aguera, A. and Fernandez-Alba, A.R. (2009) Degradation of caffeine and identification of the transformation products generated by ozonation. Chemosphere 74(6), 825-831.

Schollée, J.E., Bourgin, M., von Gunten, U., McArdell, C.S. and Hollender, J. (2018) Non-target screening to trace ozonation transformation products in a wastewater treatment train including different post-treatments. Water Res. 142, 267-278.

Schollée, J.E., Gulde, R., von Gunten, U. and McArdell, C.S. (2021) High-throughput screening of predicted ozonation transformation products in wastewater through *in silico* fragmentation. in preparation.

Staehelin, J. and Hoigne, J. (1985) Decomposition of ozone in water in the presence of organic solutes acting as promoters and inhibitors of radical chain reactions. Environ. Sci. Technol. 19(12), 1206-1213.

Sui, Q., Gebhardt, W., Schroder, H.F., Zhao, W., Lu, S. and Yu, G. (2017) Identification of New Oxidation Products of Bezafibrate for Better Understanding of Its Toxicity Evolution and Oxidation Mechanisms during Ozonation. Environ. Sci. Technol. 51(4), 2262-2270.

Tay, K.S., Rahman, N.A. and Abas, M.R. (2013) Ozonation of metoprolol in aqueous solution: ozonation by-products and mechanisms of degradation. Environ. Sci. Pollut. Res. Int. 20(5), 3115-3121.

Tay, K.S., Rahman, N.A. and Abas, M.R.B. (2011) Characterization of atenolol transformation products in ozonation by using rapid resolution high-performance liquid chromatography/quadrupole-time-of-flight mass spectrometry. Microchem. J. 99(2), 312-326.

Tentscher, P.R., Bourgin, M. and von Gunten, U. (2018) Ozonation of Para-Substituted Phenolic Compounds Yields p-Benzoquinones, Other Cyclic alpha,beta-Unsaturated Ketones, and Substituted Catechols. Environ. Sci. Technol. 52(8), 4763-4773.

von Sonntag, C. and von Gunten, U. (2012) Chemistry of ozone in water and wastewater treatment: From basic principles to applications, IWA Publishing, London.

Willach, S., Lutze, H.V., Eckey, K., Loppenberg, K., Luling, M., Terhalle, J., Wolbert, J.B., Jochmann, M.A., Karst, U. and Schmidt, T.C. (2017) Degradation of sulfamethoxazole using ozone and chlorine dioxide - Compound-specific stable isotope analysis, transformation product analysis and mechanistic aspects. Water Res. 122, 280-289.

Xu, Z., Xie, M., Ben, Y., Shen, J., Qi, F. and Chen, Z. (2019) Efficiency and mechanism of atenolol decomposition in Co-FeOOH catalytic ozonation. J. Hazard. Mater. 365, 146-154.

Zimmermann, S.G., Schmukat, A., Schulz, M., Benner, J., von Gunten, U. and Ternes, T.A. (2012) Kinetic and Mechanistic Investigations of the Oxidation of Tramadol by Ferrate and Ozone. Environ. Sci. Technol. 46(2), 876-884.

Zucker, I., Mamane, H., Riani, A., Gozlan, I. and Avisar, D. (2018) Formation and degradation of N-oxide venlafaxine during ozonation and biological post-treatment. Sci. Total Environ. 619-620, 578-586.