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Wastewater remediation of pharmaceuticals with ozone and granular active carbon: a risk-driven approach

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This study aimed to investigate the removal efficiency of (psycho)pharmaceuticals by ozonation and granular active carbon (GAC) in wastewater effluent, using risk as the metric for adequate removal instead of aqueous concentrations. Conventionally treated effluent was further treated with ozone or GAC until there was a 25% reduced UVA₂₅₄ absorbance, to allow for a direct comparison of the two treatment types. Samples were analysed using Ultra High-Performance Liquid Chromatography-Quadrupole Time of Flight-High Resolution Mass Spectrometry (UHPLC-qTOF-HRMS), where 20 (psycho)pharmaceuticals were quantified, and their risk was assessed using Predicted No Effect Concentrations (PNECs). A further assessment was performed using Quantitative Structural Activity Relationships (QSARs) for both parent compounds and their Oxidation Transformation Products (OTPs) to compare the relative toxicity of new species being formed by the ozone treatment. The total median removal efficiency across all compounds was $60 \pm 3\%$ for ozone in terms of concentration, yielding a $73 \pm 2\%$ reduction in terms of risk for the parent compounds, while the median removal efficiency for GAC is $57 \pm 9\%$ as expressed in concentration, and $46 \pm 11\%$ in terms of risk reduction. When factoring in the OTP toxicity, the median risk reduction for ozone flips to $-274 \pm 124\%$, indicating that there may be an increase in risk during ozonation. Pearson correlations on molecular descriptors indicated that ozone removal most strongly correlated with the number of activated aromatic rings ($r = 0.65$), while for GAC the topological polar surface area correlated strongest with removal ($r = 0.54$), therefore indicating that ozone and GAC target different types of molecules. The study demonstrates the merits of a risk-driven approach over concentration-based removal targets in current legislation, but also highlighted some drawbacks, especially with regards to data gaps and model accuracies.

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Water impact

Using a risk-driven approach, it was found that ozone removed (psycho)pharmaceutical parent compounds from wastewater effluent slightly better than granulated active carbon. However, due to the expected creation of oxidative transformation products the risk to the environment may actually increase after ozonation.

1. Introduction

Pharmaceuticals have become essential in modern medicine, contributing to increased physical and mental health, and to

a higher standard of living for many people around the world.¹ Psychopharmaceuticals, defined by the World Health Organisation under the Anatomical Therapeutic Class of N (ATC-N),² are a subclass of pharmaceuticals designed to treat medical conditions related to the nervous system, often by altering the neurochemistry of the human brain.^{3,4} A growing number of people use psychopharmaceuticals on a regular basis, fuelled by several factors such as growing and aging populations, a loss of social stigmas of the underlying diseases (e.g. depression), and a growing number of psychopharmaceuticals entering global markets.^{5–10}

In surface waters, psychopharmaceuticals have been shown to be a unique class of contaminants by exerting both

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While studies have shown that both adsorptive and AOP methods can yield high removals for psychopharmaceuticals^{44,46,49,75-77} there have been concerns about the lack of comparability between different advanced treatment techniques due to inconsistent experimental approaches.^{45,78} Notably, removal efficiencies between adsorptive and oxidative methods may be quite different

Advanced treatments in WWTPs include at least one additional step after conventional CAS treatment,^{39–41} and have demonstrated significantly higher removals for many micropollutants when compared to conventional treatments,⁴² albeit with increases to the capital, operation, and maintenance costs of WWTPs.^{40,43} Advanced treatment is a broad term that can refer to multiple different treatment techniques, such as Advanced Oxidation Processes (AOPs), which may use ozone or ultra-violet light (UV) in combination with H₂O₂ and other catalysts.^{44–46} Another example of advanced treatments are adsorption/biodegradation-based processes, such as sand filtration and active carbon (granular or powdered).^{47–51} In addition, so-called nature-based solutions (NBS) may also be considered advanced treatments, which refer to techniques that utilise natural systems and biota, such as natural and constructed wetlands or algae, to

Given that psychopharmaceuticals have unique modes of action, their use is increasing, and current WWTP removals are sub-optimal, further investigation into advanced treatment techniques to reduce the impact of these compounds on the aquatic environment is imperative. As such, risk assessments can be used to evaluate environmental impact and can aid in the understanding of the impact of OTPs. Therefore, the present study aimed to test the risk-based removal efficiencies for psychopharmaceuticals for ozone and GAC as two commonly used advanced treatment methods. To this end, firstly a preliminary risk assessment as an indicator for successful remediation of psychopharmaceuticals was employed, and secondly a further risk analysis was performed on OTPs from ozonation using a simple mass balance approach coupled with ecotoxicity QSAR models.

2.1. Materials and target list

After treatment, 100 ml samples were collected in dark HDPE bottles (DS2185-0004, Thermo Scientific) using grab sampling. The samples from the Ozone treatment were taken from the untreated effluent before (“Pre-Ozone”, 2 samples) and after ozonation (“Post-Ozone”) from each batch (8 in total), while the samples from the GAC treatment were taken from the untreated effluent before (“Pre-GAC”, 2 samples) and after (“Post-GAC”) each batch (4 samples total), leading to 14 samples overall. All samples were stored at -20 °C and

transported to the Institute for Biodiversity and Ecosystem Dynamics (IBED) at the University of Amsterdam (the Netherlands) for further analysis. Prior to analyses, samples were thawed overnight in 4 °C before extraction in October 2023.

2.3. Solid phase extraction

The extraction method used in this study is detailed in Davey *et al.*²⁵ Briefly, after thawing, samples were spiked with labelled standards and shaken at 90 rpm for 30 minutes. Outlets, tubes, and adapters were cleaned with ultrapure water followed by methanol. Conditioning of the Oasis HLB cartridge (150 mg, 6 cc) was done with 6 mL MeOH and 6 mL ultrapure water. After sample loading (50 mL, in duplicate), the cartridge was dried for 30 minutes under vacuum and washed with 6 mL of ultrapure water. Before elution, a 0.22 µm polypropylene syringe filter was placed in between the cartridge and SPE inlet. Elution was achieved with 2 × 5 mL methanol under vacuum. The collected elution fractions were evaporated under a gentle nitrogen flow at 37 °C to <1 mL and reconstituted to 1 mL in methanol for storage until analysis.

2.4. UHPLC-HRMS method

The analytical method used here was the same as Davey *et al.*²⁵ Briefly, a LC system (Nexera 30 Shimadzu, Den Bosch, The Netherlands) was coupled to a maXis 4G quadrupole time-of-flight HRMS (qToF/HRMS) upgraded with HD collision cell and ESI source (Bruker Daltonics, Leiderdorp, The Netherlands). The LC column used was an Acquity UPLC CSH C18 column (130 Å, 2.1 × 150 mm, 1.7 µm, Waters Corporation, Milford) kept at a temperature of 40 °C. The mobile phases consisted of ultrapure water (Milli-Q) with 0.05% acetic acid (A) and MeOH (B). The gradient started with a 7-minute equilibration at 10% B and gradually increased to 100% B in 10 minutes, held at 100% B for 5 min, and back to 10% B in 0.5 minutes, totalling 22.5 minutes. The flow rate was 0.3 mL min⁻¹ and the injection volume 20 µL. For compounds above the linear quantification range, a second round of injections was performed with an injection volume of 5 µL. The samples were analysed in both positive and negative mode, acquiring HRMS1 spectra for 20–1000 *m/z* with a resolving power of 30 000–60 000 at full width half maximum (FWHM), with a spray voltage of +3.5 kV and –3.5 kV for positive and negative modes respectively.

Qualification and quantification of psychopharmaceuticals was carried out with TASQ version 2021.0 316 (Bruker Daltonics, Leiderdorp, the Netherlands). Qualification of target compounds was based on the mass accuracy of full-scan HRMS spectra and MS/MS ions acquired in data-independent MS/MS mode (DIA), and their retention time match with the calibration series.

During quantification, only chromatograms with a retention time (RT) of >1 minute, RT tolerance of ±0.3

minutes, mass tolerance of 0.002 Da, detectable qualifier ion, mSigma of <100, and a peak intensity of >1000, were considered (see SI-1 for further details). Calibration curves for quantification were calculated by analysing ultrapure water spiked with target compounds and serially diluted to obtain 18 concentration levels. The 18 solutions were further spiked to contain 10 µg L⁻¹ of Internal Standard (IS). Concentration calibration was achieved using the calibration series in compared to calibration IS area to determine the per-sample recoveries and adjust for losses. For more information on the quantification method, see Davey *et al.*²⁵ The instrumental and methodological limits of detection and quantification (LoD/LoQ) can be found in SI-5, columns Z-AA.

2.5. Method validation

The method for 30 selected psychopharmaceuticals was validated in a previous study²⁵ while the method for an additional 5 pharmaceuticals was validated in the present study (SI-2). The method validation followed the same workflow as Davey *et al.*,²⁵ where 5 sample types were made in order estimate the matrix effects and recovery efficiency for each of the 5 new pharmaceuticals. Only recoveries and matrix effects of between 60–140% were considered acceptable. The method validation results are reported in SI-2. Two compounds (caffeine and clonidine) were removed from further study due to contamination issues for caffeine and bad calibration results for clonidine (SI-3), thus the study included 33 compounds.

2.6. Removal efficiencies and preliminary risk analysis

After quantification, blank subtractions, and dilution adjustments, concentration-based removal efficiencies per compound were calculated using eqn (1) for both advanced treatment methods (SI-4):

$$\text{Concentration Based Removal\%} = \frac{(C_{\text{Pre}} - C_{\text{Post}})}{C_{\text{Pre}}} \times 100 \quad (1)$$

where C_{Pre} is the pre-treatment concentration, C_{Post} is the post-treatment concentration, and Concentration Based Removal% is the concentration-based removal expressed as a percentage.

A second removal calculation was also performed using the sum of the concentrations of all compounds per sample to allow direct comparison to risk-based removal (eqn (4)):

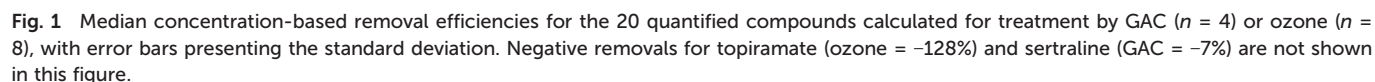
$$\text{Total Concentration Based Removal\%} = \frac{(\sum C_{\text{Pre}} - \sum C_{\text{Post}})}{\sum C_{\text{Pre}}} \times 100 \quad (2)$$

A preliminary risk analysis was performed to better understand the impact of the removal efficiencies by factoring the ecotoxicity of each compound to come to risk-based removal efficiencies. Risk quotients (RQs) were calculated per compound using eqn (3), based on the lowest available freshwater Predicted No Effect





Making direct comparisons to removal efficiencies found in literature remains difficult, due a lack of standardisation in testing methodologies^{45,78} and differences in dosages between studies.^{45–48,50,65,70,72,76,77,79–82} The dosage and exposure time used this study have been shown to produce removals of >80% for micropollutants in studies with dosages and exposure times.^{47,82,83} The results presented here for removal by ozonation are broadly in line with other literature. Similar removal rates to Bourgin *et al.*⁴⁴ after ozone treatment were found for carbamazepine, diclofenac and sulfamethoxazole (>90% removal, SI-4), and compared to Spilisbury *et al.*⁷⁰ the present study had significantly better ozone removal at a comparable dose of 0.35 g O₃ g⁻¹ DOC. However, for other compounds, removal efficiencies were slightly lower than those of other lab-, or pilot-scale experiments.^{27,44,105} For GAC, removal efficiencies found here were substantially lower than those reported in literature. This is likely due to the lower dose used in the present study



compared to literature,^{27,77} as well as the lack of a biofilm in the present study. GAC is commonly implemented as a fixed bed adsorber with biofilm development that can provide additional biodegradation over longer operation.⁷⁷ Here, virgin GAC was used as a purely adsorptive substrate, with the dosage and contact time being similar to other GAC studies.^{50,81} However, using Powdered Activated Carbon (PAC) to increase sorption surface area, or extending the operation of GAC to allow for a biofilm to form, incurring biodegradation potential may improve the removals presented here.^{48,49,77}

3.2. Risk analysis

Ecotoxicological preliminary risks for the parent compounds were calculated for all 33 compounds present in the study (Fig. 2, SI-5), since LOD and LOQ values were used as surrogates for the compounds that could not be quantified or detected, to present a worst-case scenario. Here, ibuprofen,

diclofenac, and sertraline present a risk in the untreated effluent (RQs of 21, 13, and 1.3 respectively). Diclofenac, citalopram, amitriptyline, clozapine, carbamazepine, venlafaxine, sulfamethoxazole, aripiprazole, propranolol, and lamotrigine had RQs of over 0.1, meaning they were within 1 order of magnitude to a risk. Aripiprazole was within an order of magnitude to an RQ of 1 despite all measurements being below the LOQ, due to the small distance between the potentially toxic concentrations and the LOQ. The median RQs were 0.025, 0.013, 0.023, 0.014 for Pre-Ozone, Post-Ozone, Pre-GAC, and Post-GAC respectively.

The PNECs obtained from the NORMAN database originate from diverse sources. While many were from literature, 9 out of 30 were QSAR predicted, and the assessment factors used in the NORMAN database ranged from 0 to 1000 (SI-5). The use of the NORMAN PNECs was a pragmatic choice due to their availability, however the shortcomings of these PNECs has been discussed before.²⁵ The risk assessment performed here is illustrative and not

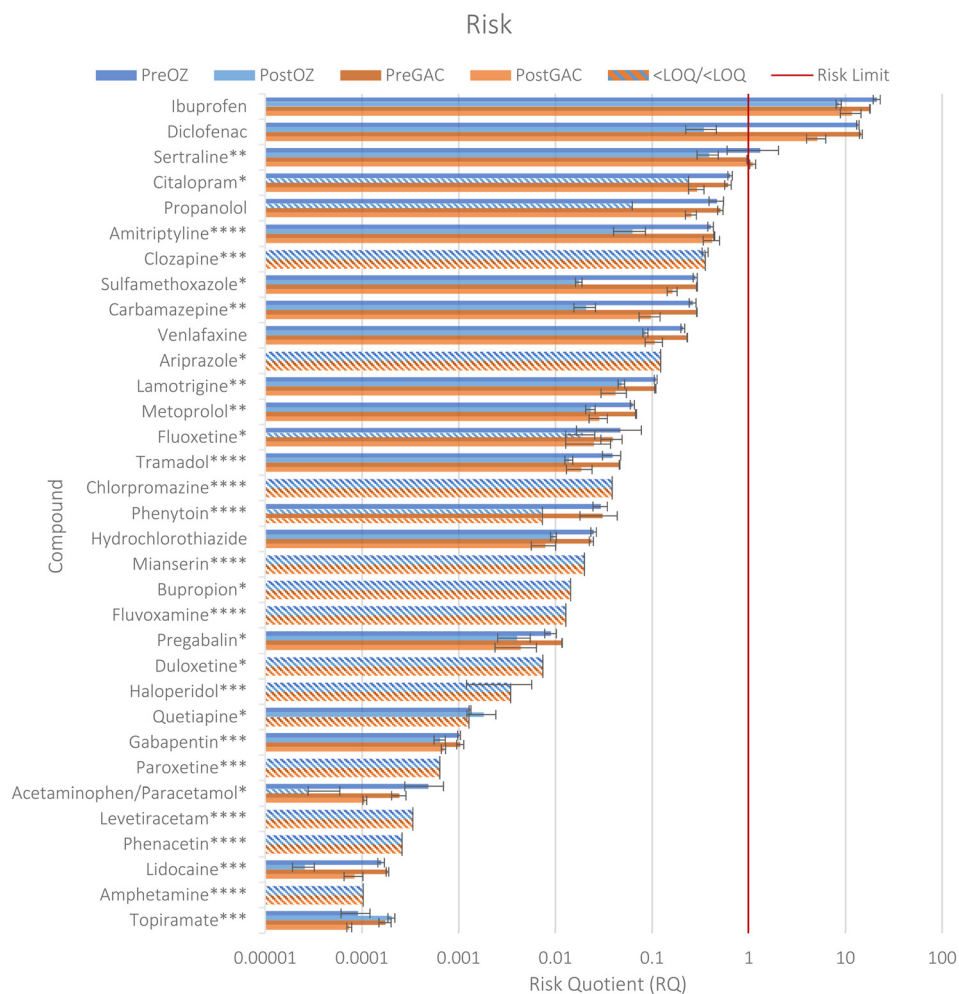


Fig. 2 Risk quotients for all 33 compounds in the study, for all 4 samples. The red line indicates where a risk is seen (i.e. $RQ > 1$) and the error bars indicate the standard deviation. Striped bars indicate that the risk assessment was based on the LOD or LOQ. Asterisks next to the names indicate the assessment factor used by the NORMAN database. * = 10, ** = 50, *** = 100, **** = 1000 (QSARS only), no asterisk indicates no assessment factor present.



designed to be compared to EMA style risk assessments, since the ecotoxicity data for those are generally lacking.^{23,24,69} For the mentioned highest risk compounds, the PNECs were based on literature data, and had assessment factors of only 10–50 applied (Fig. 2).¹⁰⁶

3.3. OTP toxicity and QSAR data gap filling

146 structures of known OTPs that might be formed by ozone treatment were obtained from literature for 17 of the 22 detected compounds (SI-6).^{58,60,63,66,88–101} The number of OTPs per parent compound range from 4 to 30. Most of the OTPs returned ChVs, with only 18 OTPs returning an EC/LC50 value and requiring an assessment factor (SI-6). Based upon the QSAR calculations, the risk of most OTPs that could be produced by the ozone treatment is greater than the risk that was removed by it, where a total of 29 OTP structures returned ecotoxicity values lower than their parents, and thus more toxic. For diclofenac, sertraline, citalopram, propranolol, amitriptyline, sulfamethoxazole, carbamazepine, venlafaxine, hydrochlorothiazide, gabapentin, and paracetamol the possible contribution of the most toxic OTP to the toxicity of the effluent was higher than the toxicity by the parent compound that was removed (Fig. 3, SI-6). In the case of paracetamol, the most toxic OTP was around 100 times more toxic than the parent compound on molar basis. As a result of the OTP analysis, citalopram, amitriptyline, carbamazepine, and hydrochlorothiazide went from no predicted risk based only on the parent compound, to a predicted risk based on parent and OTP after ozonation (Fig. 3).

The formation of ecotoxic OTPs has been discussed in literature^{58,60,62} but the exact effects of individual OTPs in these studies are difficult to unravel due to the lack of OTP standards, and most studies on OTP toxicity being broad-

scope bioassays on ozonated water.⁶² The analysis performed here attempted to bridge this gap by estimating the individual OTP toxicity using *in silico* methods and is broadly in agreement with literature that the formation of OTPs can be detrimental to the ecosystem without an additional treatment step to remove them. However, it should be reiterated that the actual concentrations of the OTPs were unknown, and thus the analysis performed and shown in Fig. 3 should be seen as a worst-case scenario which may not reflect the real situation.

When assessing the risk of pharmaceuticals, many studies neglect transformation products for practical reasons.^{21,25} The current lack of analytical standards leads to large uncertainties in quantification, and including analytes without standards is often advised against.¹⁰⁷ Additionally, the lack of standards leads to a lack of ecotoxicity data for these transformation products. While the use of QSARs on the confirmed OTP structures provided data to fill gaps, there is still many missing data to accurately estimate the risk after ozonation. Firstly, there are probably more OTP structures than those reported in literature,⁵⁹ and there were some compounds with missing literature on OTP structures (SI-6). There are also the OTP structures of human metabolites of the psychopharmaceuticals, which were available only for some pharmaceuticals,^{91,92,95,97,101} and which are not included here but could add to the risks. Secondly, there are some general limitations with the use of QSARs. Quality and performance of the models are highly dependent on the training dataset and thereby applicability domain.¹⁰⁸ However, none of the QSARs used in the present study were outside the applicability domain. Toxicological mode of action is also a limitation for QSARs. Like the PNECs, behavioural endpoints are not covered in the QSARs, although they might be vital to understanding the ecological impact of psychopharmaceuticals.^{16,109}

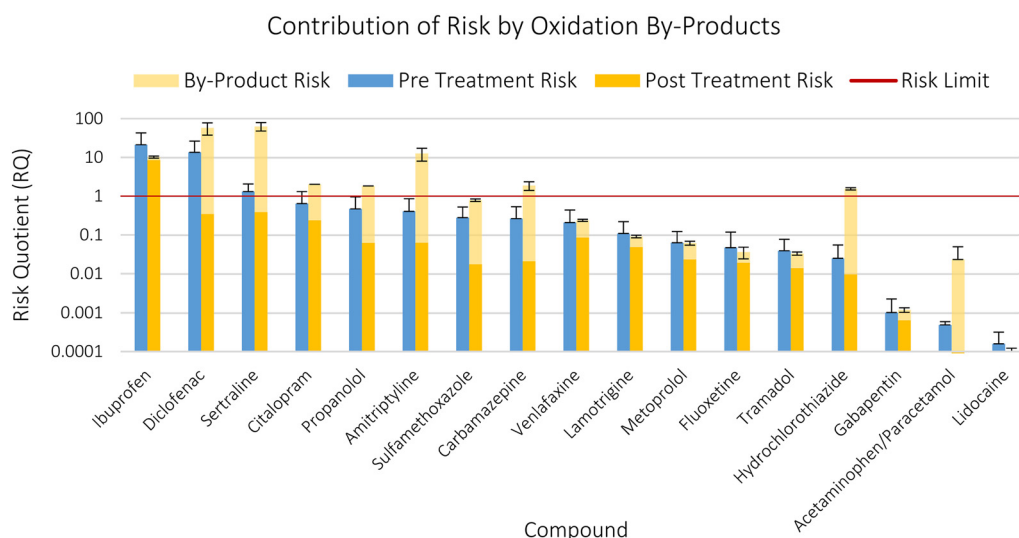


Fig. 3 Risk quotients of 17 detected parent compounds including a worst-case risk estimation of probable OTPs after ozonation. Error bars indicate standard deviation.



3.5. Implications for wastewater treatment

In this study, ozone outperformed GAC both in terms of concentration-based removal and in terms of risk-based removal (SI-5). However, the advantage of ozone may disappear due to the formation of toxic OTPs, which is also reflected in the literature.^{58,60,62} The inverse of the Pearson correlations between the two treatment types across the molecular descriptors indicates that GAC works well on compounds which ozone does not (SI-8), which has also been shown in other studies.⁷⁷ This is why in practice ozone and other AOPs are in tandem with an adsorptive technique such as GAC, PAC, or other biofiltration methods.⁶⁰ This type of tandem setup is already used in many existing and pilot advanced treatment setups for especially drinking water treatment and to a lesser extent wastewater treatment.^{65,115–119} However, there are still arguments against the deployment of widespread advanced treatments, citing the upfront and running costs of advanced treatments. This argument is compounded by the lack of tertiary treatments (e.g. CAS) in large parts of Europe,²⁷ which should be the priority in those regions. Some estimations indicate that advanced treatment costs can be up to two times the current operating costs of a WWTP, with GAC being more expensive than ozone to implement.⁴³

Lowering the operating costs of advanced treatments can lower the barrier to their adoption,¹²⁰ which may be possible by lowering dosages if it can be assured that the risks are sufficiently low, even when taking temporal variation of influent concentrations into account.¹²¹ Utilising risk-based removal, or other risk-based approaches, as have been discussed in the EU,⁷³ could provide a potential way to reduce the dose of advanced treatments. A potential workflow for reducing advanced treatment dosage could use the UVA₂₅₄ method alongside non-target and semi-quantitative methods used in tandem with OTP structure and toxicity prediction perform a preliminary risk assessment on OTPs.^{59,112,113,122} If done on a tandem ozone-GAC setup, the dosage of both ozone and GAC, as measured by UVA₂₅₄, could be raised or lowered based on predetermined risk thresholds of both parent compounds and OTP. This workflow could allow for a more dynamic system when compared to the current flat, percentage-based removal targets.⁷³

However, major knowledge gaps would need to be addressed first, such as robust ecotoxicity data,^{23,123} and concerns over the identity, quantity and toxicity of biological metabolites and OTPs of the psychopharmaceuticals. It may be possible to also use bioassays and effect-based studies as combined with effect directed analysis to aid in answering the question of ecotoxicity of OTPs after ozonation^{124–130} with an alternative being broader mesocosm-style experiments.^{17,131}

The present study investigated a risk-driven approach to directly assess the environmental risk and removal

efficiencies of two advanced wastewater treatment technologies on (psycho)pharmaceuticals, *i.e.* ozone and GAC. Ozone performed better in terms of both concentration-based removal and risk-based removal for parent compounds. When QSARs were utilised to fill ecotoxicity data gaps on OTPs in a worst-case scenario, the results give a strong indication that ozone produces toxic byproducts that not only negate the risk reduction but may even increase the toxicity of the ozonated effluent. Furthermore, analysis on molecular descriptors indicates that ozone and GAC can remove different, complementary groups of (psycho)pharmaceuticals. Combined with the concerns over OTP toxicity, a tandem ozone-GAC setups would therefore be recommended. Finally, the study demonstrates the merits of novel methodologies, namely the UVA₂₅₄ method for direct comparison of different treatment methods, and the risk-driven approach over flat concentration-based removal targets as a metric for removal. Future workflows should include semi-quantitative risk assessment for OTPs to further elucidate the potential risk of these transformation products.

Conflicts of interest

There are no conflicts of interest to declare.

Data availability

Supplementary information is available. See DOI: <https://doi.org/10.1039/D5EW00600G>.

The data supporting this article have been included as part of the supplementary information (SI).

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