Ecotoxicological profiling of sediments from the Wurm River (North Rhine-Westphalia, Germany) under different weather and wastewater treatment conditions

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Aliaksandra Shuliakevich
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Dekan:

Prof. Dr. Sven Klimpel

Goethe-Universität Frankfurt am Main

Institut für Ökologie, Evolution und Diversität

Integrative Parasitologie und Tierphysiologie

Max-von-Laue-Str. 13, D-60438 Frankfurt am Main

Gutachter:

Prof. Dr. Henner Hollert

Goethe-Universität Frankfurt am Main

Institut für Ökologie, Evolution und Diversität

Abt. Evolutionsökologie und Umwelttoxikologie

Max-von-Laue-Str. 13, DE-60438 Frankfurt am Main

Prof. Dr. Andreas Schäffer

RWTH Aachen University

Institut für Umweltforschung

Kackertstraße 10, DE-52072 Aachen

Datum der Disputation: _____

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ZUSAMMENFASSUNG

Mit dem Inkrafttreten der Europäischen Wasserrahmenrichtlinie (EU WRRL) im Jahr 2000 soll bis 2027 ein „guter ökologischer und chemischer Zustand“ der europäischen Gewässer erreicht werden. So schreibt die EU WRRL die Überwachung von 45 prioritären Stoffen vor. Diese Zahl steht in keinem Verhältnis zu der beträchtlichen Anzahl an Chemikalien im weltweiten Handel (etwa 50,000), wovon ca. 60% als human- und umwelttoxisch gelten. Aus diesem Grund reicht die alleinige Erfassung von prioritären Stoffen nicht aus, um das Gefährdungspotenzial einer komplexen chemischen Mischung in der aquatischen Umwelt abschätzen zu können.

Ursprünglich legte die EU WRRL den Schwerpunkt auf die Überwachung der Wasserphase. Sedimente stellen jedoch ebenso eine wichtige Quelle und Senke für die chemische Belastung dar. Unter stabilen hydrologischen Bedingungen sind kontaminierte Sedimente der tieferen Schichten von weniger kontaminierten Sedimenten der oberen Schichten bedeckt. Versiegelung von Grünflächen, Renaturierung von Flussbetten oder Entleerung von Regenüberlaufbecken können allerdings zu einer Freisetzung partikelgebundener Schadstoffe führen. Zusätzlich beschleunigen häufige Dürreperioden und Starkregenereignisse die Bodenerosion und den Sedimenttransport in aquatische Ökosysteme. Aus diesem Grund wurden 2008 Sedimente als eine weitere Überwachungsmatrix in die EU WRRL aufgenommen. Doch trotz aller Bemühungen haben zum jetzigen Zeitpunkt weniger als die Hälfte aller europäischen Gewässer einen guten ökologischen und chemischen Zustand erreicht.

Kläranlagen sind eine der Hauptquellen für Mikroschadstoffe in aquatischen Ökosystemen. In den letzten zehn Jahren wurden zahlreiche wissenschaftliche Initiativen zur Erforschung fortschrittlicher Behandlungsverfahren (Ozon, Aktivkohle, Membranverfahren) in Kläranlagen ergriffen. Als eine besonders vielversprechende Methode hat sich der Einsatz von Ozon herausgestellt, um eine Vielzahl von Mikroschadstoffen im Klärprozess zu eliminieren. Doch das Wissen über die dadurch potenziell erreichte Verbesserung des chemischen und ökologischen Zustands der Vorfluter ist weiterhin lückenhaft.

Die letztgenannten Aspekte waren die Hauptgründe für den Start des $DemO_3^{AC}$-Projekts im Jahr 2014. Als Untersuchungsobjekt wurde der Fluss Wurm und sein stromaufwärts gelegener Nebenfluss Haarbach in Nordrhein-Westfalen (Deutschland)

gewählt. Beide Gewässer dienen als Vorfluter für die Kläranlage Aachen-Soers bzw. Kläranlage Eilendorf. Durch Einbeziehen des stromaufwärts gelegenen Haarbaches in die Projektstudien konnte der ökotoxikologische Zustand des Haarbaches und des Ablaufes der Kläranlage Eilendorf als eine mögliche Belastungsquelle untersucht werden. Während die Kläranlage Eilendorf das ankommende Abwasser mithilfe von drei konventionellen Reinigungsstufen (mechanisch, biologisch, chemisch) behandelt, wurde die Kläranlage Aachen-Soers (Vorfluter: Wurm) im Rahmen des $DemO_3^{AC}$-Projekts um eine vierte Reinigungsstufe erweitert und eine großtechnische Ozonierung implementiert. Der Bau der großtechnischen Ozonierungsanlage auf dem Gelände der Kläranlage Aachen-Soers wurde durch umfassende ökologische und chemische Untersuchungen der beiden Vorfluter begleitet, um bestehende Wissenslücken über eine mögliche ökologische und chemische Verbesserung des Vorfluters durch die Mikroschadstoffeliminierung im Ablauf durch Ozon zu schließen. Außerdem wurde im Rahmen des $DemO_3^{AC}$-Projektes die Wasserphase als die Hauptuntersuchungsmatrix ausgewählt. Doch Starkregenereignisse, Überschwemmungen oder der Abschlag eines Regenüberlaufbeckens (40 Mal pro Jahr bei der Kläranlage Aachen-Soers) können zur Aufwirbelung und Resuspension der Sedimente und so zur Freisetzung zahlreicher absorbierter Mikroschadstoffe in die Wasserphase führen.

Aus diesem Grund wurde die vorliegende Arbeit in Anlehnung an das $DemO_3^{AC}$-Projekt durchgeführt und hatte zum Ziel, die allgemeine Sedimenttoxizität und damit verbunden den Einfluss des Sediments auf die Gewässer zu erfassen. Das Untersuchungskonzept wurde auf Grundlage des ganzheitlichen Ansatzes zur Bewertung der ökotoxikologischen Belastung von Oberflächengewässern entwickelt und auf die Sedimente übertragen: Die Erfassung eines breiten Spektrums von chemischen Stoffen in Kombination mit wirkungsbasierten ökotoxikologischen Methoden erlaubt es, mögliche Treiber der Toxizität zu identifizieren und folgerichtig auf potenzielle Belastungsquellen zurückzuführen.

Um die ökotoxikologischen Einflüsse der Sedimente zu untersuchen, wurden insgesamt zwei Probenahmen in aufeinanderfolgenden Jahren durchgeführt. Die erste Probenahme erfolgte im Juni 2017 während einer langanhaltenden Trockenperiode. Die zweite Probenahme fand genau ein Jahr später (Juni 2018) nach einer langen Regenperiode und einem damit verbundenen hohen Wasserpegel statt. Auf diese Weise wurde das ökotoxikologische Potential der Sedimente unter verschiedenen

hydrologischen Bedingungen betrachtet. Zum Zeitpunkt der zweiten Probenahme war die Vollstromozonierung auf der Kläranlage Aachen-Soers bereits ein halbes Jahr in Betrieb.

Folgende Endpunkte wurden zur Untersuchung des ökotoxikologischen Potentials der Sedimente betrachtet: Genotoxisches potenzial, endokrin-disruptive Aktivität, embryotoxisches Potential und Änderung des Schwimmverhaltens bei Zebrabärblingslarven (*Danio rerio*). Diese Endpunkte wurden anhand einer Testbatterie aus *in vitro* und *in vivo* Bioassays mit verschiedenen Zelllinien und Organismen bewertet, um verlässliche Ergebnisse auf verschiedenen biologischen Organisationsstufen zu erhalten. Das chemische Profil der Sedimente wurde mit modernen chemisch-analytischen Methoden erstellt, um mögliche chemische Ursachen für die beobachteten Wirkungen zu ermitteln.

Das genotoxische Potenzial wurde mithilfe einer Testbatterie aus einem eukaryotischen Mikrokern-Test und einem bakteriellen Ames Fluktuationstest mit *Salmonella typhimurium* Teststämmen TA98, TA100, YG1041 und YG1042 untersucht. Ein einzigartiges Merkmal der vorliegenden Studie war die Verwendung von den sog. Nicht-Standard *Salmonella typhimurium* Teststämmen YG1041 und YG1042 im Ames-Fluktuationstest. Die erhaltenen Ergebnisse wurden im *peer-review* Journal *Water Research* publiziert (s. Shuliakevich et al., 2022a oder Anhang 1). Durch die Kombination der genannten Bioassays konnte gezeigt werden, dass die Sedimente der Wurm ein mutagenes Potenzial aufwiesen und mit Frameshift-, Basenpaar-substituierenden (Pro-)Mutagenen, Nitroarenen und aromatischen Aminen belastet waren. Die vorliegende Studie zeigte eine hohe Empfindlichkeit und Anwendbarkeit der Nicht-Standard *Salmonella typhimurium* Teststämme YG1041 und YG1042 im Ames-Fluktuationstest, um die verschiedenen Klassen von chemischen Verbindungen als Ursache der beobachteten Mutagenität zu identifizieren. Hier konnten die Starkregenereignisse sowie die Entleerung des Regenüberlaufbeckens und damit verbunden eine Sedimentaufwirbelung als wesentliche Faktoren für die Erhöhung des mutagenen Potenzials identifiziert werden.

Die endokrin-disruptive Aktivität wurde mithilfe von Rezeptor-basierten *in vitro* CALUX® assays untersucht. Die erhaltenen Ergebnisse bildeten die Grundlage für die bereits publizierte Studie im *per-review* Journal *Science of the Total Environment* (s. Shuliakevich et al., 2022b oder Anhang 2). Die Studie von Shuliakevich et al., 2022b

beschreibt Schwankungen der endokrin-disruptiven Aktivität in der Wurm bei unterschiedlichen Wetterbedingungen. Unter stabilen hydrologischen Bedingungen im Juni 2017 lagen die östrogenen und antiandrogenen Aktivitäten in den Sedimenten der Wurm im Bereich von 0,03-0,1 ng E2 Äquivalente (eq.)/g Trockengewicht Sedimentäquivalente (dw SEQ) bzw. 3,0-13,9 µg Flu eq./g dw SEQ. Nach den starken Regenereignissen im Juni 2018 wurden in den Sedimenten östrogene und antiandrogene Aktivitäten im Bereich von 0,06-0,2 ng E2 eq./g dw SEQ bzw. 1,7-39,2 µg Flu eq./g dw SEQ nachgewiesen. Eine erhöhte endokrine Aktivität (bis zu 0,2 ng E2 eq./g dw SEQ in ERα- und 39,2 µg Flu eq./g dw SEQ in Anti-AR-CALUX®-Tests) in den Sedimenten flussabwärts des Regenüberlaufbeckens deutete darauf hin, dass es sich um eine mögliche Quelle der chemischen Belastung handelt. So zeigte sich, genau wie bei der Betrachtung des mutagenen Potentials, dass ein Abschlag des Regenüberlaufbeckens und der damit verbundene Eintrag von nahezu ungereinigtem Abwasser in den Vorfluter zu einer erhöhten endokrin-disruptive Aktivität führt. Zusätzlich wurde eine positive Korrelation zwischen der gemessenen partikelgebundenen antiandrogenen Aktivität und den nachgewiesenen partikelgebundenen polyaromatischen Kohlenwasserstoffen (PAKs) festgestellt.

Das embryotoxische Potenzial wurde durch die Exposition von Zebrabärblingslarven mit nativen und gefriergetrockneten Sedimenten im Sedimentkontakttest untersucht. Bevor eine mögliche neurotoxische Aktivität von Sedimenten aus der Wurm und dem Haarbach untersucht wurde, wurde das embryotoxische Potenzial und die sog.e *No-Effect-Concentration* (NOEC) untersucht. Expositionsszenarien untersuchten die potenzielle Embryotoxizität bei realistischen (native Sedimente) und *Worst-Case*-Szenarien (gefriergetrocknete Sedimente und Sedimentextrakte). Die Exposition von Zebrabärblingslarven gegenüber Sedimenten wurde mit dem Sedimentkontakttest durchgeführt. Auf Basis der Konzentration ohne beobachtete Wirkungen aus dem Fischembryotoxizitätstest (NOEC; 1,56-25 mg dw SEQ/mL) als Expositionskonzentration wurden Verhaltensänderungen in Zebrabärblingslarven im Verhaltensversuch untersucht. Die erzielten Ergebnisse wurden mit den Ergebnissen der chemischen Analyse der partikelgebundenen Schadstoffe kombiniert, um mögliche chemische Treiber für beobachtete Effekte zu ermitteln. In unterschiedlichem Ausmaß wiesen alle beprobten Sedimente ein embryotoxisches Potenzial auf, wobei die EC50-Werte zwischen 2,3 und 30,3 mg dw SEQ/mL lagen. Das Regenüberlaufbecken wurde erneut als potenzielle Kontaminationsquelle ermittelt. Die Analyse der möglichen

Ursachen für die Toxizität ergab, dass PAKs, polychlorierte Biphenyle (PCBs) und nitroaromatische Verbindungen für die beobachteten embryotoxischen Auswirkungen verantwortlich sein könnten, während andere Toxizitätsfaktoren unbekannt blieben. Die gewonnenen Ergebnisse dieser Studie wurden zur Veröffentlichung eingereicht und sind als eingereichtes Manuskript in Anhang 3 dargestellt.

Die hier kurz vorgestellten Studien lieferten nicht nur ein umfassendes ökotoxikologisches Wirkungsprofil, sondern auch ein ebenso umfassendes chemisches Profil der Sedimente (zusätzlich zu den bereits erwähnten identifizierten chemischen Treibern der Toxizität). Sie bestätigten partikelgebundene aromatische Amine, Karbazole, Indole, Nitroarene, PAKs, PCBs, polyzyklische Heteroarene und andere chemische Verbindungen (Stilbenoide, aliphatische Halogene, Phenanthroline). Alle Sedimentproben enthielten erhöhte Konzentrationen partikelgebundener chemischer Verbindungen, wobei der stärkste Anstieg flussabwärts des Regenüberlaufbeckens zu verzeichnen war (+96 %). Die am häufigsten vorkommende chemische Gruppe in den untersuchten Sedimentproben waren die PAKs (0,4-5 µg/mg OC (organischer Kohlenstoff) im Juni 2017 und 0,7-26 µg/mg OC im Juni 2018). Sie machten rund 95% der Gesamtkonzentration der in den Sedimenten nachgewiesenen organischen chemischen Verbindungen aus. Unter stabilen hydrologischen Bedingungen war die PAK-Konzentration in Sedimenten der Wurm und des Haarbaches vergleichbar mit solchen in der Donau und im Rhein. Nach starken Regenfällen stieg die PAK-Konzentration in den Sedimenten auf das 100-fache an.

Die letzte Studie in Anhang 4 enthält zusätzliche Ergebnisse zur Risikobewertung von Mikroverunreinigungen aus Kläranlagenabläufen. Zur detaillierteren Betrachtung der potenziell durch Kläranlagenabläufen eingetragenen Mikroschadstoffen in die Gewässer, wurden Proben der Kläranlagen Aachen-Soers und Eilendorf innerhalb einer europaweiten Studie zur Bewertung spezifischer Muster in Kläranlagenabläufen im Kontext einer *Joint Programm Activity* (JPA) des internationalen NORMAN-Netzwerks für neuartige Schadstoffe untersucht. In der JPA wurden im Kontext der eigenen Dissertation drei Proben zum Set der insgesamt 57 europäischen Abwasserproben beigesteuert. Aus dem jeweiligen Ablauf wurden jeweils 50 L der Kläranlagenproben mittels sog. *large volume solid phase extraction* aufkonzentriert und mittels Gas- und Flüssig-Chromatographie auf 499 chemische Verbindungen untersucht. Zusätzlich wurde das komplette Probenset in ERα- und anti-AR-CALUX©

Tests untersucht (Ergebnisse gehören nicht zu der aktuellen Arbeit). In der Diskussion und in Annex 4 ist ein bisher noch nicht publizierter Datensatz bezüglich der Identifizierung der Treiber der Toxizität für die Aachener Teilproben dargestellt. Zur Bewertung des potenziellen Risikos von Mikroverunreinigungen in Kläranlagenabläufen wurden die sog. *Toxic Units* (TU) verwendet. Ein TU ist ein Verhältnis zwischen der gemessenen Umweltkonzentration einer einzelnen Verbindung und dem Grad ihrer Wirkung auf eine Gruppe von Organismen oder eine einzelne Art. Mit dem derzeitigen Ansatz konnten keine potenziell gefährlichen chemischen Verbindungen in den Gruppen der künstlichen Süßstoffe, Perfluorkohlenwasserstoffe, Flammschutzmittel, Tenside und UV-Filter ermittelt werden. Es wurden jedoch 15 Chemikalien als potenziell gefährlich für Süßwasserorganismen identifiziert.

Die vorliegende Arbeit hat gezeigt, dass Sedimente selbst in kleinen Gewässern, wie der Wurm und des Haarbachs, auch ohne industrielle Aktivitäten im Einzugsgebiet durch verschiedene chemische Verbindungen hochgradig belastet sein können, sodass potenziell schädliche Auswirkungen auf Wasserorganismen nicht ausgeschlossen werden können. Damit unterstreichen die Ergebnisse die unzureichende Betrachtung der Sedimente in der EU WRRL und betonen die Notwendigkeit einer kontinuierlichen Sedimentüberwachung von Gewässern. Weiterhin zeigen die Ergebnisse der vorliegenden Studie, dass die toxischen Effekte nach langanhaltenden Regenereignissen ausgeprägter waren als nach anhaltender Trockenheit. Zudem konnte der Eintrag von nahezu ungeklärtem Abwasser durch das Regenüberlaufbecken der Kläranlage Aachen-Soers als potenzielle Kontaminationsquelle identifiziert werden. Jedes Starkregenereignis oder jede Entleerung des Regenüberlaufbeckens führt zur Freisetzung von partikelgebundenen Schadstoffen mit der Folge einer erhöhten toxischen Belastung für Organismen in der Wurm. Die Kläranlageabläufe wurden als Punkt-Quellen für Mikroschadstoffe in die untersuchten Gewässer bestätigt, wobei die Vollstromozonierung sich als eine effektive Behandlungsmethode in Bezug auf die Schadstoffelimination im Ablauf herausstellte. Doch im Zuge des Klimawandels steigt die Wahrscheinlichkeit des erhöhten Auftretens von Starkregenereignissen und der damit verbundenen häufigen Entleerung von Regenüberlaufbecken. Das $DemO_3^{AC}$-Projekt und insbesondere die vorliegende Arbeit leistete einen wichtigen Beitrag, um die Aufmerksamkeit auf die ökologische und ökotoxikologische Überwachung kleiner Mittelgebirgsgewässer zu lenken.

Für das Einzugsgebiet der Wurm wäre es ratsam, Retentionsflächen zu schaffen, die bei Starkregenereignissen geflutet werden können, um Sedimenttransport zu verhindern. Zusätzlich sollte das Volumen des Regenüberlaufbeckens an die Regenwassermenge angepasst werden. Die kommunale Entwässerung und die damit verbundene Ableitung großer Regenwassermengen könnte eine Lösung für das Stadtgebiet sein. Für den außerstädtischen Bereich ist dies jedoch keine geeignete Lösung. Die Regenwassermengen bedürfen einer besonderen Behandlung und einer Verringerung der Einleitung in den Vorfluter. Eine mögliche Lösung stellen Retentionsbodenfilter oder Pflanzenkläranlagen als künstliche Systeme mit natürlichen Prozessen wie in Feuchtgebieten dar. Nach unserem Kenntnisstand betreibt der Betreiber der Kläranlage Aachen-Soers den Bau eines Retentionsbodenfilters, was in Zukunft zu sichtbaren positiven Veränderungen in der Wurm führen könnte.

ABSTRACT

The European Community has set a milestone in the European water policy in 2000: all water directives and policies were united into one comprehensive document – the European Water Framework Directive (EU WFD). The EU WFD requires the monitoring of 45 priority substances, primarily in the water phase, which is not related to a substantial amount of chemicals available on the market worldwide (about 50,000). About 60% of these are human and environmentally toxic. Hence, the currently monitored 45 priority substances are not even close to being sufficient to provide a comprehensive picture of the actual chemical pollution in the aquatic environment.

Furthermore, the EU WFD in its original shape paid less attention to sediments as an important source and sink for chemical contamination. Under stable hydrological conditions, polluted old sediments are covered by less polluted younger sediments preventing erosion of deeper sediment layers and, therefore, the release of particle-bound contaminants. However, urbanization, deforestation, flooding, dredging, riverbed renaturation, and stormwater overflow basin releases can lead to an unpredictable release of particle-bound pollutants. Therefore, in 2008, sediments were added to the EU WFD as a monitoring matrix for substances that tend to accumulate there. As a result, after 18 years of the EU WFD, less than half of all European waterbodies reached a good ecological (40%) and chemical (38%) status.

One of the primary pollution sources in aquatic ecosystems are wastewater treatment plants (WWTPs). Advanced wastewater treatment by ozonation is promising to remove most micropollutants. However, the knowledge about the possible improvement of the receiving waterbody is rare. The latter aspects were the main reasons for the start of the $DemO_3^{AC}$ project in 2014. The study area was located in the federal state of North Rhine-Westphalia (Germany). The study area included the Wurm River and its tributary, the Haarbach River. Both waterbodies act as receiving waterbodies for WWTPs. One of them is the Aachen-Soers WWTP (receiving waterbody: Wurm River), upgraded by full stream ozonation as an advanced effluent treatment. Therefore, the extensive investigation program within the $DemO_3^{AC}$ project included an investigation of the ecological and chemical status of both receiving waterbodies and the investigation of a possible improvement of the Wurm River after implementing advanced effluent treatment.

The current study was a part of the $DemO_3^{AC}$ project and covered the sediment toxicity and a possible impact of the ozonation on aquatic organisms in the receiving waterbody. Time-resolved sampling campaigns allowed investigations under different hydrological conditions, mainly determined by the weather. The first sampling campaign took place in June 2017 during a prolonged dry period with low water flow in the receiving waterbodies. The second sampling campaign was performed exactly one year later (June 2018) after a long rainy period and corresponding high-water levels. Full-stream ozonation at the Aachen-Soers WWTP had been in operation for half a year. Furthermore, a wide range of organic micropollutants was investigated in the effluent of the studied WWTPs to assess a possible hazard emerging from contaminants released into the receiving waterbody.

The study design was developed based on the holistic approach to assessing the ecotoxicological pollution of surface waterbodies. It included the detection of chemical compounds combined with effect-based methods to identify possible drivers of toxicity. The sediment's ecotoxicological assessment included studies on endocrine-disrupting activity, genotoxic and embryotoxic potentials. These endpoints were evaluated using *in vitro* and *in vivo* bioassays. In addition, sediments' chemical profiling was performed using modern analytical chemistry techniques.

The genotoxic potential was investigated using the Ames fluctuation assay with *Salmonella typhimurium* bacterial strains TA98, TA100, YG1041, and YG1042, sensitive to different classes of compounds, and the Micronucleus assay as a eukaryotic assay with mammalian cells. A unique feature of the present study was the implementation of non-standard *Salmonella typhimurium* bacterial strains YG1041 and YG1042 in the Ames fluctuation assay. Moreover, a comprehensive genotoxicity ranking of chemical compounds identified in sediments was used and combined with statistical analysis to identify the drivers of genotoxicity. The results of this study were published in Shuliakevich et al. (2022a) (see also Annex 1), describing the mutagenic potential of all sampling sites, which was primarily driven by polycyclic aromatic hydrocarbons, nitroarenes, aromatic amines, and polycyclic heteroarenes. In addition, the rainwater overflow basin was identified as a significant source for particle-bound pollutants from untreated wastewater, suggesting its role as a possible source of genotoxic potential. The present study showed high sensitivity and applicability of non-standard *Salmonella typhimurium* bacterial strains YG1041 and YG1042 in the Ames

fluctuation assay to assess the different classes of mutagenic compounds. A combination of effect-based methods and a chemical analysis was shown as a suitable tool for a genotoxic assessment of freshwater sediments.

The sediments' endocrine-disruptive activity was investigated using the cell-based reporter gene CALUX® assay. A simultaneous launch of the full-scale effluent ozonation at the Aachen-Soers WWTP was used for investigation of the entrance of the ozonated effluent into the Wurm River and the endocrine-disrupting activity in the water phase. A particular focus of the present study was the unique investigation of PAHs as possible drivers of the endocrine-disrupting activity in sediments of the Wurm River. The results of this study were laid down in the publication by Shuliakevich et al. (2022b) (see also Annex 2), describing variations in endocrine-disrupting activity in the Wurm River under different weather conditions. Briefly, under stable hydrological conditions in June 2017, the estrogenic and the antiandrogenic activities in sediments of the Wurm River were within the range of 0.03-0.1 ng E2 equivalents (eq.)/g dry weight sediment equivalents (dw SEQ) and 3.0-13.9 µg Flu eq./g dw SEQ, respectively. After extensive rain events in June 2018, the sediments' estrogenic and antiandrogenic activities were detected within the range of 0.06-0.2 ng E2 eq./g dw SEQ and 1.7-39.2 µg Flu eq./g de SEQ, respectively. Increased endocrine-disruptive activity (up to 0.2 ng E2 eq./g dw SEQ in ERα- and 39.2 µg Flu eq./g dw SEQ in anti-AR-CALUX® assays) in sediments downstream of the rainwater overflow basin suggested it as a possible source of pollution. A unique result of the second study was finding a positive correlation between measured particle-bound antiandrogenic activity and detected polyaromatic hydrocarbons (PAHs).

Annex 3 comprises a study of a less investigated group of such chemical pollutants as neurotoxic substances. Before investigating possible neurotoxic activity from sediments from the Wurm and the Haarbach Rivers, embryotoxic potential and, therefore, the no-effect concentration was studied. Exposure scenarios investigated potential embryotoxicity at realistic (native sediments) and worst-case (freeze-dried sediments and sediment extracts) scenarios. Zebrafish larvae exposure to sediments was performed using the Sediment Contact Test. Behavioral changes after exposure of the zebrafish larvae to the no observed effects concentration from the fish embryotoxicity test (1.56-25 mg dw SEQ/mL) were studied in the locomotion assay. Obtained results were combined with the chemical analysis of particle-bound

contaminants elucidating possible drivers for detected effects. To a different extent, all sediments samples showed embryotoxic potential within the EC50-values range of 2.3-30.3 mg dw SEQ/mL. The rainwater overflow basin was again identified as a potential source of contamination, while further potential sources of contamination remained unknown. Analysis of the possible drivers of toxicity revealed PAHs, PCBs, and nitroaromatic compounds as possible drivers of the observed embryotoxic effects. Gained results of this study were submitted for publication and are represented as a submitted manuscript in Annex 3.

Aside from providing a comprehensive ecotoxicological effect profile, the briefly presented studies also examined the chemical profile of the sediments (in addition to the identified drivers of toxicity mentioned earlier). They confirmed particle-bound aromatic amines, carbazoles, indoles, nitroarenes, PAHs, PCBs, polycyclic heteroarenes, and other chemical compounds (stilbenoids, aliphatic halogens, phenanthrolines). All sediment samples contained elevated concentrations of particle-bound chemical compounds, with the most significant increase downstream of the rainwater overflow basin (+96%). The most abundant chemical group in the sediment samples studied were PAHs (0.4-5 µg/mg OC (organic carbon) in June 2017 and 0.7-26 µg/mg OC in June 2018). They accounted for around 95 % of the total concentration of organic chemical compounds detected in the sediments.

The last study in Annex 4 provides additional results on the risk assessment of micropollutants from WWTP effluents. For a more detailed consideration of the micropollutants potentially entering the aquatic environment through WWTP effluents, samples from the Aachen-Soers WWTP and Eilendorf WWTP were investigated within a Europe-wide study to assess specific patterns in WWTP effluents in the context of a Joint Program Activity (JPA) of the international NORMAN network for novel pollutants. In the JPA, three samples were contributed to the set of a total of 57 European wastewater samples in the context of our own dissertation. From each effluent, 50 L of the WWTP samples were concentrated by so-called large volume solid phase extraction and analyzed for 499 chemical compounds by Gas- and Liquid Chromatography. In addition, the complete sample set was analyzed in ERα- and anti-AR-CALUX© assays, however these results are not part of the current work. A previously unpublished data set regarding the identification of drivers of toxicity for the mentioned subsamples is presented in the discussion and in Annex 4. Toxic Units

(TU) were used to assess the potential risk of micropollutants in WWTP effluents. A TU is a ratio between the measured environmental concentration of a single compound and the degree of its effect on a group of organisms or a single species. The current approach did not identify any potentially hazardous chemical compounds in the groups of artificial sweeteners, perfluorocarbons, flame retardants, surfactants, and UV filters. However, 15 chemicals were identified as potentially hazardous to freshwater organisms.

Furthermore, the presented results underline the insufficient consideration of sediments in the EU WFD and emphasize the need for continuous sediment monitoring of any waterbody. The $DemO_3^{AC}$ project and especially the present study was provided an essential scientific framework to increase the attention towards the ecotoxicological monitoring of small but highly polluted waterbodies in North Rhine-Westphalia. It would be advisable for the Wurm River catchment area to create retention areas that can be flooded during heavy rain events preventing sediment transport. In addition, the volume of the rainwater overflow basin should be adjusted to the rainwater volume. Municipal drainage and the associated discharge of large volumes of rainwater could be a solution for the urban area. Additionally, retention soil filters or constructed wetlands are a possible solution as artificial systems with natural processes as in wetlands. These processes involve a combination of mechanical and physicochemical processes, as well as the activity of bacterial communities, and are commonly used to treat rain- and wastewater of all types. Retention soil filters have proven to be particularly effective in eliminating total suspended solids, bacterial agents, ammonium, and organic compounds. To the best of our knowledge, the operator of the Aachen-Soers wastewater treatment plant is currently building a retention soil filter, which could lead to visible positive changes in the Wurm River in the future.

ABBREVIATIONS

DDT: Dichlorodiphenyltrichloroethane

DNA: Deoxyribonucleic acid

DOM: Dissolved organic matter

EBM: Effect-based method

EDC: Endocrine-disrupting compound

EU WFD: European Water Framework Directive

FET: Fish embryotoxicity test

GU: Goethe University

PAH: Polycyclic aromatic hydrocarbon

PCB: Polychlorinated biphenyl

PCDD: Polychlorinated dibenzo-*p*-dioxin

PCDF: Polychlorinated dibenzofuran

PE: Population equivalents

SQS: Sediment quality strategy

TOC: Total organic carbon

UFZ: Umweltforschungszentrum

UWWTD: Urban Wastewater Treatment Directive

WWTP: Wastewater treatment plant

INTRODUCTION

Curse and blessing of the European Water Framework Directive

The European Community set a milestone in the European water policy in 2000: all water directives and policies were united into one comprehensive document – the European Water Framework Directive (EU WFD) (European Parliament and Council, 2000). According to the EU WFD, the ecological and chemical status of the European waterbodies should be improved and conserved for the following generations. With the implementation of the EU WFD into national law, all Member States have committed to achieving the EU WFD objectives by 2027.

The EU WFD prescribes the monitoring of 45 priority substances only (European Commission, 2013; European Parliament and Council, 2000). This number of chemicals is in no relation to a substantial amount of chemicals in commerce globally (about 50,000) (see Figure 1) (Bond and Garny, 2019). About 60% of these are human and environmentally toxic (Milieu Ltd et al., 2017). Thus, it should be no surprise that renowned projects such as SOLUTIONS (Brack et al., 2019), MARS (Hering et al., 2015), EDA EMERGE (Brack et al., 2013), and the Joint Danube Survey (JDS1-JDS4) (ICPDR, 2018) showed the presence of a wide range of emerging pollutants in aquatic ecosystems. These and further results were evaluated by the NORMAN network, which comprises more than 70 institutions and laboratories in Europe and North America. Thus, over 700 chemical compounds currently enter the NORMAN list of emerging substances (status: August 2021) (NORMAN, 2021). The majority of these emerging substances are not currently included in routine monitoring programs at the EU level.

Initially, the EU WFD prioritized the interception of the water phase (European Parliament and Council, 2000), even though particulate matter and sediments were also shown to serve as a significant source and sink for chemical pollution (Chapman and Hollert, 2006). Therefore, in 2008 sediments were added in an update to the EU WFD as a monitoring matrix for substances that tend to accumulate there (European Parliament and Council, 2008). Between 2012 and 2018, over 130,000 monitoring sites of surface and groundwater were recorded Europe-wide (EEA, 2018). In comparison, sediment monitoring measurements were indicated as insufficient or even absent (European Commission, 2019).

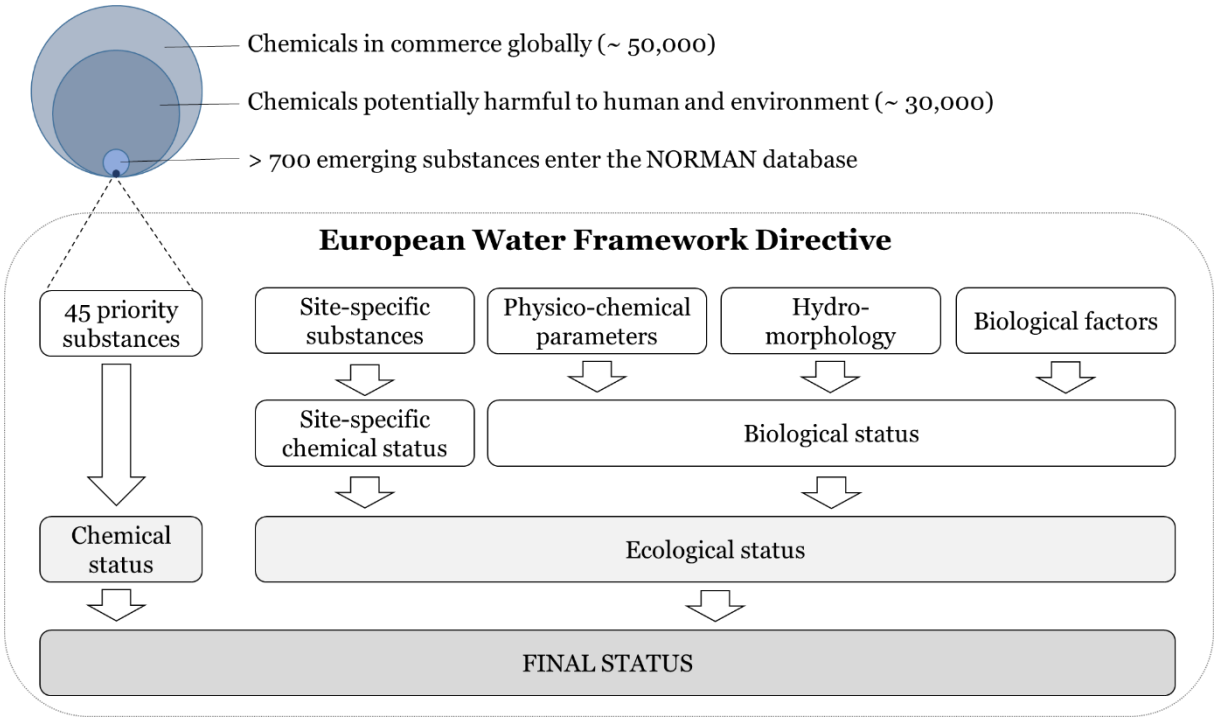


Figure 1: Procedure behind the EU WFD toward the current numbers of emerging contaminants in Europe. Adapted from: Bond and Garny (2019), European Parliament and Council (2015), (2019), Milieu Ltd et al. (2017), NORMAN (2016).

The high willingness of the Member States to contribute to clean waterbodies has been changed by the sober-looking results to date. After 18 years of effort, less than half of all European waterbodies reached a good ecological (40%) and chemical (38%) status. Multiple factors can be responsible for the failure of the EU WFD: beginning from monetary problems, over low acceptance by the society, and up to an increasing number of emerging pollutants in the environment (Bunke et al., 2019; European Commission, 2019). The pressure to still reach the goals is enormous due to the threat of infringement proceedings at the European Court if the agreed targets cannot be met.

Sediments as a matrix for binding of environmental pollutants

Sediments are an essential part of aquatic ecosystems. Sediments offer aquatic organisms a habitat and breeding space while simultaneously playing an essential role in nutrient cycles and serving as a formation material for the riverbed. Furthermore, sediments can also be seen as silent recorders of history, thus often classified as chemical timebombs (Förstner and Müller, 1974). Thus, under stable hydrological conditions, polluted old sediments are covered by less polluted younger sediments preventing erosion of deeper sediment layers and, therefore, the release of particle-

bound contaminants (Hollert et al., 2003). Different anthropological activities (e.g., urbanization, agriculture, and deforestation) and extreme ecosystem interventions (e.g., flooding, dredging, riverbed renaturation, and stormwater overflow basin releases) can lead to an unpredictable release of particle-bound pollutants. Furthermore, progressive climate change underlies the role of sediments in the assessment of pollution risk. Frequent droughts and heavy rain events accelerate soil erosion and sediment transport into aquatic ecosystems. While the water phase is fluctuating, sediments accumulate micropollutants over time.

In the 70s, several studies observed heavy metal-polluted sediments (Förstner and Müller, 1974; Stoffers et al., 1977) or petroleum-derived hydrocarbons in sediments (Giger et al., 1974). Since then, more and more chemical timebombs have been identified around the world, i.e., persistent organic pollutants such as polycyclic aromatic hydrocarbons (PAHs) were found up to 150 cm depth in sediments from the Buffalo River (USA) (Gawedzki and Forsythe, 2012); trace metals from the pre-industrial time were found in sediment cores of a 220 cm length from a special area of Conservation San Simon Bay (Spain) (Ramírez-Pérez et al., 2020); even microplastic was detected in 60 cm depth in the Beibu Gulf (China) (Xue et al., 2020).

To understand contaminants binding to sediment particles, knowledge about the structure of the sediment matrix is essential. Sediments consist of three main bricks (Chen and White, 2004):

- organic components such as decomposition materials, humic and tannic acids from vegetation and soil,
- inorganic components such as calcium carbonate, shell fragments of diatoms, and clams, and
- minerals such as clay, carbonates, sulphates, quartz, and zeolites.

The binding force and distribution of contaminants depend on multiple factors such as organic content, grain size, and functional groups. Thus, most organic contaminants bind to fine particles (< 63 µm, e.g., clay) and organic matter in oxic sediments. Contaminants in anoxic sediments build bridges with organic atoms (e.g., sulphur) (Tessier and Campbell, 1987). Furthermore, pH value and salinity can affect the binding of contaminants. Total organic carbon (TOC) frequently correlates with a contamination grade (Schumacher, 2002). The permanently presented hydrogen

donors and acceptors explain the high affinity of organic contaminants to organic matter (Perkins, 2020).

Currently, the choice of chemical compounds for sediment monitoring within the EU WFD was differentiated based on the octanol-water partitioning coefficient log kow ≥ 3 (European Commission, 2011). Following this rule, only 14 priority substances are elucidated for monitoring in the sediment phase. However, such a small number of chemicals and the evaluation method cannot adequately assess the sediment chemical status. Furthermore, as particle binding is primarily dependent on the organic content (Creusot et al., 2013; Huang et al., 2003), the adsorption coefficient koc, as a proportion between the concentration in soil/sediment and water, is a more suitable coefficient to describe the partitioning of molecules between water and sediment. Including this fact, Dulio et al. (2013) recognized sediments as a relevant matrix for molecules with the log kow > 5 and the koc_max-value > 1000 L/kg.

An indiscernible conclusion so far is that an adequate list of monitoring substances in sediments, other than the priority substances and evaluating the sediment organic content, is needed. However, where do organic micropollutants originate from? The answer is as simple as predictable: wastewater treatment plants (WWTPs) and rainwater overflows serve as the main paths for organic micropollutants into aquatic ecosystems (Commission of the European Parliament and the Council, 2019).

Wastewater treatment plants as the main point source of pollution with organic contaminants

Most households in Germany (97%, 2016) are connected to canalization (Statistisches Bundesamt (Destatis), 2018). Around 97% of collected wastewater underlies at least a secondary treatment (biological) treatment (Statistisches Bundesamt (Destatis), 2018). Almost nationwide wastewater treatment in Germany could be reached due to the national Wastewater Management Regulation of 1990 (*ger*. Rahmen-Abwasserverwaltungsvorschrift), which was replaced by the European Urban Wastewater Treatment Directive (UWWTD) 91/271/EEC (last revision: 2014) in 1991. According to the UWWTD, all WWTPs in the EU with a capacity of over 2,000 population equivalents (PE)[1] are obligated to upgrade their treatment up to the

[1] One PE is 54 gram of biochemical oxygen demand (BOD) load per person per day

secondary treatment (biological). WWTPs with a load higher than 10,000 PE should be equipped by the tertiary treatment (nutrients reduction) (Council of the European Communities, 2014).

There are two groups of wastewaters: industrial and municipal wastewaters. Industrial wastewater is unique in its composition and differs fundamentally from a municipal wastewater composition containing high concentrations of, e.g., heavy metals or dyes. Depending on quality, characteristics, and amount, industrial wastewater enters the local WWTP directly or after a specific in-plant treatment. Conventional wastewater treatment includes three initial treatment levels: mechanical, biological, and chemical (see Figure 2) (Qasim, 1999). The first step in wastewater treatment is to separate settleable solid waste from sewage. Large solid particles such as hygienic products, textiles, residual toilet paper, cigarette filters, or even animal carcasses can be separated from wastewater by a rake. However, it does not appropriate for any reuse and underlies combustion as general waste. Separation of more fine particles promotes in the sand- and fat catcher. Due to flat construction and aeration, fine massive particles like grit and sand are set to the bottom, and light particles like microplastics of low density, oils, or soap (*generic term:* scum) are floating at the water surface.

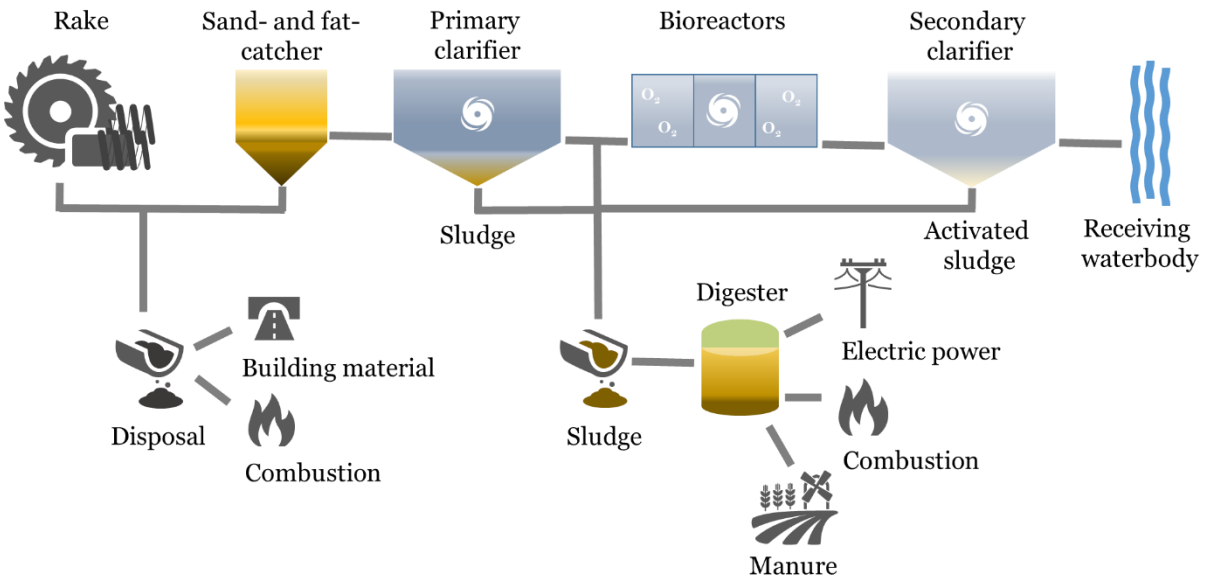


Figure 2: Scheme of conventional treatment of municipal wastewater. Created using Power-user®.

Depending on sand quality, it can be used as a building material. Otherwise, it proceeds to combust together with floating elements. Contrarily to the last treatment step, wastewater remains in the next wastewater treatment basin (primary clarifier) within

a hydraulic retention time of several hours. This time is essential for the constitution of the primary sludge from residual organic and inorganic non-dissolved solids. The next step includes the secondary treatment for removing dissolved organics by pending aerobic and anaerobic basins. Each basin has a unique targeted bacterial community for mineralizing organic nutrients from wastewater. Wastewater treated in this step promotes the secondary clarifier for recovering microorganisms (constructed sludge) and further clarification. Incurred primary and not used for the biological treatment, constructed sludge is collected in a digester. Retention and heating of sludge provide production of biogas and dehydrated sludge for manure of the agricultural field. Such comprehensive treatment steps allow eliminating nutrients, microorganisms, and in part, organic micropollutants (Knopp et al., 2016). However, well recognizable pollution patterns downstream of WWTPs still signalize the insufficiency of conventional wastewater treatment (Beckers et al., 2020; Cantwell et al., 2018; Müller et al., 2020).

Ozonation as an advanced effluent treatment strategy

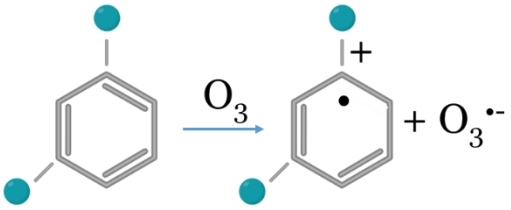
WWTP effluents were identified as sources for such micropollutants as surfactants (Beckers et al., 2020), estrogenic compounds (Adeyeye and Laub, 2020), pesticides, pharmaceuticals, and industrial chemicals (Müller et al., 2020), partitioning between water and sediment. Furthermore, due to the problem of organic micropollutants' in wastewater, additional wastewater treatment steps can be implemented (UBA, 2015):

- biological wastewater treatment such as sand filtration, wastewater pond, biofiltration,
- oxidative wastewater treatment such as ozonation, chlorination, ultrasonic treatment,
- physical wastewater treatment such as activated charcoal, ion exchangers, nano flocculation, and/or filtration.

Within the last decade, numerous scientific initiations have been dedicated to investigating advanced treatment procedures in WWTPs. The use of ozone has been proven to be promising for eliminating the most micropollutants (Abegglen et al., 2009; Schneider et al., 2020; Stalter et al., 2010a; Stalter et al., 2010b; Stalter et al., 2020; Triebskorn, 2017; Tuerk et al., 2010).

Ozone is a gas that is about ten times more soluble in water than oxygen. In addition, low temperature increases ozone solubility in water. The implementation of ozone in wastewater treatment is based on the ozone reacting with reactive molecules' sites, resulting in free radicals. Dissolved organic matter (DOM), which naturally occurs in wastewater, serves as an initiator, significantly contributing to the ozone's instability in an aqueous solution. Equitation 1 shows a site reaction of ozone with electron-reach aromatic compounds contained in DOM. Instable $O_3^{\bullet-}$ radicals decay with the formation of oxygen and reactive $O^{\bullet-}$ radicals (see Equitation 2), which further react with water molecules to hydroxyl radicals, •OH (see Equitation 3). The formation of hydroxyl radicals is a crucial reaction within ozonation tanks. Therefore, hydroxyl radicals are essential for splitting micropollutants completely (up to mineralization) or, in most cases, to less reactive, short-chain, and polar transformation products (Rodríguez et al., 2008; Sonntag and Gunten, 2012). In some exceptions, the formation of transformation products with lipophilic properties is also possible (Itzel et al., 2018).

Equitation 1



DOM-prototype

Equitation 2

$$O_3^{\bullet-} \rightleftharpoons O_2 + O^{\bullet-}$$

Equitation 3

$$O^{\bullet-} + H_2O \rightleftharpoons {}^{\bullet}OH + OH^-$$

Its strong oxidative properties make ozone a suitable option to significantly reduce the chemical burden, e.g., pharmaceuticals (Huber et al., 2005), endocrine-disrupting compounds (Wolf et al., 2022), or plant protective agents (Nasuhoglu et al., 2018) of wastewater. However, the ozone's reactivity may also have side effects and increase the toxicity of treated wastewater by forming reactive by-products (Stalter et al., 2010b) or unmasking of toxic effects (Stalter et al., 2011). The most well-studied ozonation by-product is bromate (BrO_3-), resulting from a reaction of bromide ions (Br^-) with ozone (O3). Bromate has nephrotoxic properties and is potentially carcinogenic to humans (U.S. EPA, 2001). Furthermore, conventionally treated effluent may show toxic effects caused by chemical compounds removable by ozonation. Removing these chemical compounds, toxic effects outgoing from ozonation stable chemical compounds become

unmasked, even without concentrations changes (Stalter et al., 2011). To avoid the adverse effects of ozonation, combining the ozonation with the subsequent sand filtration is appropriate (Knopp et al., 2016; Stalter et al., 2010b).

Nevertheless, modernization of WWTPs by advanced treatment steps cannot completely eliminate the problem of micropollutants in the environment. Modern canalization systems allow separating sewage and stormwater collection ('two-pipe' collector system) as it usually does not require any treatment but a controlled release in the environment (Qasim, 1999). Unfortunately, German municipalities still have an old 'one-pipe' type of canalization (about 41% in Germany) (Statistisches Bundesamt (Destatis), 2018), meaning waste- and rainwater are collected into one pipe for further treatment in the WWTP. With increased sealing of natural areas due to urban expansion, the rainwater portion in canalization increases. Low precipitation amounts may be of advantage in diluting wastewater. However, massive rain events overload a canalization system and should be consequently released untreated in the environment.

DemO$_3^{AC}$ project

80 % of Europe's rivers consist of small inland rivers (Kristensen and Globevnik, 2014). Although there is no consistent definition for small waterbodies, headwaters with a catchment area < 100 km^2, springs, flushes, ditches, small lakes, and ponds were recognized by the EU WFD as small waterbodies (European Parliament and Council, 2000). Small waterbodies play an essential role in representing habitat for flora and fauna (Biggs et al., 2017) and providing multiple ecosystem services (European Commission, 2012). Nevertheless, the flow of thousands of creeks and streams was transferred to mostly linear riverbeds. WWTP effluents and drainage runoffs notably changed the natural water flow and the water quality.

Germany's North-Rhine Westphalian State Agency for Nature, Environment and Consumer Protection (ger. LANUV) has recently investigated mixture toxicity in the German federal state North-Rhine Westphalia waters. Out of 153 analysed substances, 98 of the detected substances were mainly pharmaceuticals and plant protection products (Markert et al., 2020). One of the most frequently implemented micropollutants reduction principles is the 'reduction at the source' – i.e., at the WWTP effluent. As mentioned before, advanced wastewater treatment is a widely applied and

effective upgrade of WWTPs worldwide for more effective elimination of micropollutants (Audenaert et al., 2014). However, the knowledge regarding the subsequent potentially significant improvement of receiving waterbodies' chemical and ecological status is still rare.

Within the EU, a holistic approach was developed to assess and manage chemical pollution in surface waters. It includes coverage of all substances and mixtures combined with effect-based methods (EBMs) to identify drivers of toxicity (Brack et al., 2019; Posthuma et al., 2019). Within the last decades, EBMs have been proven to be a suitable tool for detecting adverse effects of complex mixtures in the environment, which occurred in analytically, not even quantifiable concentrations (Hollert et al. 2005; Wernersson et al. 2015). Recently, the implementation of EBMs into the EU WFD was successfully launched to monitor estrogens (Kase et al. 2018; Könemann et al. 2018).

The latter aspects were the main reasons behind starting the $DemO_3^{AC}$ project in 2014 (Brückner et al., 2018a). The project's study area is located in western Germany, the federal state of North Rhine-Westphalia, city of Aachen (see Figure 3). A small waterbody, namely the Wurm River, crosses Aachen underground, entering the riverbed in a less urban region. The Wurm River shows insufficient ecological and bad chemical status. It serves as a recipient waterbody for the Aachen-Soers WWTP, which was hypothesized as a potential source of pollution for the Wurm River. Therefore, the Aachen-Soers WWTP is suited to investigate the influence of a large-scale ozone plant on the discharging river. Upstream the Aachen-Soers WWTP, the tributary Haarbach River enters the Wurm River carrying the effluent from the Eilendorf WWTP. Therefore, this region was also included in the study area.

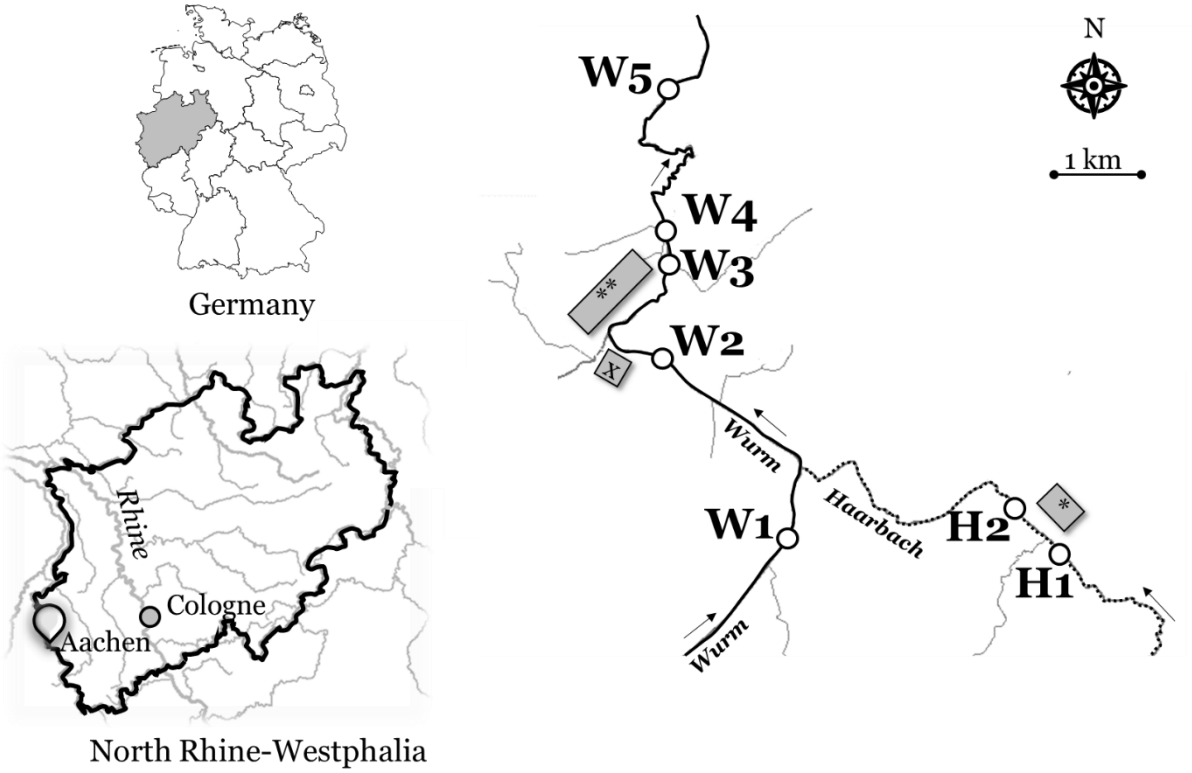


*Figure 3: Geographical position of the Wurm River with its' tributaries and local municipal WWTPs (1:36.112). *: WWTP Eilendorf (receiving waterbody: Haarbach River). **: WWTP Soers (receiving waterbody: Wurm River). X: rain overflow basin of the Aachen-Soers WWTP. Source: ELWAS-WEB v. 4.0.0, www.elwasweb.nrw.de, modified by the authors. Created using Power-user®.*

The main scopes of the $DemO_3^{AC}$ project were:

- conception and building of an ozonation plant for a full-steam effluent treatment; evaluation of the best settings for optimal removal of micropollutants,
- evaluation of the ecological, ecotoxicological, and microbiological state of the Wurm River as receiving water under conventional wastewater treatment (Phase 1) and after installation of the full-scale effluent ozonation (Phase 2),
- ecological, ecotoxicological, and microbiological investigations of the Haarbach River,
- balancing of advantages for the Wurm River ecosystem after improving the wastewater treatment under the background of potential sources of pollution located upstream.

To fulfil these goals, five institutions established an interdisciplinary collaboration for two separate phases of the project spanning a total of six years (see Figure 4). The Water Board Eifel-Rur served as an applicant and coordinator of the studied WWTPs. The Institute for Urban Water Management (*ger*. Institut für Siedlungswasserwirtschaft, ISA) of the RWTH Aachen University established all details and parameters for the future ozonation plant and coordinated its construction. The Institute for Urban Water Management analysed the elimination rates of micropollutants within the Aachen-Soers WWTP. Additionally, it investigated the water samples' ecotoxicological potential from the Wurm and the Haarbach Rivers with luminescent bacteria, algae, and daphnids. The Department 'Ecosystem Analysis' of the Institute for Environmental Research (*ger*. Institut für Umweltforschung, IfU) of the RWTH Aachen University (Phase 1 and Phase 2) and the Department 'Evolutionary Biology and Environmental Toxicology' (E3T) of the Goethe University Frankfurt (Phase 2) performed a comprehensive investigation of the water and the sediment samples using effect-based methods. In a comprehensive effect-based bioassay battery, the genotoxicity, endocrine-disrupting, and embryotoxic and teratogenic potential were investigated. Furthermore, the mentioned institution implemented *in situ* investigations with rainbow trout, gammarids, and snails. The Institute for Applied Microbiology (*ger*. Institut für Angewandte Mikrobiologie, iAMB) of the RWTH Aachen University assessed the presence of antibiotic-resistant bacteria in the effluent and the rivers and the Research Institute for Ecosystem Analysis and Assessment (*ger*. Forschungsinstitut für Ökosystemanalyse und –bewertung, gaiac) of the RWTH Aachen University investigated ecological states of the studied rivers and performed statistical analysis for data interpretation.

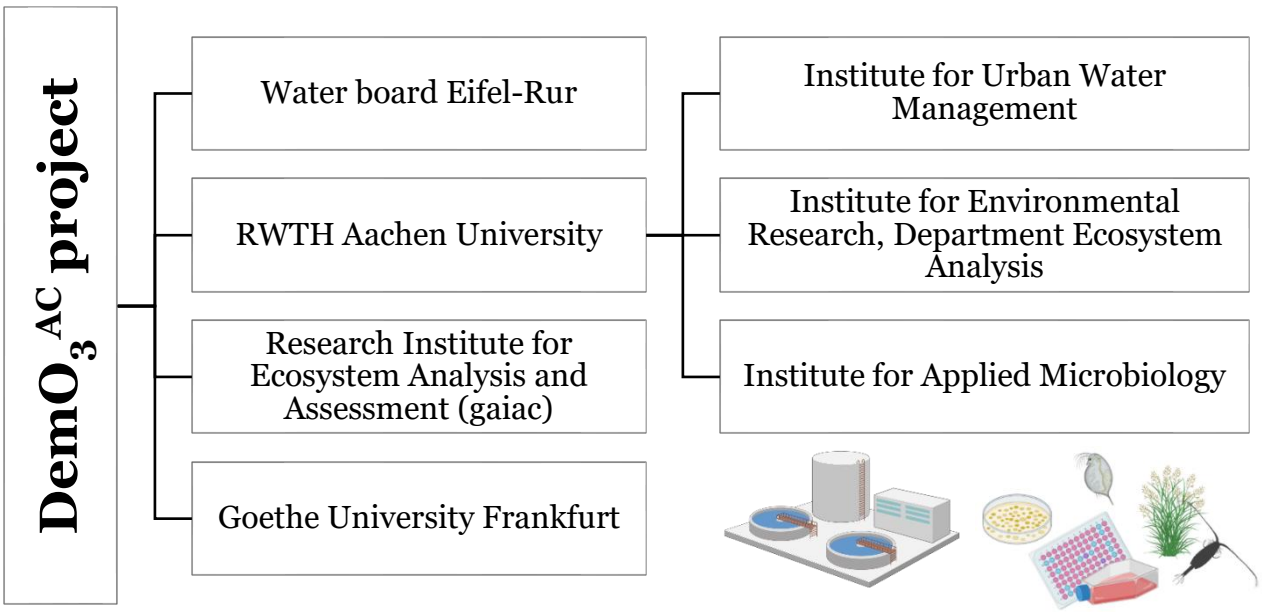


Figure 4: Major institutions within the DemO$_3^{AC}$ project. Created using Biorender©.

Categorization of the present study within the DemO$_3^{AC}$ project

The study by Wolf et al. (2022) showed that ozonation could completely reduce the endocrine-disruptive activity in the Aachen-Soers WWTP effluent. Further results from the DemO$_3^{AC}$ project showed no genotoxic or embryotoxic potential outgoing from the ozonated effluent (not published results). However, ecological investigations within the DemO$_3^{AC}$ project after the launch of the ozonation plant showed no deterioration of flora and fauna of the Wurm River. Therefore, such essential aspects as general sediment toxicity, the ecotoxicological potential of the content of the rainwater overflow basin, and organic micropollutants in the effluent of the Eilendorf WWTP were not a part of the investigation.

The present study's general objective was to bring more insights into the ecotoxicological and chemical profiling of sediments in a small but highly anthropogenic affected waterbody, the Wurm River (North-Rhine Westphalia, Germany). Temporarily resolved sampling campaigns allowed investigations at different hydrological conditions mainly driven by the weather. However, possible ecotoxicological effects of the wastewater (conventional treatment, ozonized wastewater) were also investigated, as the wastewater from the Aachen-Soers WWTP accounts for the largest part of the total water flow downstream the WWTP. The study concept was created based on the established and validated monitoring tools from the

SOLUTIONS project, Joint Danube Survey, recommendations of the international NORMAN network for monitoring emerging environmental substances, statements, and recommendations of the European Joint Research Centre, as well as comprehensive literature research. The ecotoxicological profiling of the Wurm River sediments comprised investigations of the endocrine-disrupting activity, genotoxic, and embryotoxic potentials (see Figure 5). These endpoints were evaluated using a battery of *in vitro* and *in vivo* bioassays with different cell lines and organisms to achieve reliable results at different organization stages. The sediments' chemical profiling was conducted by employing modern techniques of analytic chemistry to identify possible chemical drivers of detected effects. Ecotoxicological assessment of the possible effluent toxicity of the Aachen-Soers WWTP was conducted within the joint NORMAN-project "57 WWTP effluents" under the leadership of the Centre for Environmental Research (ger. Umweltforschungszentrum, UfZ) in Leipzig (Germany) (see Figure 5).

Therefore, a comprehensive investigation strategy in the present study serves as a basis for:

- explanation of the insufficient chemical and ecological status of the Wurm River;
- prediction of possible toxicological effects on invertebrates and fish;
- identification of possible sources of pollution;
- resilience evaluation of the Wurm River, as an example for small waterbodies;
- discussion about possible actions preventing deterioration of the ecotoxicological status of the Wurm River;
- awareness about sediment toxicity.

Objective 1: Ecotoxicological profiling and possible drivers of the genotoxic potential in sediments of the Wurm River

Numerous chemicals in the environment can directly or indirectly damage cell genetic material, thus being classified as genotoxic. Many genotoxic compounds are highly lipophilic, tending to adsorb to particulate matter and sediment (Boettcher et al., 2010; Kosmehl et al., 2004; Reifferscheid and Oepen, 2002; Seitz et al., 2008). Therefore, every detected genotoxic potential should be considered a risk for organisms or the

ecosystem as it is impossible to assess a safe genotoxic level (Belpomme et al., 2007; EFSA, 2008).

Annex 1 comprises the study on an overall sediment genotoxic potential and chemical target analysis in sediments. The genotoxic potential was investigated using the Ames fluctuation assay with different *Salmonella typhimurium* bacterial strains, sensitive to different classes of compounds, and the Micronucleus assay as a eukaryotic assay with mammalian cells. A unique feature of the current sub-study was the implementation of non-standard bacterial strains in the Ames fluctuation assay. Moreover, a comprehensive genotoxicity ranking of chemical compounds identified in sediments was used and combined with statistical analysis to identify the drivers of genotoxicity.

The study in the Annex 1 should answer the following questions:

(i) Do extensive rain events have an impact on sediment genotoxicity in the Wurm River?

(ii) Can the observed genotoxic potential in sediments be explained by the target chemical analysis?

(iii) Are additional *Salmonella typhimurium* strains suitable for sediment genotoxicity testing?

Objective 2: Endocrine-disrupting activity and PAHs as its possible drivers in sediments of the Wurm and the Haarbach Rivers

Worldwide concern rises regarding alterations in the endocrine system in humans and wildlife (UNEP and WHO, 2012). WWTPs still represent the primary source for endocrine-disrupting compounds (EDCs) in aquatic ecosystems (EEA, 2018; Eggen et al., 2014; Sonavane et al., 2018). Release of the rainwater overflow basin as a consequence of extensive rain events acts as a flood and may lead to a sediment perturbation that risks releasing particle-bound EDCs (Wolf et al., 2022). Due to humic acids' adsorption capability, such hydrophilic EDCs as steroid hormones might be detected in sediment (Hollert et al., 2005; Neale et al., 2009; Sangster et al., 2015). However, many EDCs are quite hydrophobic and tend to sorb to particles. A particular interest in this study was paid to ubiquitously present persistent PAHs. PAHs are typical pollutants of the particulate phase (Hollert et al., 2002; Keiter et al., 2008) and are known geno- (Nacci et al., 2002), terato-, and embryotoxins (Kais et al., 2017; Schiwy et al., 2015), as well as disruptors of vitellogenesis (Nicolas, 1999). However,

PAHs are still not recognized EDCs providing a long-term endocrine-disrupting potential in aquatic ecosystems.

Annex 2 comprises a study describing the distribution of the endocrine-disrupting activity in the Wurm River (water, suspended particles, and sediment) at opposite weather conditions (dry and rainy weather with the rainwater overflow basin release). The endocrine-disruptive activity was investigated using the cell-based reporter gene CALUX® assay. A simultaneous launch of the full-scale effluent ozonation at the Aachen-Soers WWTP was used for a simultaneous investigation of the entrance of the ozonated effluent into the Wurm River and the endocrine-disrupting activity in the water phase. A particular focus of the present study was the unique investigation of PAHs as possible drivers of the endocrine-disrupting activity in sediments of the Wurm River.

The study in the Annex 2 should answer the following questions:

(i) Which distribution does the endocrine-disrupting activity have in the Wurm River?

(ii) Do extensive rain events have an impact on the endocrine-disrupting activity?

(iii) Can particle-bound PAHs be possible drivers for the observed endocrine-disrupting activity?

Objective 3: Ecotoxicological profiling and possible drivers of the embryotoxic potential in sediments of the Wurm River

The study by Busch et al. (2016) showed that 13% of 426 detected in European rivers chemical compounds act neurotoxic. Currently, about 200 chemicals, including plant protection products, pharmaceuticals, heavy metals, flame retardants, and polychlorinated biphenyls (PCBs), are known neurotoxic substances (Grandjean and Landrigan, 2006; Herbstman et al., 2010; Maranho et al., 2014). Organism behavioral changes can occur as a result of exposure to even no effect concentrations in the fish embryotoxicity test (FET), representing a very sensitive toxicological endpoint (Busch et al., 2016; Melvin and Wilson, 2013).

Annex 3 comprises a study of a less investigated group of such chemical pollutants as neurotoxic substances. As the development of the nervous (Jurisch-Yaksi et al., 2020; Tegelenbosch et al., 2012; Winberg et al., 1997) and neurotransmitter systems (Haug et al., 2013) of vertebrates is evolutionarily conserved, zebrafish larvae were chosen as

a study organism. Before investigating possible neurotoxic activity from sediments from the Wurm and the Haarbach Rivers, embryotoxic potential and, therefore, the no-effect concentration was studied. Exposure scenarios investigated potential embryotoxicity at realistic (native sediments) and worst-case (freeze-dried sediments and sediment extracts). Zebrafish larvae exposure to sediments was performed using the Sediment Contact Test. Behavioral changes after exposure of the zebrafish larvae to the no observed effects concentration from the fish embryotoxicity test were studied in the locomotion assay. Obtained results were combined with the results from the chemical analysis of particle-bound contaminants elucidating possible drivers for detected effects were elucidated.

The study in the Annex 3 should answer the following questions:

(i) Does sediment in the Wurm River reveal embryotoxic potential?

(ii) Does exposure to sediment in the Wurm River can lead to behavioral alterations in the zebrafish embryo?

(iii) Can identified particle-bound target chemical compounds explain observed effects?

Objective 4: Hazard evaluation of WWTP effluents by a comprehensive target-screening: Valuable insights but also a big toxicological data gap

Up to date, about 30,000 chemicals (60%) presented on the European market are potentially harmful to humans and the environment (Bond and Garny, 2019; Milieu Ltd et al., 2017). Municipal WWTPs cannot completely eliminate hazardous chemicals that serve as the main pathway for organic micropollutants as pharmaceuticals, natural hormones, and consumer products in aquatic ecosystems (König et al., 2017).

Annex 4 comprises additional data on a chemical target analysis of 499 chemicals (parent and their metabolites) in the Aachen-Soers effluent before and after installing the full-stream ozonation. Additionally, the effluent of the Eilendorf WWTP located upstream of the Aachen-Soers WWTP was investigated to compare the chemical load outgoing from two WWTPs of different sizes and catchment areas. Obtained results were used to assess both the micropollutants elimination by ozonation and the potential risk outgoing from not eliminated micropollutants. For the latter purpose, the Toxic Units-approach (TU) was implemented (Ohe et al., 2009; Ohe et al., 2011). As the calculation of TUs is still not uniform, two approaches were compared. The so-

called Goethe University (GU)-approach from the current study was compared with the approach developed at the department of the Effect-directed Analysis of the UfZ in Leipzig (Germany; UfZ-approach) (Schulze et al., 2021). The present study was a part of the European-wide study on evaluating specific patterns within the WWTP effluents under the patronage of the NORMAN network and the leadership of the UfZ (Germany).

The study in the Annex 4 should answer the following questions:

(i) Which target chemical compounds can be detected in the studied effluents?

(ii) Which target chemical compounds can be elimination by the effluent ozonation?

(iii) Which target chemical compounds represent a potential risk to freshwater organisms?

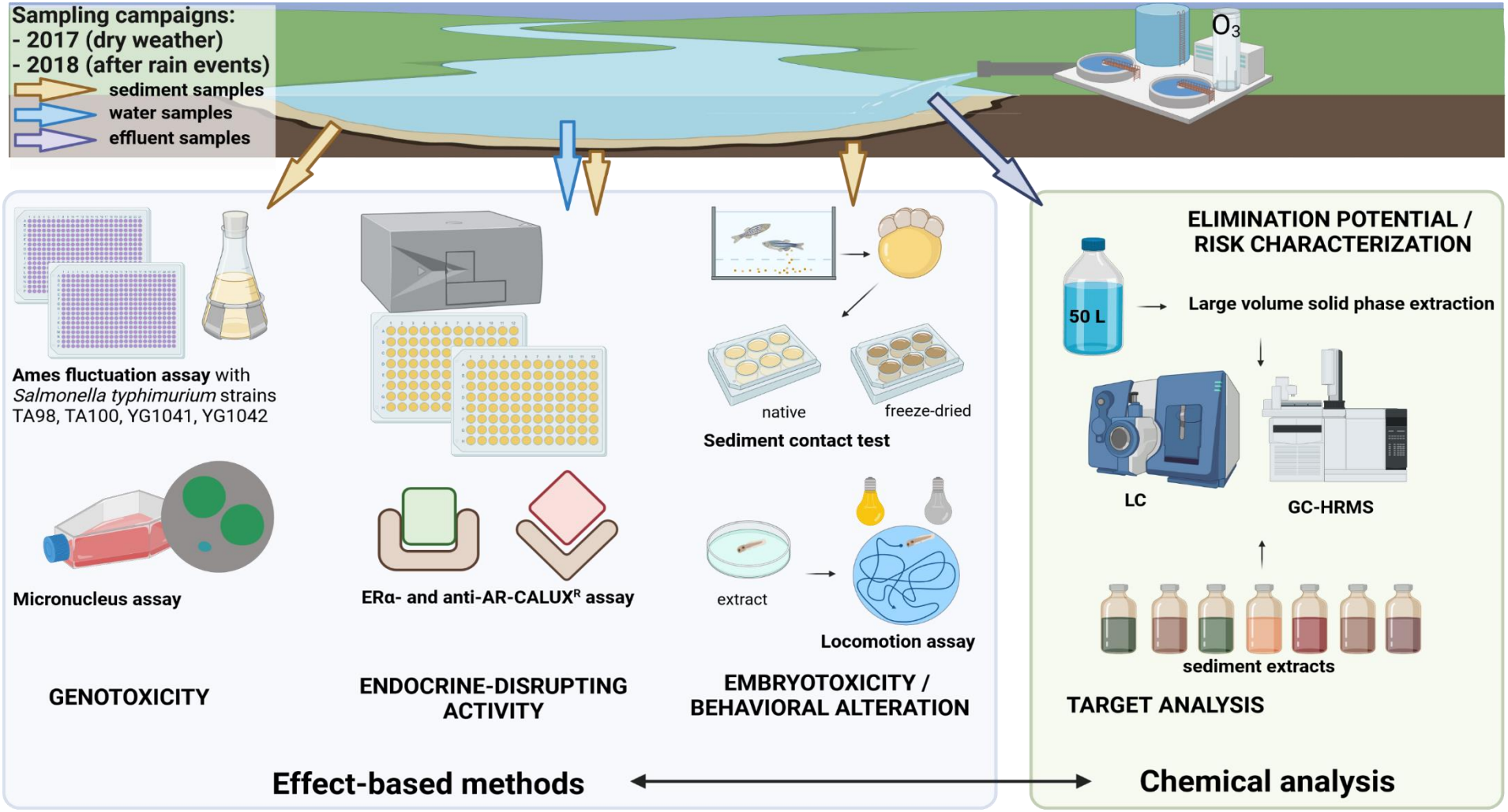


Figure 5: Concept of the present thesis. Created using Biorender©.

STUDY AREA DETAILS

Study area

The study area of the current thesis begins upstream of the Wurm River, at its tributary Haarbach River. The Haarbach River is located in the eastern part of Aachen and passes the districts Forst, Eilendorf, and Haaren (from south to north). At the Haaren district, it flows together with the Wurm River. At this point, the original flow of the Haarbach River was changed many times due to the Eilendorf WWTP and to prevent flood events (Wolf et al., 2021).

The effluent of the Eilendorf WWTP is currently released into the old flow route of the Haarbach River (see Figure 6). The Eilendorf WWTP is a small-scaled one (87,000 PE). According to the information system ELWAS-WEB for the water management administration in the federal state North Rhine-Westphalia in Germany, an average portion of wastewater from the Eilendorf WWTP during a year makes up about 70% of the total water flow in the Haarbach River (last update: 2018). Several industries release sewage water into the Eilendorf WWTP (see Table 1).

Thus, a local provider of waste disposal services, Schönmackers Umweltdienste GmbH & Co KG, discharges pre-treated mineral oil-containing sewage into the Eilendorf WWTP. Rainwater collected at the company's terrain discharges directly into the Haarbach River (ELWAS-WEB, 2020). Two industrial productions within proximity of the Eilendorf WWTP, production of paper tube and a laundry detergent production, release their sewage directly into the Eilendorf WWTP. Out of a conversation with colleagues working at the Eilendorf WWTP, the wastewater may heavily foam from time to time due to detergents.

Under 5% of households discharge their sewage untreated directly into the Haarbach River (Joerrens, 2017). Due to frequent flood events, the Haarbach River was subjected, among other things, to remediation activities (Firk, 2010). Beginning from the Eilendorf WWTP, the Haarbach River is surrounded by numerous agriculturally used fields. Parallel to the WWTP (left shore of Haarbach in the flow direction), two agricultural fields with a total area of over 20 ha are situated. The middle distance to the waterbody accounts for 30 m. Hydrological soil characteristics and soil texture determine moderate to high runoff potential.

Two sampling sites were located in the Haarbach River (abbreviation by the first letter): H1 up- and H2 downstream of the Eilendorf WWTP.

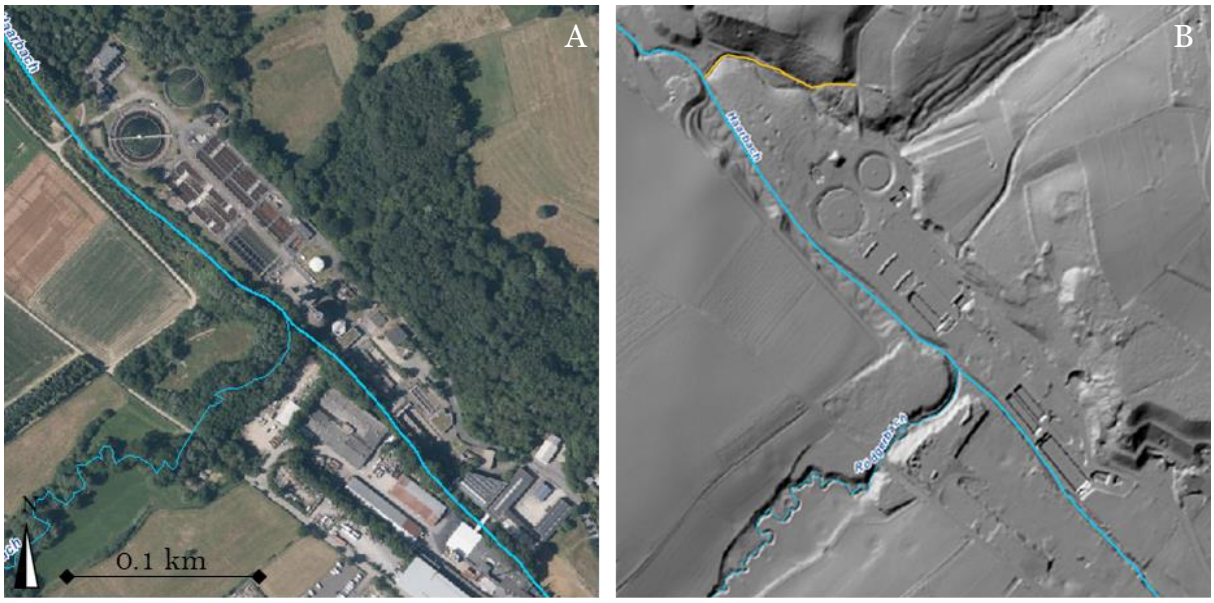


Figure 6: Aerial depiction of the Eilendorf WWTP (A) and its digital terrain model (B). Figure B depicts the old flow route of the Haarbach River by the orange line. Source: ELWAS WEB v. 4.1.0, www.elwasweb.nrw.de, modified by the author.

After the confluence of the Haarbach and Wurm Rivers, a united waterbody, namely the Wurm River, follows the north direction. As the flow conditions within two waterbodies are almost equal, direct comparisons between the Wurm and Haarbach Rivers are possible. Shortly after the confluence of the Haarbach River and the Wurm River, an agricultural field of 1.35 ha is located close to the waterbody (6.85 m). It is essential to mention this agricultural area as it is characterized as having moderate to high runoff potential.

The catchment area of the Wurm River extends over a 356 m^2 area with predominately urban and agricultural sectors. Shortly after the origin, the route of the Wurm River crosses the middle-size city of Aachen, mostly underground. Rare aboveground parts of the Wurm River are morphologically adapted to the urban landscape (e.g., by straightening) (MKULNV NRW, 2014). Morphological changes, agricultural activities, and impact by WWTPs are the main drivers for moderate to phyto- and zoobenthos' bad status and decline in the Wurm River's fish fauna. The Wurm River serves as a recipient stream for 12 municipal WWTPs. One of them is one of the biggest in Europe, the Aachen-Soers WWTP, with a daily inflow of 2000 L/s and over 458,000 population

equivalents. During dry weather, the effluent of the Aachen-Soers WWTP makes up two-thirds of the total water flow in the Wurm river (ELWAS-WEB, 2018, 2019; MKULNV NRW, 2015, 2017). A minor portion of 11% consists of the effluent from the upstream located Eilendorf WWTP (Brückner et al., 2018a; ELWAS-WEB, 2018).

The Wurm River catchment area is known as the ore district, which contained numerous mines and tunnels in the 18. and 19. Centuries. Nowadays, four mines (Altwerk, Breinigerberg, Diepenlinchen, Georg) are located in the study area's surroundings. According to the MKULNV NRW, in the presence, the four mentioned mines do not significantly impact the environment with heavy metals (MKULNV NRW, 2012). A petrol station of the Real,-SB-Warenhaus GmbH discharges mineral oil-containing sewage (pre-treated in the oil separator) into the WWTP Soers and the stormwater directly into the Wurm River. Moreover, the Aachen-Soers WWTP achieve effluents from 8 local hospitals.

At the area of the Aachen-Soers WWTP, the Wildbach River enters the Wurm River. As the last 5 km before the confluence with the Wurm River, the Wildbach River passes agricultural fields only, it is essential to include these areas into the meta-analysis of possible diffuse sources of pollution. Four agricultural areas may directly impact the Wildbach River due to the waterbody's distance between 1 m and 2 m. Together they account for over 20 ha. Over 13 ha of this area are characterized as having moderate to high runoff potential. After the effluent of the WWTP Aachen Soers, the Wurm River crosses a nature-close surrounding with no official agricultural fields or industrial dischargers present.

Sampling sites in the Wurm River were evaluated as follows (abbreviation by the first letter): W1 - before the confluence of the Haarbach and the Wurm Rivers, W2 - after, W3 - downstream the rainwater overflow basin of the Aachen-Soers WWTP, W4 - effluent site, W5 - 2.5 km downstream (see Figure 3). For more details, see Table 2.

Table 1: Industrial dischargers of wastewater into the canalization system of Aachen. Source: ELWAS WEB v. 4.1.0, www.elwasweb.nrw.de.

direct discharge into the municipal canalization	
TWA Eicherstollen/STAWAG	Discharging water: Stormwater, wastewater contaminated with heavy metals. Internal treatment: Two sedimentation tanks
Schönmackers Umweltdienste GmbH & Co. KG	Discharging water: Stormwater, wastewater contaminated with mineral oil. Internal treatment: Separator
Real,-SB-Warenhaus GmbH	Discharging water: Stormwater, wastewater contaminated with mineral oil. Internal treatment: Fuel separator
Production of paper tubes	No information
Production of laundry detergents	No information
indirect discharge into the municipal canalization	
Philippen Containerdienst GbR	Discharging water: No information. Internal treatment: Separator
Horsch GmbH & Co. KG	Discharging water: No information. Internal treatment: Separator
Schönmackers Umweltdienste GmbH & Co. KG	Discharging water: No information. Internal treatment: Separator

*Table 2: Geographical and ecomorphological description of the sampling sites. H - Hydromorphology: * low altered, ** moderately altered, *** clearly altered, **** strongly altered, ***** completely altered. E - Ecological / C - chemical status: * unsatisfactory, ** bad. Source: ELWAS WEB v. 4.1.0, www.elwasweb.nrw.de.*

	Sampling site	Description	Rating following criteria of the EU WFD H	E	C
← Stream direction	**H1**	Main waterbody, no former confluences with other waterbodies, upstream of the Eilendorf WWTP UTM: 32U 299014 563618	**	**	**
	Eilendorf WWTP	Capacity: 87,000 population equivalents, volume treated wastewater: 4.8 Mio. m³/y, applied treatments: Primary treatment, secondary treatment, N removal, P removal, sand filtration UTM: 32U 299261 5630462			
	H2	Main waterbody, former confluence with the old main branch of the Haarbach River, downstream of the Eilendorf WWTP UTM: 32U 298654 5630852	**	**	**
	W1	Main waterbody, no former confluences with other waterbodies, upstream of the Aachen-Soers WWTP UTM: 32U 297008 5630330	*****	**	**
	W2	Main waterbody, after the confluence with the Haarbach River, upstream of the rainwater overflow basin of the Aachen-Soers WWTP UTM: 32U 296118 5631884	*	**	**
	Rainwater overflow basin	About 40 releases per year UTM: 32U 295827 5631904			
	W3	Main waterbody, downstream of the rainwater overflow basin of the Aachen-Soers WWTP, upstream of the effluent UTM: 32U 296093 5632694	*	*	**
	Aachen-Soers WWTP	Capacity: 458.000 population equivalents, volume treated wastewater: 35.893.148 m³/y, applied treatments: Primary treatment, secondary treatment, N removal, P removal, sand filtration, since 2018: full-stream effluent ozonation UTM: 32U 295545 5632182			
	W4	Main waterbody, downstream of the rainwater overflow basin, effluent site UTM: 32U 296087 5632785	****	*	**
	W5	Main waterbody, 2.5 km downstream of the Aachen-Soers WWTP UTM: 32U 296047 5634164	***	*	**

Studied wastewater treatment plants (WWTPs)

The **Aachen-Soers WWTP** is one of the biggest WWTPs in Germany. The daily sewage inflow from over 458,000 population equivalents accounts for around 32 Mio. m^3 treated wastewater per year. The wastewater collection conforms to the mixed canalization characterized by a simultaneous collection of wastewater and rainwater. The Aachen-Soers WWTP meets all requirements of the European Union concerning the treatment of urban wastewater (Commision of the European Communities, 1998; Council of the European Communities, 1991) and has the following construction (Qasim, 1999):

Mixed water enters the Aachen-Soers WWTP within a channel communicating with the **rainwater overflow basin** (see Figure 7). In case the influent volume exceeds the WWTP's entry capacity, surplus mixed water will be stored in the rainwater overflow basin and forwarded later to the WWTP. In case the influent velocity exceeds the WWTP's treatment capacity, and the rainwater overflow basin is full, mixed water will be released into the recipient water untreated. The remaining particulate matter on the rainwater overflow basin ground will be removed and transported to the WWTP within a few days after its release. The Aachen-Soers WWTP has six rainwater overflow basins;

Rake keeps accompanying rough material like toilet paper or cigarette filter back;

Sand and fat catcher allows to separate waste according to their density;

Primary clarifier is for collection of settled organics (primary sludge) and its reuse in digester tank;

Bioreactor combines aerobic and anaerobic areas for optimal bacterial removal of nitrogen- and phosphorus-contained compounds. The monitoring of the correct bacteria composition occurs automatically. It can be corrected by inoculating activated sludge if required;

A secondary clarifier is a collector for activated sludge, containing numerous valuable bacteria from the biological treatment. Settled activated sludge is used to maintain the activity of the biological treatment step. Not-used activated sludge will be transported to the primary clarifier;

Full-stream ozonation is for the reduction/elimination of micropollutants, microorganisms, and dissolved organic carbon. Ozone generates from liquid oxygen and pumps using 56 diffusers installed on the ground. The average ozone dose is between 3 and 5 mg O_3/L;

Secondary nitrification is for settlement of residual organic materials and absorption of microorganisms;

Sand filtration is the last step before effluent enters the recipient waterbody.

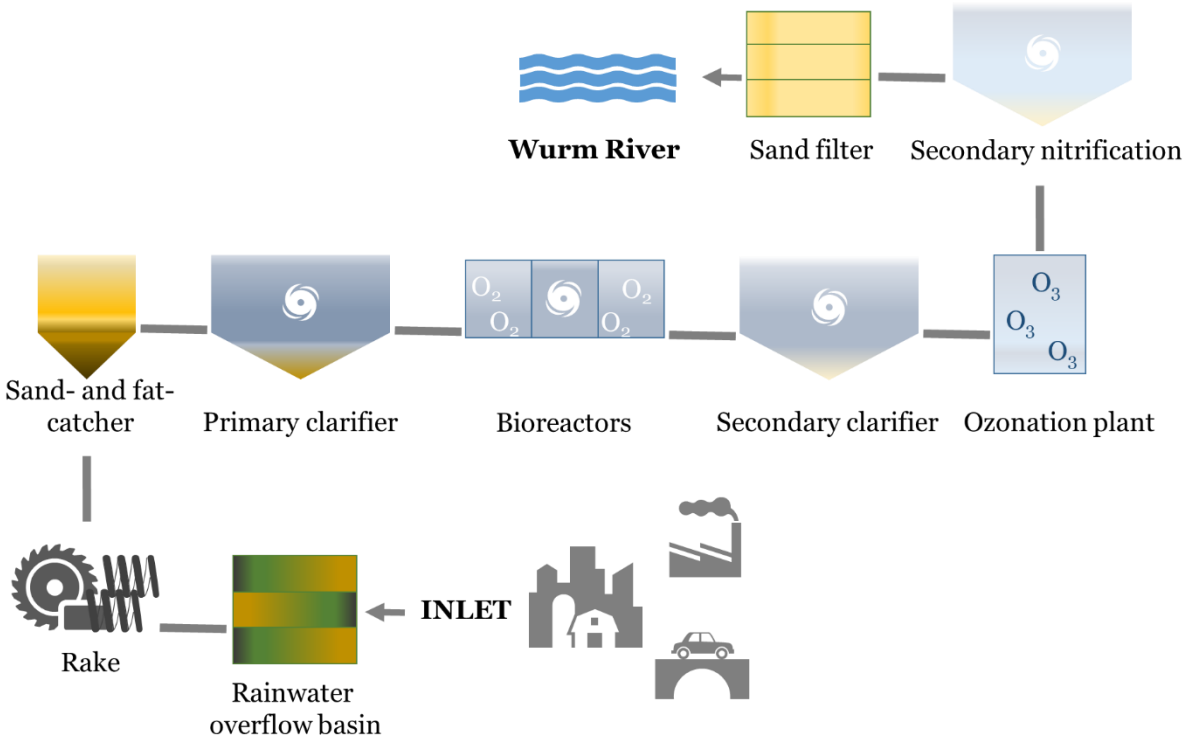


Figure 7: Scheme of the Aachen-Soers WWTP. Created using Power-user®.

The **Eilendorf WWTP** is a middle-sized WWTP with over 4 Mio m³ treated wastewater per year collected by mixed canalization. The construction of the Eilendorf WWTP is identical to this of the Aachen-Soers WWTP except for the advanced ozonation step and the secondary nitrification (steps 1-6 + 9).

Sampling campaigns

The first sampling campaign of water and sediment occurred in June 2017 under prevailing stable hydrological conditions due to arid summer. During dry weather, the average precipitation rate in Aachen within the last 21 d before the sampling campaign

accounted for 1 L/m² (WetterKontor GmbH, 2017). The Aachen-Soers WWTP used conventional wastewater.

The second sampling campaign was carried out at different dates due to the following arguments:

- to assess a possible impact of ozonated effluent on the ecotoxicological profile of the Wurm River downstream the effluent, the second water sampling took place one year after the launch of the ozonated plant (March 2019);
- to capture the possible influence of pronounced agricultural activities in the study catchment area, the second sediment sampling was conducted precisely one year later (June 2018) after extensive rain events. The average precipitation rate in Aachen within the last 21 d before the sampling campaign accounted for 5 L/m² (WetterKontor GmbH, 2018).

DISCUSSION

Summary of the main findings

The present study demonstrates ecotoxicological and chemical sediment profiling at different weather conditions in the presence of potential point sources of pollution - wastewater treatment plant (WWTP) effluents and a rainwater overflow basin. Sediment samples were collected at two subsequent years (June 2017 and June 2018) from the stretches of the Haarbach and the Wurm Rivers (North-Rhine Westphalia, Germany) up- and downstream of two WWTPs (see STUDY AREA DETAILS). A comprehensive overview of ecotoxicological and chemical sediment profiling is represented in Annexes 1 - 3. The analysis of WWTP effluents as potential sources of pollution completed the investigative strategy and is laid down in Annex 4.

The first study (Shuliakevich et al. (2022a), see Annex 1) describes sediments of the Wurm River as highly polluted by ubiquitous organic pollutants with pronounced genotoxic potential. Particle-bound genotoxic potential indicated temporal exposure of aquatic organisms in the Wurm River to a mixture of frameshift and base pairs-substituting (pro-)mutagens combined with a permanent exposure to nitroarenes and aromatic amines. Rain-initiated sediment perturbation and release of the rainwater overflow basin were identified as significant factors leading to increased genotoxic potential and elevated concentrations of particle-bound chemical compounds.

The second study (Shuliakevich et al. (2022b), see Annex 2) records fluctuations of the endocrine-disrupting activity in the Wurm River at different weather conditions. Observations of the water phase and particle matrices (sediment and suspended solid matter) indicated partial responsibility of the rainwater overflow basin on increased estrogenic and antiandrogenic activities in the Wurm River. A unique result of the second study was identifying a positive correlation between measured particle-bound antiandrogenic activity and detected polyaromatic hydrocarbons (PAHs). Moreover, the second study showed the effectiveness of advanced effluent treatment by ozonation resulting in complete elimination of estrogenic and antiandrogenic activities. However, the entrance of the ozonated effluent with at least not detectable endocrine-disrupting activity was extinguished by pronounced endocrine-disruptive activity in water and sediments of the Wurm River after extensive rain events.

The third study (see Annex 3) characterizes the sampled sediments' toxic potential regarding zebrafish larvae development and behavior. Exposure to native sediments as the most realistic scenario was inconspicuous. In contrast, freeze-dried sediments and organic sediment extracts mimicking the ecotoxicological status of sediments during flood events caused acute sublethal effects. After exposure to sediment extracts, zebrafish larvae revealed behavioral alterations below the threshold of visible deformations, emphasizing its high sensitivity. To a different extent, all investigated sediment samples showed embryotoxic potential, while some potential sources of pollution remained unknown. Rainwater overflow basin was highlighted as a possible source of pollution one more time. Target chemical analysis identified PAHs, PCBs, and nitroaromatic compounds as possible drivers for observed embryotoxic effects, whereas further toxicity drivers remained unknown.

Annex 4 comprises a chemical target analysis of 499 chemicals (parent and their metabolites) in the Aachen-Soers WWTP effluent before and after installing the full-stream ozonation (recipient waterbody Wurm River). Additionally, the effluent of the closely located Eilendorf WWTP (recipient waterbody Haarbach River), located upstream of the Aachen-Soers WWTP, was investigated to compare the chemical load outgoing from two WWTPs of different sizes and catchment areas. The Annex 4 study was a part of the European-wide study on evaluating specific patterns within WWTP effluents under the patronage of the NORMAN network and the leadership of the Helmholtz Centre for Environmental Research (*ger*. Helmholtz Umweltforschungs-zentrum, UFZ) in Leipzig (Germany).

Weather impact on the ecotoxicological and chemical profiling of sediments

Sediments collected during dry weather (sampling campaign in June 2017; historical water level: 18 cm (ELWAS-WEB, 2021b)) contained frameshift (pro-)mutagens, nitroarenes, and aromatic amines and led to a slight occurrence of sublethal effects in zebrafish embryos (edema, cardio-vascular disorders, low pigmentation). In contrast, after extensive rain events in June 2018 (water level: 70 cm (Shuliakevich et al., 2022b)) and the release of the Aachen-Soers WWTP rainwater overflow basin, the higher revertant rate in the Ames fluctuation assay, and additionally to frameshift (pro-) mutagens, nitroarenes and aromatic amines, base-pair substituting (pro-)mutagens were detected at all sampling sites. The elevated embryotoxic potential in zebrafish

embryos was clearly correlated with the sampling sites' spatial location. Thus, the effects mentioned above were especially pronounced at the sampling sites downstream of the rainwater overflow basin. The study by Thellmann et al. (2015) described embryotoxic potential in sediments of the Schussen River (Germany) downstream of the local WWTP, while Vincze et al. (2014) reported more substantial embryotoxic potential during flood events in the Neckar River (Germany).

In addition to the detected genotoxic effects, sediments of the Wurm River collected during dry weather (June 2017) showed low estrogenic and antiandrogenic activities. In contrast, after extensive rain events (June 2018), elevated endocrine-disrupting activity correlated with the spatial location of the sampling sites. Especially pronounced endocrine-disrupting activity downstream of the rainwater overflow basin contributed to the classification of the rainwater overflow basin of the Aachen-Soers WWTP as a point source of pollution. The study by Neale et al. (2020) confirmed this assumption describing increased estrogenic activity in connection with rain-initiated sewer overflows.

Identified ecotoxicological potentials in sediments of the Wurm River may have severe long-term effects on the wildlife population of studied waterbodies. Already now, the Haarbach's and the Wurm River's ecological and fish status is classified as bad. The identified genotoxic potential may hide a risk at even low concentrations (Belpomme et al., 2007). Particle-bound genotoxic pollutants can be ingested or absorbed through the gill epithelium of fish (Barceló et al., 2007), potentially leading to abnormalities in the genetic material of native fish species inhabiting the Wurm River. Grung et al. (2016) observed significantly more severe DNA damage in minnows in sedimentation ponds achieved road runoffs than minnows from a river. As the rainwater overflow basin of the Aachen-Soers WWTP achieves wastewater and runoffs from the whole city, including highway, fish exposure conditions in the Wurm River during the release of the rainwater overflow basin can be comparable with those in sedimentation ponds from the study by Grung et al. (2016). Based on investigations by Mueller et al. (2019b) on fish exposure to sediment-bound endocrine-disrupting compounds (EDCs), both benthic and pelagic living can be affected by particle-bound pollutants.

Furthermore, Mueller et al. (2019a) showed remobilization of such EDCs as nonylphenol, estrone, 17β-estradiol, and ethynylestradiol during turbulent conditions, such as in a flood event, in bioavailable ecotoxicologically relevant concentrations. The

observed reduction of the ecotoxicological potential in the ozonated effluent may positively impact the wildlife population inhabiting studied waterbodies. However, it is necessary to scrutinize not eliminated chemical compounds in the ozonated effluent (see Annex 4).

Hazard evaluation of WWTP effluents by a comprehensive target-screening: Valuable insights but also a big toxicological data gap

The ecotoxicological sediment profiling, as described in Annexes 1 - 3, recorded more pronounced toxic effects after extended rain events than during dry weather. The spatial resolution of the results helped identify the rainwater overflow basin as a potential source of pollution. Important to note that during the sampling campaign 2018, full-effluent ozonation was already launched. The study by Wolf et al. (2022) showed that ozonation could completely reduce the endocrine-disruptive activity in the effluent. Further results from the $DemO_3^{AC}$ project showed no genotoxic or embryotoxic potential outgoing from the ozonated effluent (not published results). However, the results of the present study, combined with the results of the $DemO_3^{AC}$ project, revealed a not significant improvement of the toxicological potential in the Wurm River Shuliakevich et al., 2022a; Shuliakevich et al., 2022b; Wolf et al., 2022).

On the one hand, the release of the highly polluted content of the rainwater overflow basin combined with the pollution outgoing from the upstream located and conventionally treated Eilendorf WWTP could be responsible for masking the entering of ecotoxicologically less polluted ozonated effluent into the Wurm River (see Annex 4). On the other hand, the elimination efficiency of the ozonation and toxicity of not eliminated chemical compounds should be assessed critically. For this purpose, potential risk outgoing from not eliminated micropollutants was assessed using the Toxic Units-approach (TU) (Ohe et al., 2009; Ohe et al., 2011). A TU is a ratio between the measured environmental concentration of the individual compound (in that case: the effluent concentration) and its effect level for a group of organisms or an individual species. Important to note that there is currently no uniform approach for calculating TUs. That means that the effect level can be each toxicity level ($LOEC, NOEC, EC_x, LC_x$) derived from each evaluable procedure (practical evaluation, modeling, prediction, mean of values).

The Annex 4 study prioritized chemical compounds of concern using effect values that cause effects in 50% of the exposed organisms, respectively. Additionally, the difference between species-specific and species-unspecific toxicity was conducted. The Annex 4 study used species-unspecific acute LC_{50}-values for daphnids and fish, and species-specific acute EC_{50} values for a planktonic crustacean *Daphnia magna* and a salmonid Rainbow trout *Oncorhynchus mykiss*. For a sensitivity comparison, the acute chemical hazard assessment strategy included a zebrafish *Danio rerio* as a common model species in environmental sciences. The data were chosen for freshwater organisms only. The lowest effect concentration was chosen without differentiation between the active ingredient and a formulation among all evaluable data. In the following paragraphs, the TU-approach from the current study will be called the GU-approach (GU: Goethe University) (see Table 3).

In Table 3 the GU-approach is compared with the approach developed at the Department of Effect-directed Analysis at the UFZ in Leipzig (Germany; UFZ-approach) (Schulze et al., 2021). The UFZ-approach is based on the Species Sensitivity Distributions approach comparing measured environmental concentration and the effect concentration, which caused an effect at 5% of exposed most sensitive organisms calculated based on the LC_x , EC_x , $LD_x, LOEL$, and $NOEL$ values as $EC_{05_{est}}$ (est: established value). Following organism groups were evaluated for the UFZ-approach: fish, crustacean, algae, while no difference was seen between freshwater and marine habitats. Effect values were chosen for active ingredients only. Additionally, the water solubility of the chemical compound was considered. Thus, if chosen effect data exceeded more than a half log unit above the predicted water solubility of the individual chemical compound, the effect data was replaced by the predicted water solubility (Schulze et al., 2021).

Table 3: Comparison of two approaches on the calculation of Toxic Units (TU).

	GU-approach (present thesis, Annex 4)	UFZ-approach (Schulze et al., 2021)
effect values used	lowest $LOEC, NOEC, EC_{50}, LC_{50}$	$EC_{05_{est}}$ from all effect concentrations (LC_x , EC_x , $LD_x, LOEL, NOEL$)
databases	US EPA ECOSAR ($LOEC, NOEC, EC_{50}, LC_{50}$) ChemProp 1.1.5 ($LC_{50}$) NORMAN Ecotoxicology Database ($EC_{50}, LOEC, NOEC$)	US EPA ECOTOX ChemProp 6.7.1
Organisms	acute toxicity, species-specific approach: - Zebrafish *Danio rerio* - Rainbow trout Oncorhynchus mykiss - planktonic crustacean *Daphnia magna* chronic toxicity, species-unspecific approach: - Daphnids - fish	acute toxicity, species-unspecific approach: - fish (all species, exception: bone fishes) - crustacean - daphnids (only daphnid type species) - algae (all algae species, exception blue-green algae, and other cyano-species)
Habitat	freshwater	freshwater, marine
Substance type	active ingredients, formulations	active ingredients
Test time	acute: <=120 h chronic: > 120 h	acute: <=120 h
Extra	-	if measured/predicted ecotoxicological data greater than a half log unit above the predicted water solubility, then replacement of the measured/predicted ecotoxicological value by the predicted water solubility
Data availability	acute toxicity: - *Danio rerio*: 31% - *Oncorhynchus* mykiss: 8% - Daphnia magna: 61% chronic toxicity: 100%	acute toxicity: 100%
Number of chemical compounds potentially hazardous to organisms	acute toxicity: 12 chronic toxicity: 3	0

Implementation of the GU-approach could not identify potentially hazardous chemical compounds in groups of artificial sweeteners, perfluorocarbons, plastic additives, flame retardants, surfactants, and UV filters. However, conventionally treated effluents of the Eilendorf and the Aachen-Soers WWTPs emitted 10 and 12 chemical compounds, respectively, being prioritized as potentially hazardous to freshwater organisms, mainly fish. The most exceedance of species-specific TU was observed for a zebrafish *Danio rerio* due to its status as a model organism (Briggs, 2002; Lessman, 2011; Segner, 2009). In contrast, three chemical compounds were detected in concentrations potentially toxic or hazardous to the crustacea. However, after the ozonation step, the fungicide trifloxystrobin and the pharmaceutical telmisartan still occurred in concentrations toxic to fish.

As the UFZ-approach did not observe species-specific acute toxicity, results from both approaches can be compared based on chronic data for fish only. Implementation of the UFZ-approach for the concentrations of chemical compounds identified in effluents of the Eilendorf and the Aachen-Soers WWTPs did not classify any compound as potentially hazardous to water organisms. However, the GU-approach recognized effluent concentrations of 2,4-dichlorophenol (industrial chemical) and telmisartan (pharmaceutical) as potentially chronically toxic in fish. Furthermore, phenyl benzimidazole sulfonic acid (UV-filter) was prioritized as potentially hazardous to crustacea.

One possible reason mainly responsible for the different assessment outcomes is the use of different effect values. The established effect values in the UFZ-approach are more reliable due to a uniform calculation approach. As the GU-approach was calculated manually, it was impossible to check every study, which provided the lowest effect data. It is likely that some data sources were outdated and performed not according to good laboratory practice or guidelines. Another point of discussion is the substance type referred to for TU evaluation.

In many cases, it is known that some formulations are more toxic than the active ingredient (Beggel et al., 2010; Schmuck et al., 1994). The UFZ-approach did not include this aspect. Although the present study was more conservative and evaluated many chemical compounds as potentially hazardous, it is essential to treat the effect data uniformly. It is urgently necessary to develop a user-friendly and time-efficient standardized approach for calculating the TUs to make future studies more consistent.

Chemical compounds identified as potentially hazardous in Eilendorf and the Aachen-Soers WWTP effluents completely matched each other. Although the effluent ozonation at the Aachen-Soers WWTP was sufficient to reduce 41% of investigated chemical compounds, the upstream located Eilendorf WWTP still treats incoming wastewater conventionally, meaning potentially positive effect by the entrance of the ozonated effluent the Wurm River can be mimicked.

Role of ubiquitously distributed pollutants on sediment toxicity

The most prominent result of the present study was the identification of the genotoxic potential in sediments throughout the investigative area. However, further studies recorded cases of similar genotoxic potential in sediments of the upper Danube (Higley et al., 2012; Keiter et al., 2006) and the Spittelwasser (Reifferscheid et al., 2011) in Germany, the Taquari River in Brazil (Gameiro et al., 2018), the Yongdinghe watershed (Ma et al., 2003) and several sites within the Three Gorges Reservoir in China (Floehr et al., 2015). Suggesting a potential ubiquitous distribution of particle-bound genotoxic potential, one of the possible genotoxicity drivers should ubiquitously occur and reveal lipophilic properties. Particle-bound PAHs, but also nitroarenes, and aromatic amines are known ubiquitous genotoxic compounds (Brack et al., 2005; Reifferscheid et al., 2011) and perfectly meet these criteria. Indeed, PAHs were the most abundant chemical group detected in the studied sediments. PAHs made up between 94.5% (at the sampling site H2 during the sampling campaign 2018; ≡ 5.9 µg/mg OC) and 99.4% (at the sampling site W1 during the sampling campaign 2018; ≡ 18.7 µg/mg OC) of the total concentration of organic target pollutants detected in sediments. Molecular indices of particle-bound PAHs indicated their pyrogenic origin being typical for urban areas with high traffic rates (Shuliakevich et al., 2022b). This fact is true for Aachen as a city on the border to the Netherlands and Belgium. As the rainwater overflow basin collects rainwater from urban areas, similar PAHs composition could be expected in the rainwater overflow basin as well.

The area around Aachen is not classified as industrialized or impacted by known hazard agents. Nevertheless, the PAH concentration in sediments of the Haarbach and the Wurm Rivers during the sampling campaign in June 2017 under stable hydrological conditions was comparable with those in from two big waterways in Europe, the Danube River (Keiter et al., 2008) and the Rhine River (Gocht et al., 2001) (see Figure 8). After extensive rain events in June 2018, the PAHs concentration in

sediments even rose to 100-times. Consequently, adverse effects on wildlife of the Haarbach and the Wurm Rivers caused by particle-bound PAHs could not be excluded. PAHs have multiple metabolic pathways and can bioaccumulate in the organism. Moreover, PAHs become more potent after the metabolic activation by liver enzymes (Lam et al., 2018; Sievers et al., 2013). Predominantly hydrophobic PAHs can freely pass a cell membrane binding to DNA (IARC, 1983). PAHs are highly affine to receptors of the cytochrome P450 (CYP) enzyme group genes (Ikenaka et al., 2013), which expression the PAHs biotransformation is generating more hydrophilic but also more bioactive PAH-adducts (Barranco et al., 2017). PAHs are well-known embryotoxic compounds causing various organ dysfunction in zebrafish embryos like edemas and cardio-vascular disorders (Cunha et al., 2020; Zhai et al., 2020). Several studies observed after the PAH exposure of fish such adverse effects as disruption of vitamin metabolism and signalling (Berntssen et al., 2015), immunotoxicity (Reddam et al., 2017; Reynaud and Deschaux, 2006), genotoxicity and carcinogenicity (Nacci et al., 2002; Rose et al., 2000; Stegeman and Lech, 1991), teratogenicity and embryotoxicity (Schiwy et al., 2015; Seiler et al., 2014), vitellogenesis disruption (Nicolas, 1999) and reduction of sex hormones concentration in plasma (Kennedy and Smyth, 2015), poor health (Grung et al., 2016), and behavioral alterations (Vignet et al., 2014).

However, further particle-bound pollutants and their mixture toxicity should be taken into account. Brack et al. (2005) investigated mutagenic potential in sediment extracts from a creek in the Neckar River basin (Germany). Thus, beneath priority PAHs, also nonpriority pollutants 11H-indeno[2,1,7-cde]pyrene, a methylbenzo[e]pyrene, and a methylperylene were identified as possible drivers of mutagenicity (Brack et al., 2005). Furthermore, Reifferscheid et al. (2011) identified a number of mutagenic heterocyclic and nitrogen-substituted aromatic compounds in sediments from an area of concern in Elbe river basins (Spittelwasser creek).

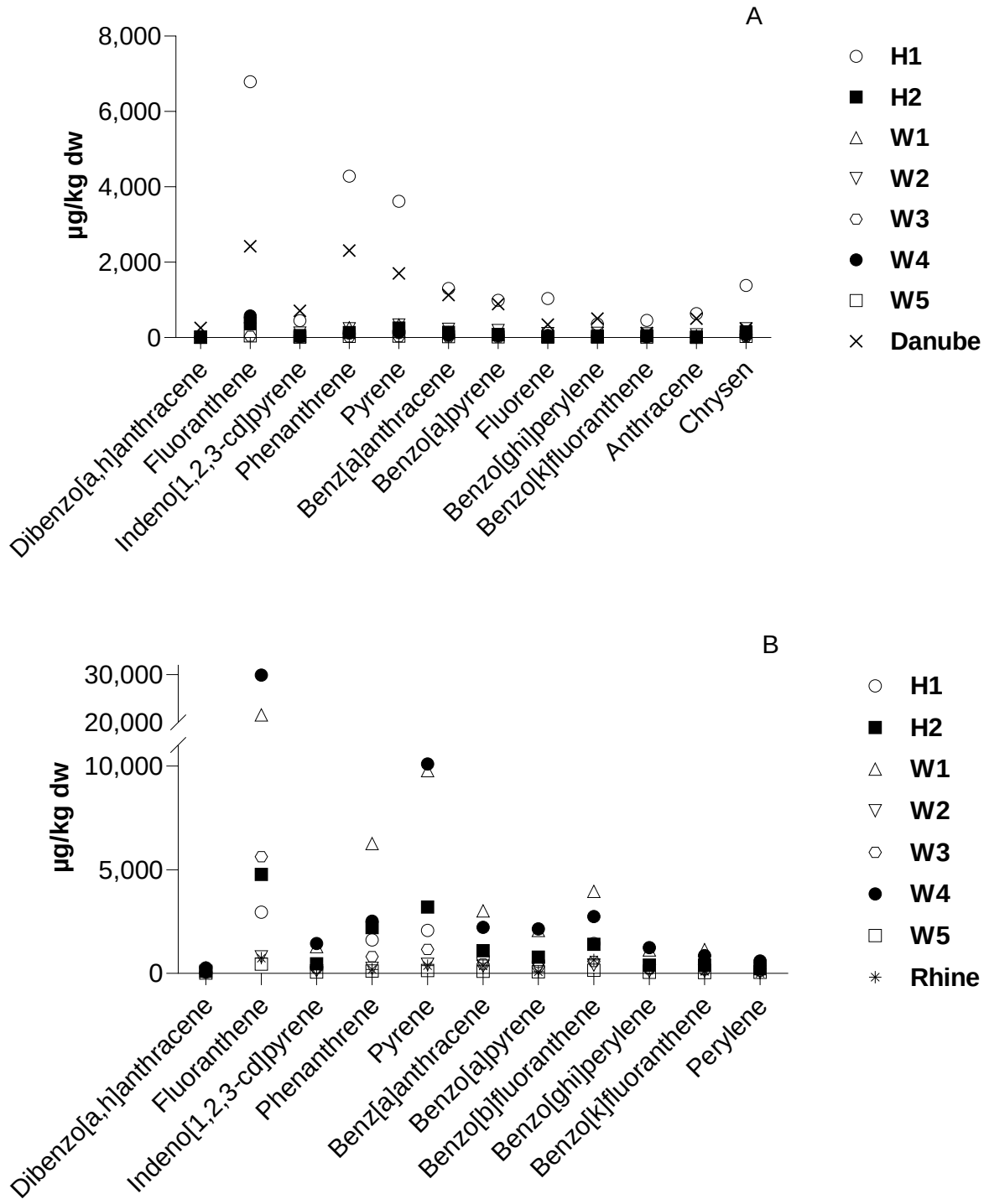


Figure 8: Detected PAH concentrations in sediments of the Haarbach and the Wurm Rivers in June 2017 at dry weather (A) and in June 2018 after prolonged rain events (B) (own data, cf. Shuliakevich et al. (2022a), Annex 1) compared to the PAH concentrations in sediments of the Danube River (A) (Keiter et al., 2008)[1] and the Rhine River (B) (Gocht et al., 2001)[2], respectively. [1]: upper part of the Danuber River; sampling depth: 0-5 cm; average concentration at eight sampling sites; [2]: Hessisches Ried, Germany; sampling depth: 0-12 cm; single measurements.

Indication of possible measurements based on the sediments chemical profiling

Chemical sediment profiling showed particle-bound aromatic amines, carbazoles, indoles, nitroarenes, PAHs, PCBs, polycyclic heteroarenes, and other chemical compounds (stilbenoids, aliphatic halogens, phenanthrolines) (cf. Annex 3). No pyrethroids and flame retardants were found in sediment samples. A remarkable trend was visible when comparing the total chemical burden in sediments at different hydrological conditions: Sediments at every sampling site showed a significant increase of the total chemical concentration excepting the sampling site H1 (upstream of the Eilendorf WWTP; -72% in 2018) (see Figure 9). The Haarbach River sampling sites (H1 and H2) are located at the river stretch with recent remediation activities. Thus, the water flow was adapted to its natural course, meandering with natural floodplain areas. However, at sampling site H1, these activities were finished before the first sampling campaign in 2017. From the personal communication with colleagues from the Eilendorf WWTP, the flooding management at the sampling site H2 was evaluated as insufficient. Thus, the sampling site H2 was restored between two sediment sampling campaigns (2017 and 2018). It could be possible that such a substantial reduction of the total chemical burden occurred due to successful flood prevention activities, resulting in a reduction of the streaming velocity upstream of the sampling site H2. However, prolonged remediation activities at the background of continuous effluent entrance could lead to sediment perturbation at H2, which was mirrored in increased chemical burden (+73% in 2018).

Each sampling site of the Wurm River showed an increased concentration of particle-bound target chemicals (+48% - +96%), with the most substantial increase at the sampling site W4 (+96%), located directly downstream the effluent of the Aachen-Soers WWTP. However, the lowest chemical concentration was found at the sampling site, located 2.5 km downstream of the Aachen-Soers WWTP effluent: 0.4 µg/mg OC in 2017 and 0.7 µg/mg OC in 2018. The more obvious reason is the meandering watercourse, which reduces further transport of particulate matter.

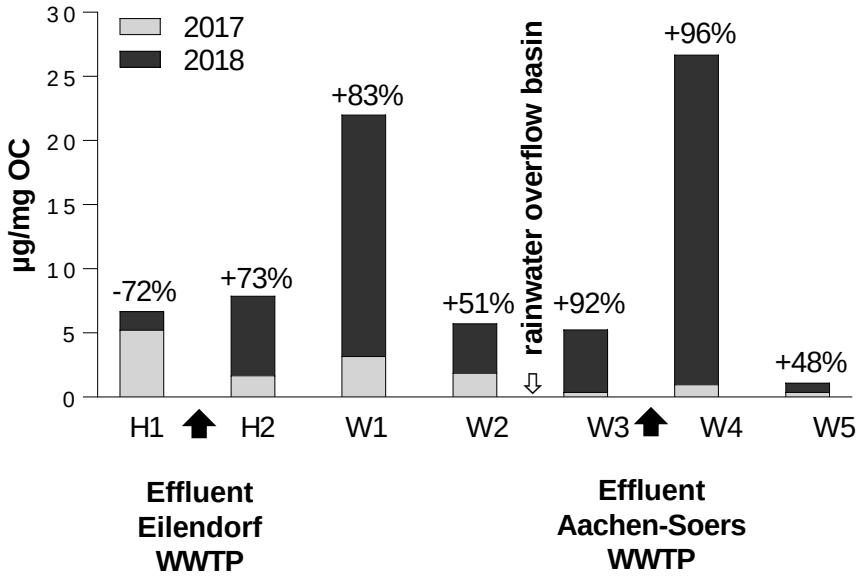


Figure 9: All target chemicals such as aromatic amines, carbazoles, indoles, nitroarenes, PAHs, PCBs, polycyclic heteroarenes, etc. (cf. Shuliakevich et al. (2022a), Annex 1), identified in sediment samples during the sampling campaigns 2017 and 2018.

Recapitulatory, the chemical burden at the sampling sites H1 and W5 indicated positive effects in a natural reduction of the streaming velocity by a meandering watercourse and forming a floodplain environment. Floodplains as flood-prone areas represent the transition between land and freshwater ecosystems providing essential ecosystem services such as nutrient and water retention, carbon sequestration, and unique habitats (Tockner and Stanford, 2002). Behind Sweden, Finland, and France, Germany occupies place 4 in the EU, actively supporting restoration measurements (EEA, 2020). Thus, Germany allocates 7.6% of the total area for such essential sediment retention areas as floodplains (EEA, 2020).

Important to notice that retained sediments still contain particle-bound pollutants. Floodplains prevent further distribution of particle-bound contaminants but do not eliminate them. Götz et al. (2007) described a high concentration of highly toxic polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) in sediments of floodplain areas of the Elbe River near Pevestorf and Heuckenlock (Germany). As a source of pollution, the upstream located region of Bittterfeld-Wolfen could be identified. Despite the high distance from the region of Bittterfeld-Wolfen to Pevestorf and Heuckenlock of 225 and 340 km, respectively, deeper sediment layers recorded

that the region of Bittterfeld-Wolfen had been a significant source of Elbe River pollution since the 1940s (Götz et al., 2007). Furthermore, Schwartz et al. (2006) described the effectiveness of the Spittelwasser floodplain near Jeßnitz (Germany) for pollutant retention close to their primary source.

The formation of sediment retention areas in the Wurm River could provide localized contaminant-concentration reduction and the formation of potentially contaminated floodplains. These would represent a restricted area of increased contamination level where monitoring and restoration activities can be specifically applied.

Complex evaluation of the sediment quality

For quality characterization of sediment samples from the studies sampling sites, the author applied the sediment quality strategy (SQS) according to Ahlf et al. (2002), adjusting it to the current study design by a combination of the chemical target analysis for the substances with established threshold values (PCBs, EPA-PAHs, dichlorodiphenyltrichloroethane (DDT)) and ecotoxicological test battery from the studies in Annexes 1-3. The SQS represents a multifactorial procedure for sediment classification with the desirable goal of $Class_{Chemistry}$ and $Class_{Ecotox}$ II. If at least one class is characterized as III or worse (IV, V) further ecotoxicological and ecological investigations are required to cover all possible adverse effects and, therefore, potentially affected organisms. Furthermore, the SQS helps identify potential "hot spots" enclosing monitoring areas and controlling sources of pollution or remediation results.

Application of the SQS to the present study results required some modifications due to the different scopes of both. The present study was created for initial deep insights into the ecotoxicological potential of sediments. At the same time, the SQS requires for the first assessment application of non-receptor-based bioassays such as algal growth inhibition test, bioluminescence bioassay, and bacterial contact test. Additionally, not all chemical compounds and heavy metals of the SQS were covered within the target analysis of the present study (cf. Annex 3). Consequently, to implement the SQS to the results of the present study, all chemical and ecotoxicological results were combined within the once and only assessment step.

The threshold values for chemical sediment quality classes were updated according to the position paper of the German Federal/State Working Group on Water (*ger*. LAWA)

'Integrated sediment management in river basins' (LAWA, 2019). Based on quantified concentrations of chemicals target compounds, nearly all sediments sampled during dry weather (June 2017) revealed a desirable $Class_{Chemistry}$ for PCBs, PAHs, and DDT of II (see Table 4). At least the sampling site H1 showed PAH concentrations above the desirable $Class_{Chemistry}$ of 4 mg/kg dw. After extended rain events and under fluctuating hydrological conditions (June 2018), the sampling sites H2, W1, W3, and W4 showed PCB- and PAH concentrations higher than the desirable 20 µg/kg dw and 4 mg/kg dw, respectively. The sampling sites H1, W2, and W5 maintained even after prolonged rain events a desirable $Class_{Chemistry}$. The DDT was found at four sampling sites at concentrations out of concern, probably explained by its ban and continuous environmental degradation (Ricking and Schwarzbauer, 2012).

Assessment of the ecotoxicological status was provided based on four endpoints:

1) genotoxicity (micronucleus assay; cf. Annex 1),

2) mutagenicity (Ames fluctuation assay; cf. Annex 1),

3) endocrine-disrupting activity (CALUX® assay; cf. Annex 2),

4) embryotoxicity (FET; cf. Annex 3),

5) behavioral alterations (locomotion assay; cf. Annex 3).

The sampling sites W2-W5 investigated during dry weather in June 2017 revealed $Class_{Ecotox}$ III and above regarding their mutagenic potential and endocrine-disrupting activity. The embryotoxic potential corresponded to $Class_{Ecotox}$ II was identified at the sampling site H1 during both sampling campaigns. However, the sampling sites H2-W5 showed continuously $Class_{Ecotox}$ above II regarding embryotoxic potential. Behavioral alterations ($Class_{Ecotox}$ III) were observed at the sampling sites H1, W2, and W4 during the sampling campaign in June 2017 (dry weather). One year later (sampling campaign in June 2018), the sampling sites H2, W1, W2, and W4 revealed $Class_{Ecotox}$ III regarding their potential to alter the behavior of fish larvae.

Chemical screening for a limited number of chemicals (PCBs, PAHs, and DDT) showed a clear temporal difference between the two sampling campaigns highlighting the sampling sites H2, W1, W3, and W4 as sites with increased contamination levels. Due to pronounced differences between two sampling campaigns: prolonged dry vs.

prolonged rainy weather with the release of the rainwater overflow basin - identified sediment contamination could be a consequence of the sediment perturbation and runoffs from urban and agricultural areas. However, investigation of ecotoxicological effects resulted in much more complex results, highlighting continuous sediment contamination in the Haarbach and the Wurm Rivers. Indeed, the current study applied a highly sensitive bioassay battery to test whole sediment extracts. Back to the introduction of the current thesis, whole sediment extracts represented a mixture of different chemical compounds potentially being responsible for the detected effects. By combining all ecotoxicological effects from two sampling campaigns that covered natural and urban sites, sediments of the Haarbach and the Wurm Rivers were continuously categorized as toxic. Already identified single point sources of pollution such as a rainwater overflow basin and WWTP effluents seem not to play a primary role in the sediment pollution. Transferring this result to the whole catchment area of the Wurm River, similar toxicity in all sediments can be expected.

Outlook for the catchment area of the Wurm River

For the catchment area of the Wurm River, it would be advisable to create sediment retention areas, which can be flooded during extensive rain events preventing sediment transport and perturbation in the downstream located river stretches. Additionally, the rainwater overflow basin volume should be adjusted to the amount of rainwater. Municipal drainage and the consequent release of massive rainwater amounts could be a solution for the urban area. However, it is not an appropriate solution for the extra-urban area. The rainwater amounts need special treatment and a discharge reduction into the recipient waterbody. The possible solution represents retention soil filters or constructed wetlands as artificial systems that use processes occurring in natural wetlands. These processes include a combination of mechanical, physico-chemical processes and activity of bacterial communities as well, and are widely used to treat rain- and wastewater of all kinds (Kadlec and Wallace, 2009). Currently, the area of the Aachen-Soers WWTP will be extended by a constructed wetland close to the rainwater overflow basin.

The State North-Rhine Westphalia financially supports each state WWTP when installing advanced effluent treatment steps (MULNV NRW and LANUV NRW, 2020). A feasibility study for the Eilendorf WWTP showed ozonation as the best option for an

advanced effluent treatment (Brückner et al., 2018b). Furthermore, a significant emissions reduction from the Eilendorf WWTP is suggested in the near future.

Continuous sediment contamination of the Haarbach and the Wurm River uncovered significant diffuse sources of pollution. Within the River Basin Management Plan 2022-2027, the State North-Rhine Westphalia identified agriculture as the primary diffuse source of pollution of aquatic ecosystems (MULNV NRW and LANUV NRW, 2020). Indeed, 50% of the State North-Rhine Westphalia is in agricultural use. Therefore, to achieve the EU WFD goals, the State North-Rhine Westphalia provides different projects and cooperation to reduce emissions and soil erosion from agriculturally used areas (MULNV NRW and LANUV NRW, 2020).

Positive experiences from Germany as a new hope for the Wurm River

The present thesis showed a small but highly anthropogenic impacted waterbody as the Wurm River is polluted by different chemical compounds and less resilient to extensive rain events. Several measurements were suggested or will be already implemented to prevent further deterioration of the chemical and ecotoxicological status of the Wurm River. Furthermore, the present thesis contributed to knowledge and awareness about sediment toxicity. The author believes that using scientific data and already implemented renaturation measurements in other waterbodies in Germany, the Wurm can improve its ecological and chemical status. Further examples show already implemented measurements in other German waterbodies, which can also be transferred to the catchment area of the Wurm River.

Probably the most prominent but also the most drastic case study is the renaturation of the Emsche River and its tributaries (Germany). In the 19th and beginning of the 20th century, the Emscher River was trapped in the fast-growing area between coal mining, steel, and chemical industry transporting wastewater only. However, a large-scale restoration strategy and high financial investments since the 1990s paid off (Gerner et al., 2018). As a result, t Emscher River got a new stream profile with multiple floodplains offering natural flood prevention, habitat diversity, and multiple societal and social benefits (Gerner et al., 2018). Despite a short time after finishing the main restoration activities, the first resident fish species start to reinhabit the Emscher River (Hempel et al., 2020). Such comprehensive measurements as during the restoration of

the Emscher River are not needed and not possible for the Wurm River, less expensive but effective measurements can bring the Wurm River into the near-natural status.

The joint research project Wilde Mulde aims at the implementation of concrete revitalization measures at the Mulde River (Germany) by investigating their effects on the provision of ecosystem functions. Thus, whole trees with root plates were already placed in the Mulde River. Furthermore, river banks were restored, and the Mulde River's sidearm was hydraulically activated (Schulz-Zunkel et al., 2019).

The joint research project Lebendige Luppe recreates a floodplain landscape of the Luppe River in Germany by comprehensive technical and ecological measurements. Since the beginning of the project in 2012, scientists have observed the recovery of amphibians and native flora populations (Riedel et al., 2017).

Since 2015 the Fechenheimer Mainbogen in the east of Frankfurt has been a place of diverse habitats for flora and fauna in an area of approximately 90 ha. The new floodplain landscape has been restored, offering refuges for fish, amphibia, birds, frogs, and alluvial flora (Hinrichs, 2016).

Up to date, the Wurm River serves as an object of scientific investigations (Brückner et al., 2018a; Buchty-Lemke and Lehmkuhl, 2018; Hagemann et al., 2018; Hagemann et al., 2020; Wolf et al., 2021). Bundling of available data in the background of already implemented measurements and combining with knowledge gained from the other restoration projects, as mentioned before, the Wurm River has an excellent opportunity to become a resilient waterbody with acceptable chemical and ecological status.

Table 4: Evaluation of the sediment quality classes according to Ahlf et al. (2002) modified. The desirable goal of the chemical sediment quality is class II. EDA: endocrine-disruptive activity. '-' not identified (below the limit of detection). '/' not performed due to the current study design. For a legend, see Table 5.

	Sampling campaign June 2017 (dry weather)							Sampling campaign June 2018 (after extended rain events)						
	H1	**H2**	**W1**	**W2**	**W3**	**W4**	**W5**	**H1**	**H2**	**W1**	**W2**	**W3**	**W4**	**W5**
PCBs	II	II	II	II	II	II	II	II	IV	III	II	III	III	II
EPA-PAHs	III	II	II	II	II	II	II	II	III	V	II	III	V	II
DDT	II	II	-	-	-	II	-	-	-	-	-	-	-	II
Genotoxicity	/	/	/	II	II	II	II	/	/	/	II	II	II	II
Mutagenicity	/	/	/	III	V	III	III	/	/	/	III	V	V	V
Endocrine-disruptive activity	/	/	/	III	III	III	III	/	/	/	III	IV	IV	IV
Embryotoxicity	II	IV	V	IV	IV	IV	III	II	IV	IV	IV	III	III	III
Behavioral alterations	III	II	II	III	II	III	II	II	III	III	III	II	III	II
$Class_{Chemistry}$	**III**	**II**	**II**	**II**	**II**	**II**	**II**	**II**	**IV**	**V**	**II**	**III**	**V**	**II**
$Class_{Ecotox}$	**III**	**IV**	**V**	**IV**	**V**	**IV**	**III**	**II**	**IV**	**IV**	**IV**	**V**	**V**	**V**

Table 5: Sediment quality class according to Ahlf et al. (2002), modified. The desirable goal of the chemical sediment quality is class II. '/' not performed due to the current study design. NC: negative control. LOQ: limit of quantification. NOEC: no observed effect concentration. 'St. sign.': statistically significant.

	I	II	III	IV	V	VI	Reference
PCBs, µg/kg		<20	<100	<500	>500		LAWA (2019)
EPA-PAHs, mg/kg	<1	<4	<10	<20	<35	>35	Ahlf et al. (2002)
DDT, ng/g	<2	<5	<20	>50			Ahlf et al. (2002)
Genotoxicity (25 mg dw SEQ/mL in test)		no effects	st. sign. higher induction of micronuclei compared to the NC	st. sign. higher induction of micronuclei at one sampling site during different sampling campaigns	st. sign. higher induction of micronuclei among all sampling sites		Ahlf et al. (2002), modified
Mutagenicity (500 mg dw SEQ/mL in test)		no effects	st. sign. higher revertant number compared to the NC	st. sign. higher revertant number at one sampling site during different sampling campaigns	st. sign. higher revertant number among all sampling sites		Ahlf et al. (2002), modified
Endocrine-disruptive activity (25 mg dw SEQ/mL in test)		no effects	effects above the LOQ	st. sign. higher EDA at one sampling site during different sampling campaigns	/		Ahlf et al. (2002), modified
Embryotoxicity (25 mg dw SEQ/mL in test)		no effects	sublethal effects above the NC, no increased mortality	sublethal effects above the NC and/or lethal effects above the NC	100% lethal effects		Ahlf et al. (2002), modified
Behavioral alterations (NOEC from the FET)		no effects	st. sign. higher/lower activity compared to the NC				Ahlf et al. (2002), modified

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ANNEX 1

Title: Assessing the genotoxic potential of freshwater sediments after extensive rain events – Lessons learned from a case study in an effluent-dominated river in Germany

Journal: Water Research

Contributing authors: Aliaksandra Shuliakevich, Melis Muz, Jörg Oehlmann, Laura Nagengast, Katja Schröder, Yvonne Wolf, Ira Brückner, Riccardo Massei, Werner Brack, Henner Hollert, Sabrina Schiwy

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	Melis Muz	10%
	Werner Brack	10%
	Henner Hollert	15%
	Sabrina Schiwy	25%
Conducting tests and experiments	Aliaksandra Shuliakevich (Ames fluctuation assay with sediment extracts from the sampling campaigns SCarid and SCrain, Micronucleus assay with sediment extracts from the sampling campaigns SCarid)	75%
	Melis Muz (chemical analysis)	10%
	Laura Nagengast (Micronucleus assay with sediment extracts from the sampling campaigns SCrain)	15%
	Katja Schröder (Ames fluctuation assay with sediment extracts from the sampling campaigns SCarid)	5%
	Yvonne Wolf (Ames fluctuation assay with sediment extracts from the sampling campaign SCarid)	5%
Compilation of data sets and figures	Aliaksandra Shuliakevich (compilation of data sets and figures of all bioassays and supplementary information)	55%
	Melis Muz (compilation of data sets of the chemical analysis)	5%
	Laura Nagengast (supportive compilation of data sets from the Micronucleus assay with sediment extracts from the sampling campaigns SCrain)	5%
	Katja Schröder (supportive compilation of data sets from the Ames fluctuation assay)	5%
	Yvonne Wolf (supportive compilation of data sets from the Ames fluctuation assay with sediment extracts from the sampling campaign SCarid)	5%
	Sabrina Schiwy (compilation of data sets and figures of all bioassays, chemical analysis, and supplementary information)	15%
Analysis and interpretation of data	Aliaksandra Shuliakevich (analysis and interpretation of all data generated in the Ames fluctuation assay and the Micronucleus assay, supportive analysis and interpretation of data generated during chemical analysis)	50%
	Melis Muz (analysis and interpretation of data generated during chemical analysis)	5%
	Laura Nagengast (supportive analysis and interpretation of data generated in the Micronucleus assay)	5%
	Yvonne Wolf (supportive analysis and interpretation of data generated in the Ames fluctuation assay)	5%
	Henner Hollert (interpretation of data)	15%
	Sabrina Schiwy (interpretation of data)	20%

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Drafting of manuscript	Aliaksandra Shuliakevich	55%
	Melis Muz	2.5%
	Jörg Oehlmann	2.5%
	Laura Nagengast	2.5%
	Katja Schröder	2.5%
	Yvonne Wolf	2.5%
	Ira Brückner	2.5%
	Riccardo Massei	2.5%
	Werner Brack	5%
	Henner Hollert	10%
	Sabrina Schiwy	12.5%

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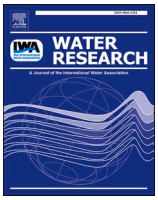

Assessing the genotoxic potential of freshwater sediments after extensive rain events – Lessons learned from a case study in an effluent-dominated river in Germany

Aliaksandra Shuliakevich [a], Melis Muz [b], Jörg Oehlmann [c], Laura Nagengast [d], Katja Schröder [d], Yvonne Wolf [d], Ira Brückner [e], Riccardo Massei [f], Werner Brack [a,b], Henner Hollert [a,*], Sabrina Schiwy [a]

[a] Department Evolutionary Ecology and Environmental Toxicology, Goethe University Frankfurt/Main, Max-von-Laue-Strasse 13, 60438 Frankfurt/Main, Germany
[b] Department of Effect-Directed Analysis, Helmholtz Centre for Environmental Research – UFZ, Permoserstrasse 15, 04318 Leipzig, Germany
[c] Department Aquatic Ecotoxicology, Goethe University Frankfurt/Main, Max-von-Laue-Strasse 13, 60438 Frankfurt/Main, Germany
[d] RWTH Aachen University, Institute of Biology V, Worringerweg 1, 52074 Aachen, Germany
[e] Eifel-Rur Waterboard (WVER), Eisenbahnstr. 5, 52354 Düren, Germany
[f] Department of Bioanalytical Ecotoxicology, Helmholtz Centre for Environmental Research – UFZ, Permoserstrasse 15, 04318 Leipzig, Germany

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ABSTRACT

Wastewater treatment plant effluents and releases from rainwater overflow basins can contribute to the input of genotoxic micropollutants in aquatic ecosystems. Predominantly lipophilic genotoxic compounds tend to sorb to particulate matter, making sediment a source and a sink of pollution. Therefore, the present study aims to investigate the genotoxic potential of freshwater sediments (i) during the dry period and (ii) after extensive rain events by collecting sediment samples in one small anthropogenically impacted river in Germany up- and downstream of the local wastewater treatment plant. The Micronucleus and Ames fluctuation assays with *Salmonella typhimurium* strains TA98, TA100, YG1041, and YG1042 were used to assess the genotoxic potential of organic sediment extracts. For evaluation of possible genotoxicity drivers, target analysis for 168 chemical compounds was performed.

No clastogenic effects were observed, while the genotoxic potential was observed at all sampling sites primarily driven by polycyclic aromatic hydrocarbons, nitroarenes, aromatic amines, and polycyclic heteroarenes. Freshwater sediments' genotoxic potential increased after extensive rain events due to sediment perturbation and the rainwater overflow basin release. In the present study, the rainwater overflow basin was a significant source for particle-bound pollutants from untreated wastewater, suggesting its role as a possible source of genotoxic potential. The present study showed high sensitivity and applicability of the bacterial *Salmonella typhimurium* strains YG1041 and YG1042 to organic sediment extracts to assess the different classes of genotoxic compounds. A combination of effect-based methods and a chemical analysis was shown as a suitable tool for a genotoxic assessment of freshwater sediments.

1. Introduction

The European Water Framework Directive (EU WFD) obligates the Member States to achieve a good chemical status in the European water bodies by 2027 (European Parliament and Council, 2000). However, exceeding the environmental quality standards for the priority water pollutants in the water phase is one reason for the insufficient chemical status of more than half of European water bodies (European Commission, 2019). Furthermore, with the development of the integrated approach for assessing sediment toxicity (Chapman, 1989; Chapman and Hollert, 2006; Hollert et al., 2002), and the formation of the European Demand-Driven Sediment Research Network "SedNet" in 2001, the problem of contaminated sediments in Europe received increased attention. However, obligatory sediment monitoring entered the EU WFD not before 2008 and is limited by the analytical testing for at least 21 priority substances (European Parliament and Council, 2008).

Genotoxic chemicals are of primary concern for aquatic ecosystems

* Corresponding author.
E-mail address: Hollert@bio.uni-frankfurt.de (H. Hollert).

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Abbreviations

APCI	Atmospheric pressure chemical ionization
CCRIS	Chemical Carcinogenesis Research Information System
DDT	Dichlordiphenyltrichloroethane
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dw	Dry weight
EU WFD	European Water Framework Directive
GC	Gas chromatography
HRMS	High-resolution mass spectrometry
LC	Liquid chromatography
MNC	Micronuclei
mRNA	Messenger ribonucleic acid
OC	Organic carbon
PAH	Polycyclic aromatic hydrocarbon
PCB	Polychlorinated biphenyl
SEQ	Sediment-equivalent
TDU	Thermal desorption unit
WWTP	Wastewater treatment plant

as there is no environmental safety level hiding a risk at even low concentrations (Belpomme et al., 2007; EFSA, 2008). Multiple studies showed adsorption of genotoxic compounds to particulate matter (Boettcher et al., 2010; García-Nieto et al., 2019; Heinrich et al., 2017; Hudjetz et al., 2014; Ouanes-Ben Othmen et al., 2019; Seitz et al., 2008). In addition, particle-bound pollutants can be ingested or absorbed through the gill epithelium of fish (Barceló and Petrovic, 2007), leading to a decline of whole fish populations (Bickham et al., 2000; Corredor-Santamaría et al., 2016; Keiter et al., 2006a; Theodorakis, 2001).

Due to the broad spectrum of genotoxic compounds, the possible entry pathways into the environment are diverse. Some genotoxic compounds such as PAHs and carbazoles result from incomplete combustion of organic material and material alteration processes entering the aquatic environment by atmospheric deposition and runoffs (Callén et al., 2013; Murkovic, 2004). Potentially genotoxic pesticides diclofop, lindane (Mesi and Kopliku, 2013), diuron, azoxystrobin (Bony et al., 2008), irgarol, and metolachlor (Mai et al., 2012) can enter the aquatic environment via runoff from agricultural fields and traffic areas (applied for surfaces and building protection), or via wastewater (Bolognesi, 2003). However, most genotoxic chemicals are industrially manufactured and released into the environment with wastewater treatment plant (WWTP) effluents (Filipic and Toman, 1996).

Severe ecosystem interventions and floods (Crawford et al., 2022; Hollert et al., 2000; Mueller et al., 2019) as a consequence of extensive rain events (Vincze et al., 2014) may lead to the release of particle-bound genotoxic contaminants (Brinkmann et al., 2010). Additionally, mixed sewer systems collecting municipal waste- and rainwater (Allinson et al., 2012; Jolibois and Guerbet, 2005) and following discharges from rainwater overflow basins of WWTPs due to insufficient capacities (Müller et al., 2020; Nickel and Fuchs, 2019) are important pathways for the discharge of particle-bound genotoxic chemicals.

The studied sector of the Wurm River receives the effluent and rainwater basin overflows of one of the biggest WWTPs in Germany, the Aachen-Soers WWTP. The WWTP effluent makes up about 90% of the total water flow of the Wurm River during dry weather. At the same time, the latter regularly receives the content of the rainwater overflow basin after extensive rain events. The study by Könemann et al. (2019) showed the accumulation of wastewater-born chemicals in benthic living organisms like *Gammarus pulex* collected in the Wurm River downstream of the rainwater overflow basin and at the effluent site of the Aachen-Soers WWTP.

The present study focuses on assessing the genotoxic potential in freshwater sediments from the Wurm River. To investigate different factors impacting the sediments' genotoxic potential, sampling campaigns were conducted at different weather - dry weather and after extensive rain events combined with the rainwater overflow basin release. The assessment strategy included a combination of effect-based methods (EBMs) and a chemical target screening. This approach is a well-established strategy (Brack et al., 2009, 2019) for evaluation of the genotoxic risk of complex environmental chemicals mixtures (Altenburger et al., 2010) in different matrices such as wastewater (Altenburger et al., 2015; Di Paolo et al., 2016) and sediment (Hollert et al., 2009a, 2009b). The choice of the genotoxic EBMs test battery was driven by the research findings of the last decades. Reifferscheid and Grummt (2000) developed a graduated test battery comprising one bacterial test (Ames fluctuation test or *umu* test) and one eukaryotic test (comet assay). Kirkland et al. (2011) modified this test battery using the Ames fluctuation assay with different bacterial strains, sensitive to different classes of compounds, and the Micronucleus assay as an eukaryotic assay with mammalian cells.

While *in vitro* bioassays described an overall sediment genotoxic potential, chemical target analysis allowed the authors to prioritize its possible drivers in the Wurm River and answer the following questions: (i) How strong do extensive rain events impact sediment genotoxicity? (ii) Can the observed genotoxic potential in sediments be explained by the target chemical analysis? (iii) Are additional *Salmonella typhimurium* strains suitable for the sediment genotoxicity testing?

2. Material and methods

2.1. Materials and chemicals

Solvents: Acetone (≥ 99.9%, UV/IR grade for analysis), methanol (Reag. Ph. Eur. for analysis, ACS, ISO), dichloromethane (Chromasolv®, for HPLC, ≥ 99.8%), dimethylsulfoxide (99.5%, for the synthesis, DMSO), ethyl acetate (hyper grade for LC-MS LiChrosolv®). All solvents were purchased from AppliChem GmbH.

Materials for the sediment extraction: Fat-free extraction quartz sand (0.3–0.9 mm), bottom cellulose filters (12.7 mm), top cellulose filters (20 mm) – were purchased from BÜCHI Labortechnik AG.

Chemicals for the Micronucleus assay: Cyclophosphamide hydrochloride (CAS Reg. No.: 50–18–2), ethyl-methane sulfonate (CAS Reg. No.: 62–50–0). Chemicals for the Ames fluctuation assay: 2-Aminoanthracene (CAS Reg. No.: 613–13–8), nitrofurantoin (CAS Reg. No.: 67–20–9), 4-nitro-o-phenylenediamine (CAS Reg. No.: 99–56–9), 2-nitrofluoren (CAS Reg. No.: 607–57–8). All chemicals were purchased from Merck KGaA.

2.2. Case study area - Wurm river

The studied stretch of the Wurm River is presented in Fig. 1. The present study was a part of the $DemO_3^{AC}$ project, investigating multiple sites of the Wurm River. The four most relevant sampling sites were evaluated for the current case study. The first sampling site was W2 located close to the urban area of Aachen. The following sampling site W3 was located downstream of the rainwater overflow basin of the Aachen-Soers WWTP, representing the possible impact of untreated wastewater and rainwater discharges. The sampling site W4 was directly at the effluent of the Aachen-Soers WWTP. The last sampling site, W5, was located 2.5 km downstream of the effluent at near-natural conditions without known significant sources of pollution.

The first sampling campaign took place in June 2017 and was characterized by a long dry period and stable hydrological conditions (SC_{arid}). During dry weather, the average precipitation rate in Aachen within the last 21 d before the sampling campaign accounted for 1 L/m^2 (WetterKontor GmbH, 2017). The Aachen-Soers WWTP used conventional wastewater treatment by mechanical, biological, and chemical

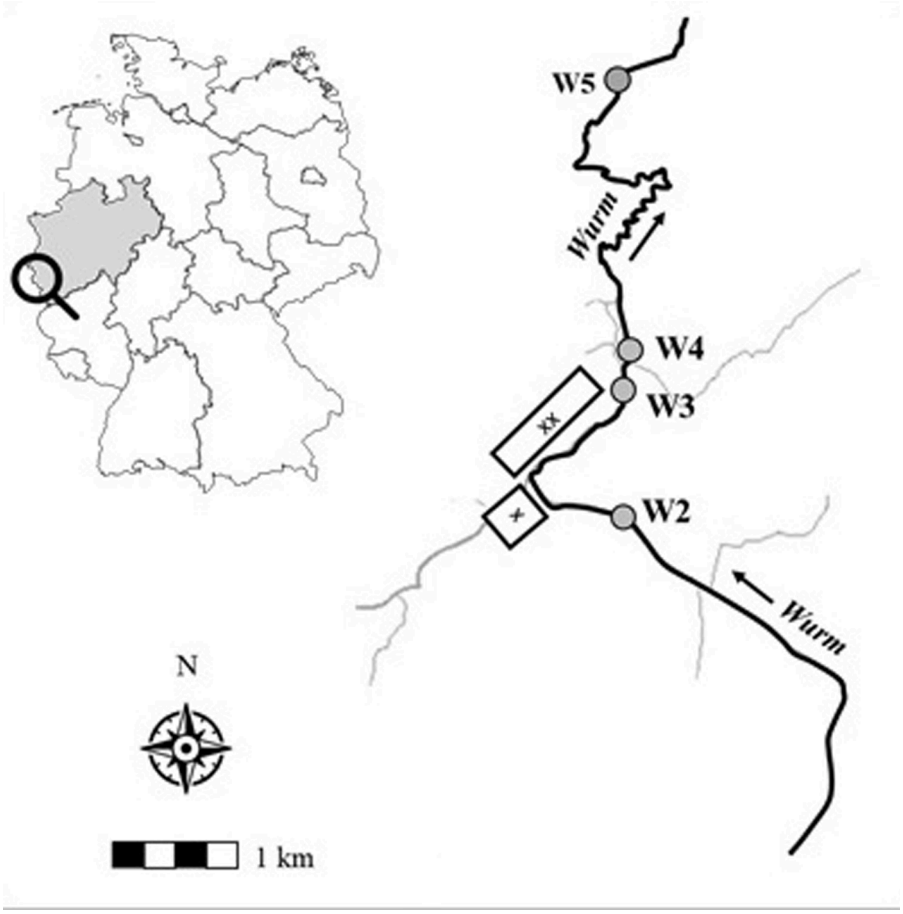


Fig. 1. Studied area of the Wurm River. x: Rainwater overflow basin of the Aachen-Soers WWTP (xx). Source: ELWAS-WEB v. 4.0.0, www.elwasweb.nrw.de, modified by the authors.

steps with the subsequent sand filter. During dry weather, the effluent water portion in the Wurm River accounts for 90% (ELWAS-WEB, 2021). The second sampling campaign was performed in June 2018, after extensive rain events (SC_{rain}). The average precipitation rate in Aachen within the last 21 d before the sampling campaign accounted for 5 L/m^2 (WetterKontor GmbH, 2018). Six months before the SC_{rain}, the wastewater treatment of the Aachen-Soers WWTP was upgraded by full-stream ozonation.

2.3. Sampling and sample preparation procedure

Sediment samples were collected from the first 10 cm in the cross and longitudinal profiles and united to one grab sample. For the subsequent biotesting, 25 g of freeze-dried, sieved (2 mm) and homogenized sediment samples were extracted using the solid phase speed extractor (SpeedExtractor E-914/E-916, BUCHI Corporation) with methanol and dichloromethane (first cycle: 100:0, v/v, 100 °C, max. pressure 100 bar; second cycle: 0:100, v/v, 100 °C, max. Pressure 100 bar) according to previous extraction methods (Giorgio et al., 2011; Grung et al., 2011; Lübcke-von Varel et al., 2012). Quartz sand was handled in the same way as sediment samples (sample preparation procedure, biotesting, chemical analysis) and served as process control. Finally, the organic extracts were evaporated (Multivapor^{TM} P-6, BUCHI Corporation). The solvent was exchanged to DMSO to achieve a final sample concentration of 25 g dry weight sediment-equivalent (dw SEQ)/mL DMSO.

2.4. Micronucleus assay

The Micronucleus assay is a cytogenetic test for detecting chemicals with clastogenic or aneugenic potential. Micronuclei are fragments of chromosomes that are not correctly transferred to the cell pole during the anaphase. The Micronucleus assay was performed with the V79 cell line (Chinese hamster lung cells) with and without metabolic activation (+/-S9) according to the ISO guideline 21,427–2 (ISO, 2004) with some modifications (see Supplementary information, SI, S1) and as detailed in Reifferscheid et al. (2008). Organic sediment extracts were tested in three independent replicates (differing condition days) in the concentration of 25 g dw SEQ/mL with a total DMSO concentration of 0.1% in the test. For each application day, quality controls were carried out by preparing one plate with DMSO as a negative control and one plate for the respective positive control substance.

The validity criteria of statistically significant differences between the negative and the positive control within single testing approaches were approved using the *t*-test ($p<0.05$). For the data evaluation procedure, a percentage rate of micronuclei per 1000 cells was calculated. Statistically significant differences were evaluated using two-way ANOVA with a post-hoc Tukey test ($p<0.05$). All statistical testing procedures were performed with the GraphPad Prism software (v. 9 for Windows; GraphPad Software, Inc.).

2.5. Ames fluctuation assay

The bacterial Ames fluctuation assay was performed according to the ISO guideline 11,350 (International organization for Standardisation, 2012) and as detailed in Reifferscheid et al. (2012). *Salmonella typhimurium* strains are modified by a point mutation in the histidine gene. Repeated mutations in the histidine gene can cause reversion to the wild-type characterized by the own production of the growth-essential

histidine.

Salmonella typhimurium strains TA98 and TA100 were used (see SI, S2). Due to the limitation of the enzymes found in a typical S9-mix in non-detection of such ubiquitously distributed promutagens as nitroarenes (IARC working group on the evaluation of carcinogenic risks to humans, 2014) and aromatic amines (IARC working group on the evaluation of carcinogenic risks to humans, 2012), this gap was closed by adding additional bacterial strains YG1041 and YG1042. The latter have elevated levels of *O*-acetyltransferase and nitroreductase, making YG1041 and YG1042 more sensitive when these compound classes are present (see SI, S2).

Sediment extracts were tested in three independent replicates (differing condition days). For each application day, quality controls were carried out by preparing one plate with DMSO as a negative control and one plate for the respective positive control substance in six dilution steps. The resulting revertant counts at each dilution step (1:2-step, 48 revertants) were assessed with reference to the revertant count at the negative control plate as (i) negative, in case of no concentration-dependent increase; (ii) reproducible, in case of a visible concentration-dependent increase, and (iii) statistically significant, in case of differences to the negative control evaluated by a statistical test (International Organisation for Standardisation, 2012). Revertant numbers from three independent replicates within the range of six concentrations were tested against the revertant numbers in the negative control plate using two-way ANOVA with a post-hoc Tukey test ($p<0.05$). Based on achieved revertant numbers, induction factors were calculated. The induction factor represents a proportion between the revertant count in the respective concentration and the revertant count within the inner plate negative control. An induction factor approach uniforms data, making multiple comparisons possible. For this purpose, Seitz et al. (2008) developed an approach based on calculating concentration-dependent induction factors. A concentration-dependent induction factor value represents the relationship between the induction factor and the respective concentration. Therefore, concentration-dependent induction factor values are independent of the tested concentrations and can be used for environmental samples' mutagenicity ranking (Seitz et al., 2008). All statistical procedures were carried out with GraphPad Prism software (v. 9 for Windows; GraphPad Software, Inc.).

2.6. Target chemical analysis

For the chemical analysis, freeze-dried, sieved (2 mm), and homogenized sediments in the amount referred to 100 mg organic carbon (see SI, S3) rather than on dry weight (Massei et al., 2018) was extracted using the procedure described in chapter 2.3. Before the analytical procedure, the gained extracts were cleaned up using flash chromatography (see SI, S4). Organic sediment extracts for the analysis utilizing Gas Chromatography High-Resolution Mass Spectrometry (GC—HRMS) were adjusted to 500 μL ethyl-acetate and spiked with 1 μg/mL internal standard mix in ethyl acetate. Organic sediment extracts for the analysis utilizing Liquid Chromatography (LC) were adjusted to 500 μL methanol and spiked with 1 μg/mL internal standard mix in methanol.

Chemical analysis of PAHs, PCBs, pyrethroids, brominated flame retardants, polycyclic heteroarenes was performed with the GC—HRMS (QExactive, Thermo Fisher Scientific, Germany) in Full Scan mode (res. 60,000). Method matched calibration standards were run at 13 levels (0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, 100, 200, 500, 1000 ng/mL). Data were evaluated using the software TraceFinder 5.1. The detection limits were taken as the lowest calibration point where these criteria were fulfilled. For more details, see SI, S5. Chemical analysis of the substituted PAHs, quinones, azaarenes, and nitro-compounds was performed with the LC-QExactive Plus with an atmospheric pressure chemical ionization (APCI) source. Separate runs were conducted in positive and negative ion modes. Data evaluation was done with TraceFinder version 5.1. Compounds were confirmed by exact mass, isotopic pattern, retention time, and at least one fragment. For more details, see SI, S6. The chemical analysis of aromatic amines and carbolines was performed with the LC-QExactive Plus with a heated electrospray ionization source in positive mode. Method matched calibration standards were run at 13 levels (0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, 100, 200, 500, 1000 ng/mL). Data evaluation was done with TraceFinder version 5.1. Compounds were confirmed by exact mass, isotopic pattern, retention time, and at least one fragment. Details of the method can be found in Muz et al. (2017) and in the SI, S7. The list of analyzed chemical compounds with respective internal standards can be found in SI, S8.

2.7. Evaluation of genotoxic compounds

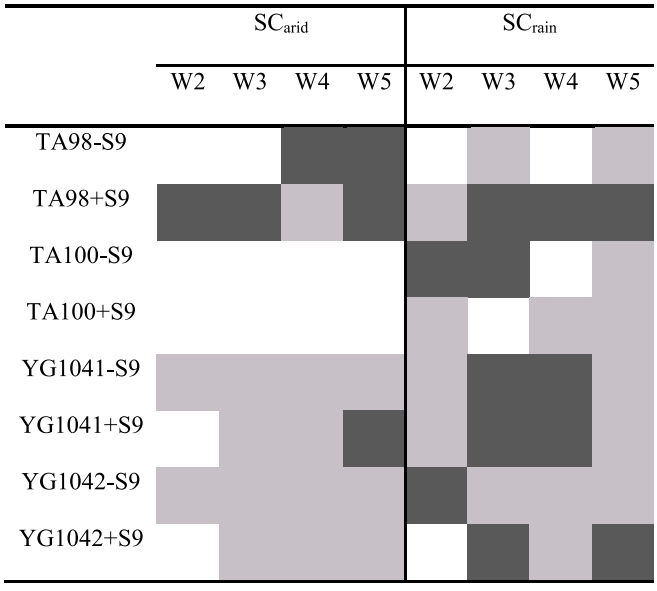
For linking detected chemicals to observed genotoxic effects, endpoint-specific classification of target substances was performed. For this purpose, the following databases and QSAR software were used: (1) PubChem with integrated data search algorithm within the Chemical Carcinogenesis Research Information System (CCRIS) and the Genetic Toxicology Data Bank (GENE TOX) for mutagenicity, carcinogenicity, and reproductive toxicity; (2) Tox tree (v. 3.1.0, Ideacoinsult Ltd.) prediction software for prediction of active binding DNA sites; (3) VEGA *in silico* platform (v. 1.1.5) using predicted and experimental data for the prediction of mutagenicity and micronuclei induction activity. For more details, see SI, S9.

3. Results

3.1. Assessment of the genotoxic potential using effect-based methods

Organic sediment extracts revealed no statistically significant increase (two-way ANOVA, $p>0.05$) in micronuclei (MNC) in comparison to the negative control (see SI, S10). The Ames fluctuation assay results with *Salmonella typhimurium* tester strains TA98 (+/-S9) indicated particle-bound frameshift mutagens in all sediment samples collected during dry weather (SC_{arid}). However, the sediment samples collected

Table 1
Mutagenic potential of the sediment samples in the Ames fluctuation assay with *Salmonella typhimurium* strains (+/-S9) based on a visible reproducible increase in revertant counts without statistical significance (gray) and with statistical significance (dark gray shadow, two-way ANOVA with post-hoc Tukey test, $p<0.05$) at single dilution steps referred to the revertant number in the negative control. White shadow boxes describe results without a visible reproducible increase in revertant counts compared to the negative control. All samples were tested in six dilution steps and three independent replicates ($n = 3$).



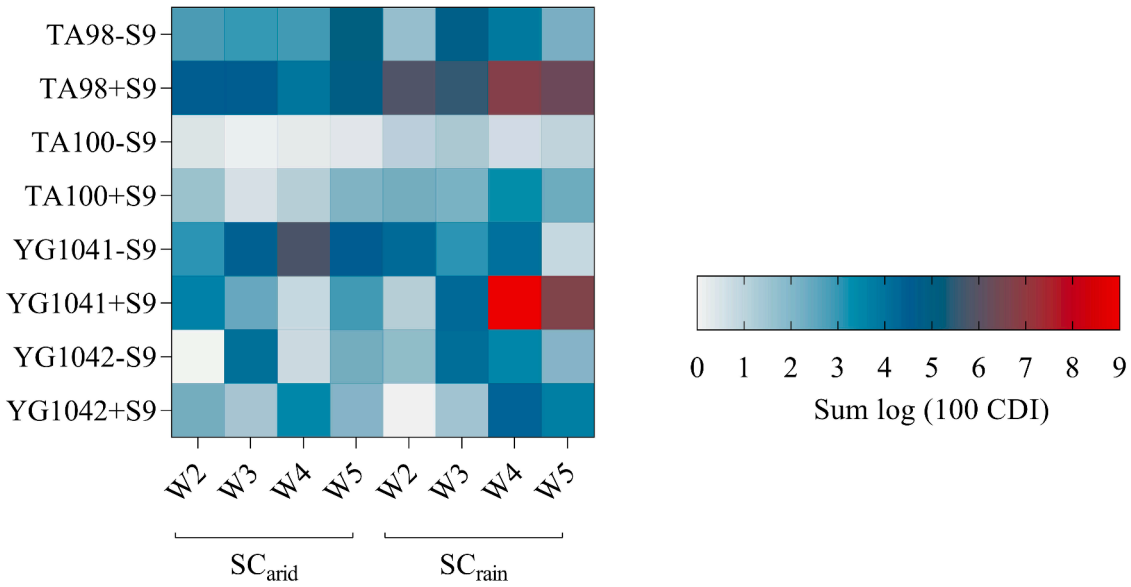


Fig. 2. Average concentration-dependent induction factor values from the Ames fluctuation assay with *Salmonella typhimurium* strains TA98, TA100, YG1041, and YG1042 (+/- S9). The results are represented as the logarithmic sum of concentration-dependent induction factors (CDI) multiplicated by 100 from three independent replicates ($n = 3$).

after extensive rain events (SC_{rain}) showed a strong increase of the particle-bound mutagenic potential caused by both frameshift and base pair mutagens at all sampling sites (see Table 1). In addition, exposure of YG1041 and YG 1042 (+/-S9) to organic sediment extracts indicated nitroarenes and aromatic amines at all sampling sites during all sampling campaigns.

Analysis of the revertant count using a concentration-dependent induction factors method showed mutagenic potential in sediment

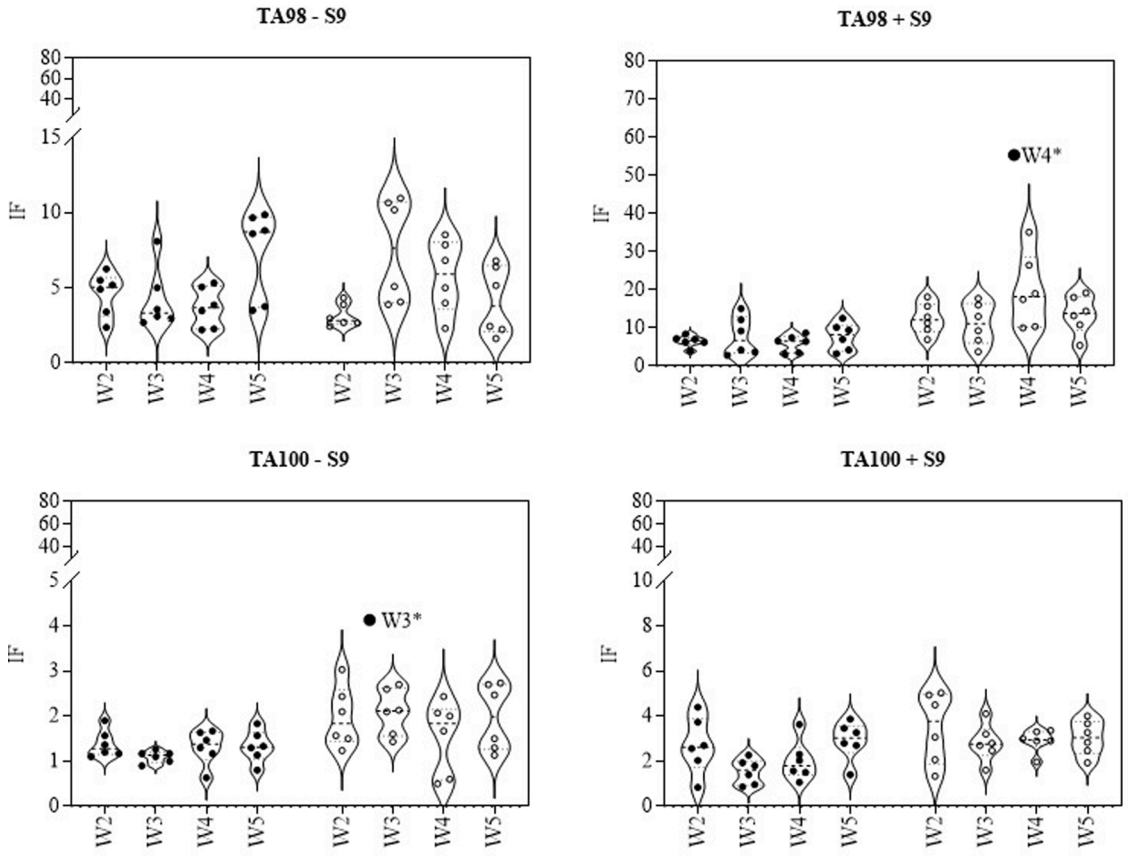


Fig. 3. Distribution of induction factors (IF; single dots) evaluated by exposure of TA98 and TA100 (+/-S9) to organic sediment extracts from the Wurm River at six dilution steps (stock concentration: 25 mg dw SEQ/mL, 1:2 steps). Black dots: Induction factors of sediment extracts taken during dry weather, SC_{arid}. White dots: Induction factors of sediment extracts taken after prolonged rain events, SC_{rain}. The sampling site signed with Wn* is statistically significant different (two-way ANOVA with post-hoc Tukey test, $p<0.05$) to the sample Wn collected during the SC_{arid} (black) or SC_{rain} (white).

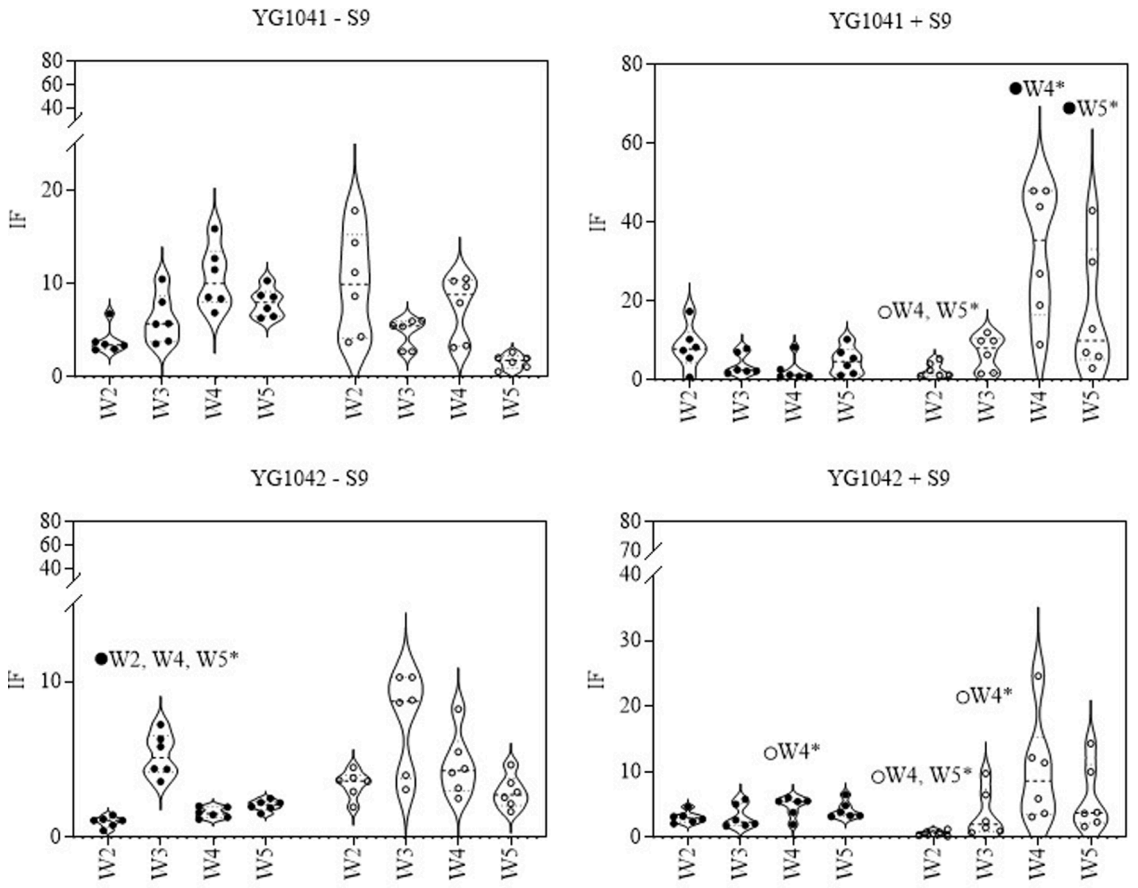


Fig. 4. Distribution of induction factors (IF; single dots) evaluated by exposure of YG1041 and YG1042 (+/-S9) to organic sediment extracts from the Wurm River at six dilution steps (stock concentration: 25 mg dw SEQ/mL, 1:2 steps). Black dots: Induction factors of sediment extracts taken during dry weather, SC_{arid}. White dots: Induction factors of sediment extracts taken after prolonged rain events, SC_{rain}. The sampling site signed with Wn* is statistically significant different (two-way ANOVA with post-hoc Tukey test, $p<0.05$) to the sample Wn collected during the SC_{arid} (black) or SC_{rain} (white).

Table 2
Identified chemical compounds in sediments of the Wurm River at different weather conditions. Other compounds: 1-Phenylnaphthalin, p-benzyldiphenyl, 3,3′-dichlorobenzidine, methyltolysulfone.

	SC_{arid} / SC_{rain} [ng/mg OC],% difference			
	W2	W3	W4	W5
Aromatic amines	1.0 / 0.9 (↓ 9.7%)	0.2 / 1.0 (↑ 75.5%)	0.2 / 1.2 (↑ 81.1%)	0.3 / 0.3
Carbazoles	6.1 / 6.7 (↑ 8.1%)	1.2 / 4.8 (↑ 74.0%)	3.1 / 6.5 (↑ 52.9%)	2.0 / 3.7 (↑ 47.4%)
Nitroarenes	2.5 / 1.5 (↓ 42.9%)	2.6 / 2.3 (↓ 12.9%)	3.2 / 24.3 (↑ 86.6%)	1.0 / 1.3 (↑ 22.9%)
PAHs	1840 / 3694 (↑ 50.2%)	378 / 4812 (↑ 92.1%)	968 / 25,526 (↑ 96.2%)	369 / 710 (↑ 48.1%)
PCBs	4.3 / 134 (↑ 96.8%)	0.3 / 7.4 (↑ 96.3%)	4.7 / 52.5 (↑ 91%)	1.1 / 3.2 (↑ 65.9%)
Polycyclic heteroarenes	16.5 / 19.5 (↑ 15.1%)	3.7 / 24.2 (↑ 84.8%)	7.6 / 77.6 (↑ 90.2%)	4.8 / 9.8 (↑ 51.1%)
Other compounds	1.6 / 0.9 (↓ 44.2%)	0.1 / 3.2 (↑ 96.1%)	0.7 / 6.9 (↑ 90.4%)	0.4 / 1.4 (↑ 74.8%)
Total (sum compounds)	**1873 / 3857 (↑ 51.4%)**	**386 / 4855 (↑ 92%)**	**987 / 25,695 (↑ 96.2%)**	**378 / 730 (↑ 48.2%)**

samples primarily outgoing from frameshift mutagens (see Fig. 2). A stronger expression was observed after the metabolic activation. Mutagens causing base pair substitutions were less represented in the sediment.

In *Salmonella typhimurium* strains TA98 (+S9) and TA100 (-S9), significantly higher (two-way ANOVA with post-hoc Tukey test, $p<0.05$) revertant induction was detected at the sampling site W4 (at the effluent) and W3 (downstream of the rainwater overflow basin), respectively, during the SC_{rain} (see Fig. 3). In contrast, the sampling site W2, located upstream of the Aachen-Soers WWTP, showed significantly lower induction factors in the *Salmonella typhimurium* strain YG1041 (+S9) than at W4 and W5 (see Fig. 4). The *Salmonella typhimurium* strain YG1042 (-S9) showed significantly higher revertant numbers at the sampling site W3 than at the other sampling sites during the dry weather. Furthermore, the metabolic activation by the S9-mix led to a significantly higher revertant number and induction factors at W4 during SC_{rain} than during SC_{arid}. The sampling sites downstream of the Aachen-Soers WWTP (W4 and W5) during SC_{rain} revealed significantly higher revertant numbers than at the sampling site W2 upstream of the Aachen-Soers WWTP.

3.2. Chemical target screening

168 target substances were analyzed in organic sediment extracts, and out of these 94 target compounds could be quantified (see SI, S9), including aromatic amines, carbazoles, nitroarenes, PAHs, PCBs,

Table 3
Chemical burden of genotoxic compounds in the Wurm River at different weather conditions. Other compounds: 1,1-Dichloro-2,2-bis(4-methoxyphenyl)ethan, azobenzene, 2-phenylindole, 1,10-phenanthroline-5,6-dion, xanthone.

	SC_{arid} / SC_{rain} [ng/mg OC],% difference			
	W2	W3	W4	W5
Aromatic amines	0.4 / 0.8 (↑ 42.9%)	0.2 / 0.9 (↑ 76.2%)	0.2 / 1.1 (↑ 84.2%)	0.2 / 0.2
Carbazoles	0.3 / 0.1 (↑ 50.1%)	0.2 / 0.6 (↑ 67.5%)	0.3 / 1.1 (↑ 69.9%)	0.5 / 1.7 (↑ 72%)
Nitroarenes	0.6 / 0.1 (↓ 90.9%)	2.0 / 0.1 (↓ 95.8%)	1.9 / 0.1 (↓ 92.4%)	0.3 / 0.1 (↓ 83.3%)
PAHs	1751 / 3599 (↑ 51.4%)	363 / 4630 (↑ 92.2%)	923 / 25,120 (↑ 96.3%)	352 / 673 (↑ 47.7%)
Polycyclic heteroarenes	12.7 / 12.6 (↓ 0.7%)	2.9 / 16.2 (↑ 82.3%)	5.5 / 41.1 (↑ 86.6%)	3.2 / 6.2 (↑ 47.9%)
Other compounds	0.1 / 0.5 (↑ 70.1%)	0.1 / 0.6 (↑ 91%)	0.1 / 0.8 (↑ 84.8%)	0.1 / 0.5 (↑ 76.8%)
Total (sum compounds)	**1765 / 3613 (↑ 51%)**	**368 / 4649 (↑ 92%)**	**931 / 25,164 (↑ 96.3%)**	**357 / 682 (↑ 47.7%)**

phenanthrolines, polycyclic heteroarenes, plant protective agents (dicofol, diphenyl sulfone, xanthone, 4,4′-DDMU), and 2.4-dinitroaniline (see Table 2). Extensive rain events (SC_{rain}) significantly (*t*-test, $p<0.05$) enhanced the total chemical burden (as summarized single concentrations) at the sampling sites W3 and W4, which were located downstream of the rainwater overflow basin and the effluent of the Aachen-Soers WWTP, respectively. At the sampling site W3, the total chemical burden during SC_{arid} increased from 386 ng/mg OC to 4855 ng/mg OC during SC_{rain}, mainly driven by PAHs and polycyclic heteroarenes. At the sampling site W4, a mixture of trans-stilbene, nitroarenes, PAHs, PCBs, and polycyclic heteroarenes contributed to a pronounced higher chemical burden during SC_{rain} (25,695 ng/mg OC) than the situation a year ago (987 ng/mg OC).

3.3. Assessment of possible drives for genotoxicity

For evaluating chemical compounds that potentially contributed to detected mutagenic effects, 59 quantified chemicals with expected genotoxicity were evaluated (see SI, S9). Due to the postulation that genotoxic compounds have no effect level (Belpomme et al., 2007), detected concentrations were directly related to the analyzed sampling sites.

The chemical burden of genotoxic compounds at the sampling site W2, located upstream of the Aachen-Soers WWTP and the rainwater overflow basin, increased after extensive rain events due to PAHs (+51.4%), aromatic amines (+42.9%), and other compounds (+70.1%) (see Table 3), while the concentration of nitroarenes decreased by 90.9%. The concentration of genotoxic chemical compounds in the sediment of the sampling site W3, located after the rainwater overflow basin, strongly increased due to aromatic amines (+76.2%), carbazoles (+67.5%), PAHs (92.2%), polycyclic heteroarenes (82.3%), and other compounds (+91%). Again, a substantial decrease of nitroarenes by 95.8% was observed at the sampling site W3. The effluent sampling site W4 showed the most substantial concentration increase for genotoxic compounds (+96.3%). This tendency could be at least partially found at the sampling site W5, located 2.5 km downstream of the Aachen-Soers WWTP. Thus, increased concentration of carbazoles (+72%), PAHs (47.7%), polycyclic heteroarenes (+47.9%), and other compounds (+76.8%) could be observed after prolonged rain events (SC_{rain}). The concentration of genotoxic nitroarenes decreased by 83.3% during SC_{rain} in comparison to the SC_{arid}.

4. Discussion

The present study was conceptualized as a case study investigating the genotoxic potential in freshwater sediments from a small receiving waterbody in Germany under different weather conditions. The Wurm River as a study object exemplary represents multiple small water bodies in Europe struggling with the EU WFD's goals of achieving a good chemical status. The studied river's stretch comprised four sampling sites up- and downstream of the Aachen-Soers WWTP effluent discharge, contributing 90% of the total water flow during dry weather (sampling site W4). The time resolution of the sampling campaigns 2017 and 2018 allowed monitoring of sediments under different weather conditions and WWTP functionality (effluent treatment, rainwater overflow basin release). Combining effect-based methods with a chemical target analysis under the mentioned environmental changes, a complex evaluation of the genotoxic potential in the Wurm River's sediments was possible.

4.1. Do weather conditions have an impact on sediment genotoxicity?

The present results showed a potential impact of weather conditions on the mutagenic potential of sediment samples in the Ames fluctuation assay. In contrast, no impact on chromosomal aberrations in the *in vitro* Micronucleus assay was observed. This result was not in line with other studies showing *in vitro* micronuclei induction after the exposure of eucaryotic cells to PAHs (Brinkmann et al., 2014) and sediment extracts (Boettcher et al., 2010; Keiter et al., 2009). However, *in situ* investigations of micronuclei formation in erythrocytes of juvenile rainbow trouts at the same sampling sites also revealed no chromosomal aberrations (data not shown).

The exposure of the *Salmonella typhimurium* standard strains TA98 and TA100 to organic sediment extracts from the Wurm River collected during a prolonged dry period, SC_{arid}, showed the presence of frameshift mutagens only. Additionally, the mutagenic potential at the sampling sites upstream of the effluent (W2 and W3) was characterized by promutagens, which require metabolic activation by mammal liver enzymes (S9-mix) to become mutagenic. At the effluent site (W4) and 2.5 km downstream (W5), sediment samples collected during dry weather (SC_{arid}) contained frameshift promutagens. The additional inclusion of *Salmonella typhimurium* strains YG1041 and YG1042 strains could elucidate nitroarenes and aromatic amines in all sediment samples during dry weather. Giorgio et al. (2011) showed positive results in the *Salmonella typhimurium* strain TA98 (+S9) after exposure to non-polar and polar sediment extracts from the Berre lagoon (France), while the *Salmonella typhimurium* strain YG1041 showed positive results after exposure to the polar extract only. In the study by Reifferscheid et al. (2011), highly polluted sediments (middle-polar and polar fractions) from the Spittelwasser creek (Germany) were investigated, showing positive results in the *Salmonella typhimurium* strain TA98 (+/-S9). In addition, they confirmed a high sensitivity of the metabolically competent *Salmonella typhimurium* strains YG1041 and YG1042 (+/-S9).

In contrast to dry weather, intensified water flow, and sediment perturbation due to extensive rain events during the sampling campaign (SC_{rain}) showed frameshift, base pair substituting promutagens, including nitroarenes aromatic amines in all investigated sediment samples. In particular, sediment samples collected at the sampling site W3, located downstream of the rainwater overflow basin, showed significantly higher revertant numbers than the sampling campaign during dry weather due to base pair substitution mutagens. Remarkable was the effluent sampling site W4 indicating a significantly higher genotoxic potential outgoing from frameshift mutagens (TA98+S9), nitroarenes, and aromatic amines (YG1041+S9). In addition, the sampling site 2.5 km downstream of the effluent (W5) showed significantly higher genotoxic potential after rain events due to nitroarenes and aromatic amines (YG1041+S9). Interestingly, similarly high genotoxic

potential independent of weathering conditions was already observed in sediments from large water bodies with pronounced anthropogenic activities in near surroundings. For example, sediments from the upper Danube River (Higley et al., 2012) and the Spittelwasser creek (Reifferscheid et al., 2011) in Germany, Taquari River in Brazil (Gameiro et al., 2018), Yongdinghe Watershed (Ma et al., 2003), and several sites within the Three Gorges Reservoir (Floehr et al., 2015) in China united the presence of particle-bound frameshift and base pair direct mutagens and frequently promutagens. The identification of promutagens was driven by ubiquitously presented lipophilic particle-bound contaminants as PAHs require metabolic activation before acting mutagenic (Phillipson and Ioannides, 1989).

The present result indicated ubiquitous genotoxic potential in studied sediments, while sediment perturbation as a consequence of rain events or rainwater overflow basin release led to its stronger pronouncement increasing risk to organisms contacting to sediment.

4.2. Can the observed genotoxic potential in sediments be explained by the target chemical analysis?

Quantified chemical target compounds (94) in the investigated sediment samples comprised the groups of aromatic amines, carbazoles, nitroarenes, PAHs, polycyclic heteroarenes, and other chemical compounds. 59 of them were classified as potentially genotoxic. The total chemical burden in studied sediment samples remarkably increased after extensive rain events (SC_{rain}), mainly due to PAHs classified as genotoxic. Thus, the average concentration of 13 PAHs detected in sediments from the Wurm River rose by a factor of 7 (5 ± 3 mg/kg dw during SC_{arid} and 34 ± 38 mg/kg dw during SC_{rain}) (Cornelissen et al., 2008). This result correlated with high mutagenic potential in the *Salmonella typhimurium* strain TA98 (up to induction factor 50) and YG1041 (up to induction factor 75) after the metabolic activation (Mestankova et al., 2014). PAHs occur in the environment as combustion products of fossil fuels and can be involved in forming nitroarenes (reaction with air nitrogen oxides) (Rosenkranz and Mermelstein, 1983; Tokiwa and Ohnishi, 1986).

SC_{rain} took place four days after releasing over 75,000 m^3 of a particle-rich rainwater-wastewater mixture from the rainwater overflow basin. Thus, each rainwater overflow basin release acts as a flash flood with subsequent sediment perturbation, which may bear the risk of releasing particle-bound chemicals (Schertzinger et al., 2019; Shuliakevich et al., 2022; Wolf et al., 2022). However, after extensive rain events, the concentration of nitroarenes strongly decreased at each sampling site. This fact did not correlate with a significantly higher induction in the Ames fluctuation assay (YG1041+S9) at the sampling sites W4 and W5 than during $SC_{arid,}$ indicating other drivers of toxicity. Thus, ubiquitously presented polycyclic heteroarenes were identified at each sampling site. Polycyclic heteroarenes as toxic metabolites of industrial chemicals, petroleum hydrocarbons, tire wear, waste materials from the mining industry, and wood-preserving facilities could be a historical burden from the mining activity in the ore mining area North Eifel (incl. Aachen) (MKULNV NRW, 2012). Additionally, two possible point sources of pollution are known to discharge their wastewater contaminated with mineral oil directly into the municipal canalization system of the Aachen-Soers WWTP (ELWAS-WEB, 2020b, 2020a). The total amount of tire wear particles entering surface waters in Germany from runoffs, the rainwater overflow basin, and effluents is assumed for up to 20,000 t/a (Baensch-Baltruschat et al., 2021).

The negative result in the conducted Micronucleus assay was probably based on the nature of sediment extracts as a complex mixture with chemical compounds that inhibited genotoxic effects of single compounds with clastogenic activity (Brack et al., 2005).

The observed presence of genotoxic drivers in sediments from the Wurm River indicated a temporal exposure of fish in the Wurm River to a mixture of frameshift and base pair substituting promutagens in case of sediment perturbation combined with a permanent exposure to nitroarenes and aromatic amines. Identified genotoxic compounds might contribute to the current insufficient fish fauna status in the studied sector of the Wurm River (Staatliches Umweltamt Aachen, 2005). Thus, frameshift mutations and base pair substitutions can lead to faulty protein synthesis, disrupted cell biochemistry, and organic dystrophy. The organismic level's consequences extend from inconspicuous effects to cell death, severe organic disruptions, and cancer (Flora et al., 1993). On the population level, possible consequences could include a population shift to adult fish due to embryotoxic effects accompanying genotoxicity (Koske et al., 2019) and a population decline (Keiter et al., 2006b). For additional information about the quality characterization of sediments according to Ahlf et al. (2002), see SI, S11.

4.3. Are additional Salmonella typhimurium strains suitable for the sediment genotoxicity testing?

A combination of the Micronucleus and the Ames fluctuation assays is a proven strategy for recognition of a representative number of *in vitro* and *in vivo* genotoxic compounds (Eastmond et al., 2009) in sediment and suspended particulate matter (Aouadene et al., 2008; Biruk et al., 2017; Giorgio et al., 2011; Keiter et al., 2009; Rigaud et al., 2012). Moreover, this EBMs-combination was suggested for its inclusion into the EU WFD.

The present study showed the application of additional bacterial strains in the Ames fluctuation assay as a sensitive method for simultaneously detecting the genotoxic potential and respective chemical drivers. Moreover, the present results concluded less sensitivity of TA100 than TA98, which was already described previously (Kosmehl et al., 2004; Reifferscheid et al., 2011; Sato et al., 1985). Additionally, previous studies implementing YG-strains described a higher sensitivity of the *Salmonella typhimurium* strain YG1041 than its corresponding strain TA98 (45-times – 200-times) (Azuma et al., 1997; Černá et al., 1999). However, our study showed a 2-fold higher sensitivity of the *Salmonella typhimurium* stain YG1041 without metabolic activation by the S9-mix. Such sensitivity discrepancy could be explained by a different complexity and pollution grade of the tested matrixes (air-born particles in both studies vs. sediment samples in the current study). Thus, the present study confirmed the application of the metabolic-active *Salmonella typhimurium* strains YG1041 and YG1042 as reliable and suitable for non-fractionated organic sediment extract testing.

5. Conclusion

The present study described sediments from a small anthropogenically impacted river without significant industrial impact as highly polluted by ubiquitous organic pollutants with strong genotoxic potential, particularly contributing to failing the EU WFD. The observed presence of genotoxic drivers in sediments from the Wurm River indicated in case of sediment perturbation a temporal exposure of fish in the Wurm River to a mixture of frameshift and base pair substituting promutagens combined with a permanent exposure to nitroarenes and aromatic amines. Furthermore, rain-initiated sediment perturbation and release of the rainwater overflow basin were elucidated as significant factors in increasing the genotoxic potential and concentration of chemicals in sediments.

The applied combination of the effect-based methods was shown as informative and reliable. Especially, implementation of the metabolic-active *Salmonella typhimurium* strains YG1041 and YG1042 for non-fractionated organic sediment extract testing contributed to gaining necessary information about commonly occurring genotoxic compounds.

Further sediment investigations at different weather conditions and implementation of effect-directed analysis for the identification of unknown genotoxicity drivers are necessary. Additionally, a desirable goal

for further investigation is monitoring the fish population in the Wurm River.

CRediT authorship contribution statement

Aliaksandra Shuliakevich: Conceptualization, Methodology, Investigation, Writing – original draft, Data curation, Formal analysis, Visualization. **Melis Muz:** Conceptualization, Methodology, Investigation, Writing – original draft, Data curation, Formal analysis. **Jörg Oehlmann:** Methodology, Writing – review & editing, Resources, Funding acquisition. **Laura Nagengast:** Writing – review & editing, Investigation, Data curation, Formal analysis, Visualization. **Katja Schröder:** Writing – review & editing, Investigation, Data curation. **Yvonne Wolf:** Writing – review & editing, Methodology, Investigation, Data curation, Formal analysis. **Ira Brückner:** Resources, Supervision, Funding acquisition, Writing – review & editing. **Riccardo Massei:** Writing – review & editing. **Werner Brack:** Conceptualization, Resources, Supervision, Writing – review & editing. **Henner Hollert:** Conceptualization, Resources, Supervision, Project administration, Funding acquisition, Writing – review & editing. **Sabrina Schiwy:** Conceptualization, Resources, Supervision, Project administration, Funding acquisition, Data curation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

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Supplementary Information

Title: Assessing the genotoxic potential of freshwater sediments after extensive rain events – Lessons learned from a case study in an effluent-dominated river in Germany

Contributing authors: Aliaksandra Shuliakevich[a], Melis Muz[b], Jörg Oehlmann[c], Katja Schröder[d], Laura Nagengast[d], Yvonne Wolf[d], Ira Brückner[e], Riccardo Massei[f], Werner Brack[a,b], Henner Hollert[a*], Sabrina Schiwy[a]

[a]: Goethe University Frankfurt/Main, Department Evolutionary Ecology and Environmental Toxicology, Max-von-Laue-Strasse 13, 60438 Frankfurt/Main, Germany

[b]: Helmholtz Centre for Environmental Research – UFZ, Department of Effect-Directed Analysis, Permoserstrasse 15, 04318 Leipzig, Germany

[c]: Goethe University Frankfurt/Main, Department Aquatic Ecotoxicology, Max-von-Laue-Strasse 13, 60438 Frankfurt/Main, Germany

[d]: RWTH Aachen University, Institute of Biology V, Worringerweg 1, 52074 Aachen, Germany

[e]: Eifel-Rur Waterboard (WVER), Eisenbahnstr. 5, 52354 Düren, Germany WVER

[f]: Helmholtz Centre for Environmental Research – UFZ, Department of Bioanalytical Ecotoxicology, Permoserstrasse 15, 04318 Leipzig, Germany

*Corresponding author: Hollert@bio.uni-frankfurt.de. Goethe University Frankfurt, Max-von-Laue-Str. 13, 60438 Frankfurt am Main, Germany

S1 Modifications in the procedure of the Micronucleus assay

For the cultivation of V79 cells, double volumes (10 mL each) of Hanks Balanced Salt Solution without Ca2+ and Mg2+ for washing cells and of culture Dulbecco's Modified Eagle's Medium (DMEM) with 8.2 % fetal calf serum and 0.9 % penicillin-streptomycin antibiotics solution (PenStrep; 10,000 E/10,000 µg/mL) were used. Culture media for treatments with and without S9 mix were composed as followed: DMEM with 2.4% L-alanyl-L-glutamine-dipeptide-solution and 1.2 % PenStrep as well as DMEM with 8% FCS, 1.8% Gibco® GlutaMAXTM and 0.9 % PenStrep, respectively.

S2

Table S2: Characteristics of *Salmonella typhimurium* strains implemented in the Ames fluctuation assay.

Salmonella typhimurium strain	Type of the point mutation	Characteristics	Potential mutagens	Reference
TA98	frameshift	deletion or insertion of several DNA nucleotides (3N±1) followed by the change of the reading frame and mRNA translation different to the original	DNA intercalators (e. g., polycyclic compounds with reactive side groups)	Crick et al. (Ames et al., 1972; 1961; Isono and Yourno, 1974)
TA100	base pair substitution	deletion or insertion of one DNA nucleotide	alkylating / deaminating agents (e. g., anticancer drugs)	Gee et al. (1994)
YG1041	frameshift + *O*-acetyl-transferase and nitroreductase activity	frameshift point mutation and overexpression of enzymes metabolizing nitroarenes and aromatic amines	nitroarenes / aromatic amines	Hagiwara et al. (1993), Watanabe et al. (1990), Watanabe et al. (1989)
YG1042	base pair substitution + *O*-acetyl-transferase and nitroreductase activity	base pair substitution point mutation and overexpression of enzymes metabolizing nitroarenes and aromatic amines	nitroarenes / aromatic amines	Hagiwara et al. (1993), Watanabe et al. (1990), Watanabe et al. (1989)

S3

Table S3: Sediment amount containing 100 mg TOC, g dw. Data from the CHN-analyzer (Vario EL III).

	SC_{arid}	SC_{rain}
W2	16.7	11.7
W3	7.4	22.7
W4	16.7	26.4
W5	8.9	26.5

S4

Clean-up with flash chromatography was performed with a pre-packed silica gel cartridge with a particle size of 40–63 µm (Chromabond Flash RS4 SiOH, Macherey Nagel) using an Agilent 1200 pump. The cartridge was preconditioned with DCM, and the sediment extract (100 mg TOC eq./500 µL DCM) was loaded on the cartridge using a syringe. DCM was pumped through the cartridge according to the following program: The DCM flow was increased from 0 to 10 mL/min in 0.5 min, followed by a constant flow of 10 mL/min until the 2. min and brought back to initial conditions within an additional 0.1 min. The collected eluent was evaporated until 0.5 mL and transferred into new glass tubes by rinsing with ethyl acetate (EtAc) to perform the complete solvent exchange. Under the nitrogen stream, DCM was removed, the volume of the extract in EtAc was reduced to 0.1 mL and then adjusted to 0.5 mL EtAc. Glass tubes were then kept at -20°C for 1 h in order to achieve precipitation of lipids. Afterward, the extracts were carefully filtered using a 0.2 µm PTFE syringe filter. 100 µL aliquots were pipetted into a glass vial with an insert, and the internal standard mix was spiked (1 µg/mL in EtAc). All aliquots were kept at -20°C until further chemical analysis.

S5

A Trace 1310 GC was linked with a Thermal Desorption Unit (TDU). A Cooled Injection System (CIS) 4 (Gerstel, Mülheim/Ruhr, Germany) was coupled to the QExactive. Aliquots of 2 µL extract were taken with the Gerstel MultiPurpose autosampler into a TDU tube. The desorption temperature of the TDU was set to 300 °C (720 °C /min heating rate) and held for five minutes. The transfer line between the TDU and the CIS system was kept at 320 °C. A baffled, deactivated glass liner was used for refocusing at -10 °C, the temperature increased with a rate of 12 °C/min to 300 °C (10 min holding time), and the analytes were injected in splitless mode (splitless time of 2 min). The QExactive was operated in the electron ionization (EI) mode at 70 eV in positive full-scan mode (70-810 m/z) and a nominal resolution of 60,000 (FWHM at m/z 200).

S6

Chromatographic separation was performed on a Kinetex 2.6 µm EVO C18 (50×2.1mm, Phenomenex) column equipped with a pre-column (C18 EVO 5×2.1 mm) and an inline filter. For positive mode, methanol and water containing 0.1 % formic acid, and for negative ion mode, water and methanol containing 1 mM ammonium formate were used as mobile phases. The sample injection volume was 10 µl. APCI source was set 10 µA (pos) and 15 µA (neg), sheat gas 20 arbitrary units (au), auxiliary gas flow 5 au and sweep gas flow, 5 au; APCI heater temperature 350 ^{o}C, ion transfer capillary temperature 300 ^{o}C. The nominal resolving power in the full scan experiments was 70,000 (referenced to 200 m/z). For data-dependent dd-ms2 experiments, an inclusion list was prepared, the nominal resolving power was 35,000 (referenced to 200 m/z), and a stepped higher collisional dissociation was operated at 55 % and 95 % normalized collision energy.

S7

A phenyl-hexyl column (AccucorePhenylHexyl 150×3 mm, 2.6 µm particle size, Thermo) was used for chromatographic separation at 40 °C with methanol and water containing 0.1 % formic acid as a mobile phase. Full scan spectra were acquired in the mass range of 100-1000 m/z at a nominal resolving power of 70,000 (at 200 m/z). In addition, product ion spectra (MS/MS) were acquired with a data-dependent method triggered from the inclusion list of masses of interest at a nominal resolving power of 35,000 using stepped collision energies of 55 % and 95 % normalized collision energy.

S8

Table S8: List of analyzed chemical compounds. Information on substance groups, molecular formulas, CAS-numbers, exact masses, and the log kow values were derived from the PubChem® open-source, Merck© KGaA internet platform, Thermo Fischer Schientific Inc.© internet platform, Exact Mass Calculator of the Scientific Instrument Services© internet platform by Adaptas Solutions and Wikipedia.

#	Compound	Substance group	Molecular formula	CAS	Exact mass, g/mol	log kow	Analytic procedure	Internal standard	LOD, ng/mL
1	trans-Stilben (trans-/trans-Methyl-Stilben)	acyclin olefin (stilbenoid)	C14H12	103-30-0	180.093.900	4.8	LC-ESI pos	Hexachlorobenzene-13C6	2
2	1,1-dichloro-2,2-bis(4-methoxyphenyl)ethan	aliphatic halogen	C16H16Cl2O2	7388-31-0	310.052.736	4.5	LC-APCI neg	Carbendazim-D4	0.2
3	4-Chloroaniline	aniline	C6H6ClN	106-47-8	127.018.877	1.9	LC-APCI pos	Carbendazim-D4	5
4	1-Aminoanthraquinone	aromatic amine	C14H9NO2	82-45-1	223.063.329	3.2	LC-APCI neg	Bezafibrate-D4	0.2
5	2-Aminoanthracene	aromatic amine	C14H11N	613-13-8	193.089.149	3.5	LC-APCI pos	Benzophenone-3-D5	10
6	2-Bromoaniline	aromatic amine	C6H6BrN	615-36-1	170.968.360	2.1	LC-APCI neg	Imidacloprid-D4	10
7	4-Aminopyrene	aromatic amine	C16H11N	17075-03-5	217.089.149	4.3	LC-APCI pos	Diclofenac_d4	10
8	6-Aminochrysene	aromatic amine	C18H13N	2642-98-0	243.104.799	5	LC-APCI pos	Tri-n-butylphosphate-D27	0.2
9	Dioctyldiphenylamine	aromatic amine	C28H43N	101-67-7	393.339.549	11.6	GC	Perylene-D12	1
10	1,3-Diaminopyrene	aromatic amine	C16H12N2	92821-64-2	232.100.048	3.6	LC-APCI neg	Tri-n-butylphosphate-D27	5
11	1,6-Diaminopyrene	aromatic amine	C16H12N2	14923-84-3	232.100.048	3.6	LC-APCI neg	Tri-n-butylphosphate-D27	5
12	1-Amino-4-bromoanthraquinone	aromatic amine	C14H8BrNO2	81-62-9	300.973.840	3.4	LC-APCI neg	Diclofenac_d4	0.5
13	1-Methylaminoanthraquinone	aromatic amine	C15H11NO2	82-38-2	237.078.979	3.9	LC-APCI neg	Diclofenac_d4	0.2
14	1-Naphthylamine	aromatic amine	C10H9N	134-32-7	143.073.499	2.2	LC-APCI neg	Sulfamethoxazole-D4	5
15	2,4-Dichloroaniline	aromatic amine	C6H5Cl2N	554-00-7	160.979.905	2.9	LC-APCI pos	Carbamazepine-D10	1
16	2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)	aromatic amine	C13H12N4	105650-23-5	224.106.196	2.2	LC-APCI neg	p-Toluene-sulfonamide-D4	1
17	2-Amino-3,8-dimethylimidazo-[4,5-f]quinoxaline (MeIQx)	aromatic amine	C11H11N5	77500-04-0	213.101.445	1	LC-APCI neg	Diglyme-D6	0.2
18	2-Aminoanthraquinone	aromatic amine	C14H9NO2	117-79-3	223.063.329	2.7	LC-APCI neg	Bezafibrate-D4	1
19	2-Aminobiphenyl	aromatic amine	C12H11N	90-41-5	169.089.149	2.8	LC-APCI neg	Carbamazepine-D10	0.2
20	2-Aminofluorene	aromatic amine	C13H11N	153-78-6	181.089.149	3.1	LC-APCI pos	Tebuconazole-D9	5
21	3-Aminoacetophenon	aromatic amine	C8H9NO	99-03-6	135.068.414	1.2	LC-APCI pos	Atenolol-D7	10
22	3-Aminofluoranthene	aromatic amine	C16H11N	2693-46-1	217.089.149	4.2	LC-APCI neg	Diclofenac_d4	5
23	4-Isopropylaniline	aromatic amine	C9H13N	99-88-7	135.104.799	2.2	LC-APCI pos	p-Toluene-sulfonamide-D4	5
24	Benzidine	aromatic amine	C12H12N2	92-87-5	184.100.048	1.3	GC	Cotinine-D3	1
25	Phenoxazine	Aromatic amine	C12H9NO	135-67-1	183.068.414	3.9	LC-ESI pos	Mecoprop-D3	1
26	Michler's ketone	aromatic dialkylamine	C17H20N2O	90-94-8	268.157.563	3.9	GC	Benzophenone-3-D5	1
27	Phenazine	azaarene	C12H8N2	92-82-0	180.068.748	2.8	LC-ESI pos	Atrazine-13C3	0.5
28	Azobenzene	azobenzene	C12H10N2	103-33-3	182.084.398	3.8	GC	Tri-n-butylphosphate-D27	1
29	Aniline Yellow	azobenzene	C12H11N3	60-09-3	197.095.297	3.4	LC-APCI pos	DEET-D7	0.2
30	2,7-Dibromocarbazole	carbazole	C12H7Br2N	136630-39-2	322.894.521	4.8	LC-APCI neg	Laurylsulfate-D25	0.2
31	2-Chlorocarbazole	carbazole	C12H8ClN	10537-08-3	304.970.151	4.5	LC-APCI neg	Diclofenac_d4	0.2
32	3,6-Dibromocarbazole	carbazole	C12H7Br2N	6825-20-3	322.894.521	4.8	LC-APCI neg	Laurylsulfate-D25	0.2
33	3,6-Dichlorocarbazole	carbazole	C12H7Cl2N	5599-71-3	234.995.555	5.4	LC-APCI neg	Laurylsulfate-D25	0.2
34	3,6-Diiodocarbazole	carbazole	C12H7I2N	57103-02-3	418.866.803	4.7	LC-APCI neg	Laurylsulfate-D25	0.2
35	3-Bromocarbazole	carbazole	C12H8BrN	1592-95-6	244.984.010	4.4	LC-APCI neg	Diclofenac_d4	0.2
36	3-Bromocarbazole	carbazole	C12H8BrN	1592-95-6	244.984.010	4.4	LC-APCI pos	PCB118_13C12	0.1
37	3-Iodocarbazole	carbazole	C12H8IN	16807-13-9	292.970.151	4.4	LC-APCI pos	Diclofenac_d4	0.2
38	Carbazole	carbazole	C12H9N	86-74-8	167.073.499	3.7	GC	Clarithromycin-D3	5
39	1,3,6,8-Tetrabromocarbazole	carbazole	C12H5Br4N	55119-09-0	478.715.543	6.2	LC-APCI neg	Laurylsulfate-D25	5
40	2-Hydroxycarbazole	carbazole	C12H9NO	86-79-3	183.068.414	3.5	LC-APCI neg	Carbamazepine-D10	5
41	Pentachloronitrobenzene	chrorinated hydrocarbons	C6Cl5NO2	82-68-8	292.837.169	4.2	LC-ESI pos	Laurylsulfate-D25	20
42	BDE-153	flameretardant	C12H4Br6O	68631-49-2	637.536.230	7.6	GC	13C12 Decachlorobiphenyl	2
43	BDE-154	flameretardant	C12H4Br6O	207122-15-4	637.536.230	7.6	GC	13C12 Decachlorobiphenyl	5
44	BDE-47	flameretardant	C12H6Br4O	5436-43-1	481.715.208	6.2	GC	PCB180_13C12	0.1
45	BDE-99	flameretardant	C12H5Br5O	60348-60-9	559.625.720	6.9	GC	BDE99_13C12	1
46	Hexabromobenzene	flameretardant	C6Br6	87-82-1	545.510.016	6.1	GC	Chrysene-D12	5
47	4,4'-DDMU	halogenated PAH / PPP	C14H9Cl3	1022-22-6	281.976.984	6.1	LC-APCI pos	PCB101-13C12	0.2
48	1,10-Phenanthrolin-5,6-dion	heterocyclic PAH	C12H6N2O2	27318-90-7	210.042.928	0.5	LC-APCI neg	Diclofenac_d4	5
49	1,10-Phenanthroline	heterocyclic PAH	C12H8N2	66-71-7	18.006.878	1.8	LC-APCI neg	DEET-D7	0.5
50	2-Phenylindole	indole	C14H11N	948-65-2	193.089.149	3.8	LC-APCI neg	Benzophenone-3-D5	20
51	7-Azaindole	indole	C7H6N2	271-63-6	118.053.098	1.8	LC-APCI pos	Imidacloprid-D4	5
52	Harmine	indole alkaloid compound	C13H12N2O	442-51-3	212.094.963	3.6	GC	Imidacloprid-D4	0.2
53	Harman	indole alkaloid ompound	C12H10N2	486-84-0	182.084.398	3.6	GC	Benzotriazole-D4	0.2
54	N-Phenyl-1-naphthylamine	Naphthalene	C16H13N	90-30-2	219.104.799	4.4	GC	Progesterone-D9	0.2
55	2,4-Dinitroaniline	nitro aromatic amine	C6H5N3O4	97-02-9	183.028.007	1	LC-APCI neg	Bentazone-D6	0.5
56	4-Nitrothiophenol	nitroamine	C6H5NO2S	1849-36-1	155.004.101	2	LC-APCI pos	Bezafibrate-D4	0.2
57	1-Nitropyrene	nitroarene	C16H9NO2	5522-43-0	247.063.329	5	LC-APCI neg	Laurylsulfate-D25	0.5
58	3-Nitrofluoranthene	nitroarene	C16H9NO2	892-21-7	247.063.329	4.7	LC-APCI neg	Laurylsulfate-D25	0.5
59	4-Nitropyrene	nitroarene	C16H9NO2	57835-92-4	247.063.329	4.9	LC-APCI pos	Laurylsulfate-D25	0.2
60	6H-Benzo(cd)pyrene-6-one	nitroarene	C20H12O6	3074-00-8	348.063.390	4.9	LC-APCI pos	Triclosan-D3	0.5
61	6-Nitrobenzo[a]pyrene	nitroarene	C20H11NO2	63041-90-7	297.078.979	6.6	LC-APCI neg	Laurylsulfate-D25	1
62	9-Nitroanthracene	nitroarene	C14H9NO2	602-60-8	223.063.329	4.8	LC-APCI pos	Diclofenac_d4	0.2
63	1,3-Dinitropyrene	nitroarene	C16H8N2O4	75321-20-9	292.048.408	4.7	LC-APCI neg	Laurylsulfate-D25	5
64	1,6-Dinitropyrene	nitroarene	C16H8N2O4	42397-64-8	292.048.408	4.7	LC-APCI neg	Triclosan-D3	0.5
65	2-Nitroanthracene	nitroarene	C14H9NO2	3586-69-4	223.063.329	4.5	LC-APCI neg	Diclofenac_d4	0.2
66	2-Nitrofluorene	nitroarene	C13H9NO2	607-57-8	211.063.329	3.7	LC-APCI neg	Diclofenac_d4	0.5
67	3-Nitrobenzanthrone	nitroarene	C17H9NO3	17117-34-9	275.058.244	4.1	LC-APCI pos	Diclofenac_d4	1
68	6-Nitrochrysene	nitroarene	C18H11NO2	08.02.7496	273.078.979	5.5	LC-APCI pos	Laurylsulfate-D25	0.2
69	7-Nitrobenz[a]anthracene	nitroarene	C18H11NO2	20268-51-3	273.078.979	6	LC-APCI pos	Laurylsulfate-D25	0.2
70	2-Nitrotoluene	nitrotoluene	C7H7NO2	88-72-2	137.047.679	2.3	LC-APCI neg	Diclofenac_d4	1
71	1,2-Dihydroxyanthraquinone	PAH	C14H8O4	72-48-0	240.042.260	3.2	LC-APCI neg	Diclofenac_d4	1
72	1,3-Dihydroxyanthraquinone	PAH	C14H8O4	518-83-2	240.042.260	3.2	LC-APCI neg	Diclofenac_d4	0.5
73	1,7-Phenanthroline	PAH	C12H8N2	230-46-6	180.068.748	2.5	LC-APCI neg	Imidacloprid-D4	1
74	1,8-Dihydroxyanthraquinone	PAH	C14H8O4	117-10-2	240.042.260	3.2	LC-APCI neg	Diclofenac_d4	0.5
75	1,9-Benz-10-anthrone	PAH	C17H10O	82-05-3	230.073.165	4.3	LC-APCI neg	Diclofenac_d4	0.2
76	10-Azabenzo(a)pyrene	PAH	C19H11N	189-92-4	253.089.149	5.3	LC-APCI neg	Tri-n-butylphosphate-D27	0.5
77	2-Acetylfluorene	PAH	C15H12O	781-73-7	208.088.815	3.5	LC-APCI neg	Metolachlor-D6	5
78	2-Acetylphenanthrene	PAH	C16H12O	5960-69-0	220.088.815	4.1	LC-APCI neg	Diclofenac_d4	0.5
79	2-Hydroxyanthraquinone	PAH	C14H8O3	605-32-3	224.047.345	3	LC-APCI neg	Bezafibrate-D4	0.5
80	2-Methylanthraquinone	PAH	C15H10O2	84-54-8	222.068.080	3.9	LC-APCI neg	Diclofenac_d4	0.2
81	3-Acetylphenanthrene	PAH	C16H12O	2039-76-1	220.088.815	4.1	LC-APCI neg	Diclofenac_d4	0.5
82	4H-Cyclopenta[def]phenanthrene	PAH	C5H10	203-64-5	70.078.250	4.4	LC-APCI pos	PCB52_13C12	0.2
83	9-Acetylanthracene	PAH	C16H12O	784-04-3	220.088.815	4.2	LC-APCI pos	Benzophenone-3-D5	0.5
84	9-Fluorenone	PAH	C13H8O	486-25-9	180.057.515	3.6	LC-APCI pos	Diclofenac_d4	5
85	9-vinylanthracene	PAH	C16H12	2444-68-0	204.093.900	5.5	LC-APCI pos	PCB52_13C12	0.2
86	Anthracene	PAH	C14H10	120-12-7	178.078.250	4.4	LC-APCI pos	Phenanthrene-D10	1
87	Anthraquinone	PAH	C14H8O2	84-65-1	208.052.430	3.4	GC	Bezafibrate-D4	5

88	Benz(a)anthracene-7,12-dione	PAH	C18H10O2	2498-66-0	258.068.080	4.4	GC	Laurylsulfate-D25	0.2
89	Benz[a]anthracene	PAH	C18H12	56-55-3	228.093.900	5.8	GC	Chrysene-D12	2
90	Benzo(b)fluorene	PAH	C17H12	243-17-4	216.093.900	5.8	GC	PCB118_13C12	2
91	Benzo[a]fluorene-11-one	PAH	C17H12	238-84-6	216.093.900	5.2	GC	Triclosan-D3	0.2
92	Benzo[a]pyrene	PAH	C20H12	50-32-8	252.093.900	6	GC	benzo[a]pyrene-d12	20
93	Benzo[b]fluoranthene	PAH	C20H12	205-99-2	252.093.900	6	GC	benzo[a]pyrene-d12	10
94	Benzo[b]fluorene-11-one	PAH	C17H12		216.093.900		GC	Diclofenac_d4	0.2
95	Benzo[e]pyrene	PAH	C20H12	192-97-2	252.093.900	6.4	GC	benzo[a]pyrene-d12	10
96	Benzo[ghi]perylene	PAH	C22H12	191-24-2	276.093.900	6.6	GC	Dibenz[a,h]anthracene D14	2
97	Benzo[k]fluoranthene	PAH	C22H12	207-08-9	276.093.900	6.6	GC	benzo[a]pyrene-d12	2
98	Chrysene	PAH	C18H12	218-01-9	228.093.900	5.7	GC	Chrysene-D12	0.2
99	Cyclopenta[cd]pyrene	PAH	C18H10	27208-37-3	226.078.250	5.5	GC	Chrysene-D12	20
100	Dibenz[a,h]anthracene	PAH	C22H14	53-70-3	278.109.550	6.5	GC	Dibenz[a,h]anthracene D14	0.2
101	Fluoranthene	PAH	C16H10	206-44-0	202.078.250	5.2	GC	Pyrene-D10	10
102	Fluorene	PAH	C13H10	86-73-7	166.078.250	4.2	GC	Acenaphthene-D10	20
103	Indeno(1,2,3-cd)fluoranthene	PAH	C22H12	193-43-1	276.093.900	2.7	GC	Dibenz[a,h]anthracene D14	0.2
104	Indeno[1,2,3-cd]pyrene	PAH	C22H12	193-39-5	276.093.900	7	GC	Dibenz[a,h]anthracene D14	2
105	m-Terphenyl	PAH	C18H14	92-06-8	230.109.550	5.6	GC	Pyrene_D10	0.2
106	O-Terphenyl	PAH	C18H14	84-15-1	230.109.550	6	GC	PCB28_13C12	0.2
107	Perylene	PAH	C20H12	198-55-0	252.093.900	5.8	LC-ESI pos	Perylene-D12	2
108	Phenanthren-9,10-dione	PAH	C14H8O2	84-11-7	208.052.430	2.5	LC-ESI pos	Atrazine-13C3	10
109	Phenanthrene	PAH	C14H10	85-01-8	178.078.250	4.5	LC-ESI pos	Phenanthrene-D10	20
110	p-Terphenyl	PAH	C18H14	92-94-4	230.109.550	5.6	LC-ESI pos	Pyrene_D10	0.2
111	Pyrene	PAH	C16H10	129-00-0	202.078.250	4.9	LC-ESI pos	Pyrene_D10	2
112	1,4-Dihydroxyanthraquinone	PAH	C14H8O4	81-64-1	240.042.260	3.7	LC-APCI neg	Diclofenac_d4	0.2
113	1,5-Dihydroxyanthraquinone	PAH	C14H8O4	117-12-4	240.042.260	3.7	LC-APCI neg	Diclofenac_d4	0.2
114	1,8-Dichloroanthraquinone	PAH	C14H6Cl2O2	82-43-9	275.974.486	4.1	LC-APCI neg	Diclofenac_d4	0.5
115	2,6-Dihydroxyanthraquinone	PAH	C14H8O4	84-60-6	240.042.260	2.2	LC-APCI neg	Bezafibrate-D4	0.2
116	5-Carboline	PAH	C11H8N2	244-69-9	168.068.748	2.3	LC-APCI pos	Diglyme-D6	0.2
117	9-Acetylphenanthrene	PAH	C16H12O	2039-77-2	220.088.815	4.1	LC-APCI pos	Benzophenone-3-D5	0.5
118	Carboline	PAH	C11H8N2	244-76-8	168.068.748	2.9	GC	Verapamil-D6	0.2
119	Dibenzo(a,e)pyrene	PAH	C24H12	192-65-4	300.093.900	7.3	GC	Dibenz[a,h]anthracene D14	10
120	Norharmane (β-Carboline)	PAH	C11H8N2	244-63-3	168.068.748	3.2	GC	Desisopropylatrazine-D5	0.5
121	PCB 101	PCB	C12H5Cl5	37680-73-2	323.883.390	6.5	GC	PCB101_13C12	1
122	PCB 118	PCB	C12H5Cl5	31508-00-6	323.883.390	7.1	GC	PCB118_13C12	0.2
123	PCB 138	PCB	C12H4Cl6	35065-28-2	357.844.418	7.2	GC	PCB138_13C12	2
124	PCB 149	PCB	C12H4Cl6	38380-04-0	357.844.418	7.1	GC	PCB118_13C12	2
125	PCB 153	PCB	C12H4Cl6	35065-27-1	357.844.418	7.2	GC	PCB138_13C12	0.2
126	PCB 170	PCB	C12H3Cl7	35065-30-6	391.805.446	7.9	GC	PCB180_13C12	5
127	PCB 18	PCB	C12H7Cl3	37680-65-2	255.961.334	5.6	GC	PCB28_13C12	1
128	PCB 180	PCB	C12H3Cl7	35065-29-3	391.805.446	7.9	GC	PCB180_13C12	1
129	PCB 194	PCB	C12H2Cl8	35694-08-7	425.766.474	8.6	GC	BDE99_13C12	5
130	PCB 28/31	PCB	C12H7Cl3	7012-37-5 / 16606-02-3	255.961.334	5.60 / 5.80	GC	PCB28_13C12	0.5
130	PCB 44	PCB	C12H6Cl4	41464-39-5	289.922.362	5.8	LC-ESI pos	PCB52_13C12	0.2
131	PCB 52	PCB	C12H6Cl4	35693-99-3	289.922.362	6.1	LC-ESI pos	PCB52_13C12	2
132	PCB 209	PCB	C12Cl10	2051-24-3	493.688.530	8.3	GC	PCB209_13C12	20
133	4,7-Phenanthroline	phenanthroline	C12H8N2	230-07-9	180.068.748	2.1	LC-APCI pos	Desisopropylatrazine-D5	20
134	1H-Benzo[g]indole	polycyclic heteroarene	C12H9N	233-34-1	167.073.499	3.3	LC-APCI neg	Clarithromycin-D3	5
135	Acridone	polycyclic heteroarene	C13H9NO	578-95-0	195.068.414	3	LC-APCI pos	Mecoprop-D3	0.2
136	Anthracene-1,4-dione	polycyclic heteroarene	C14H8O2	635-12-1	208.052.430	3	LC-APCI pos	Diclofenac_d4	0.5
137	Benz[a]acridine	polycyclic heteroarene	C17H11N	225-11-6	229.089.149	4.6	GC	Carbamazepine-D10	5
138	Dibenz[a,j]acridine	polycyclic heteroarene	C21H13N	224-42-0	279.104.799	6	GC	Tri-n-butylphosphate-D27	0.2
139	Benzo[h]quinoline	polycyclic heteroarene	C13H9N	230-27-3	179.073.499	3.4	GC	Diazinon-D10	5
140	beta-Naphthoflavone	polycyclic heteroarene	C19H12O2	6051-87-2	272.083.730	4.4	GC	Tri-n-butylphosphate-D27	0.2
141	Dicofol	plant protective agent	C14H9Cl5O	115-32-2	367.909.605	4.3	GC	PCB52_13C12	0.2
142	Diphenyl sulfone	plant protective agent	C12H10O2S	127-63-9	218.040.152	2.4	GC	PCB52_13C12	20
143	Xanthone	plant protective agent	C13H8O2	90-47-1	196.052.430	3.4	LC-ESI pos	Clarithromycin-D3	1
144	Clopyralid	plant protective agent	C6H3Cl2NO2	1702-17-6	190.954.085	1	GC	Hydrochlorothiazide-13C6	50
145	Hexabromocyclododecane	plant protective agent	C12H18Br6	134237-50-6	635.650.866	7.1	GC	Laurylsulfate-D25	50
146	Iprodione	plant protective agent	C13H13Cl2N3O3	36734-19-7	329.033.398	3	GC	Diclofenac_d4	10
147	Nitrofen	plant protective agent	C12H7Cl2NO3	1836-75-5	282.980.300	4.3	GC	Triclosan-D3	0.5
148	Procymidone	plant protective agent	C13H11Cl2NO2	32809-16-8	283.016.685	3	LC-ESI pos	Diclofenac_d4	5
149	Acrinathrin	pyrethroids	C26H21F6NO5	101007-06-1	541.132.392	6.8	LC-APCI pos	PCB180_13C12	0.5
150	Allethrin	pyrethroids	C19H26O3	584-79-2	302.188.195	4.8	LC-APCI pos	PCB101_13C12	20
151	Bifenthrin	pyrethroids	C23H22ClF3O2	82657-04-3	422.126.042	6	GC	Chrysene-D12	0.2
152	Chlorpyrifos	pyrethroids	C9H11Cl3NO3PS	2921-88-2	348.926.288	5.3	GC	PCB52_13C12	0.1
153	Cyfluthrin	pyrethroids	C22H18Cl2FNO3	68359-37-5	433.064.778	6.2	GC	BDE99_13C12	2
154	Cyhalothrin	pyrethroids	C23H19ClF3NO3	68085-85-8	449.100.556	6.1	GC	PCB180_13C12	0.2
155	Cypermethrin	pyrethroids	C22H19Cl2NO3	52315-07-8	415.074.200	6	GC	benzo[a]pyrene-d12	2
156	Deltamethrin	pyrethroids	C22H19Br2NO3	52918-63-5	502.973.166	6.2	GC	Dibenz[a,h]anthracene D14	5
157	Esfenvalerate	pyrethroids	C25H22ClNO3	66230-04-4	419.128.822	6.2	GC	Perylene-D12	1
158	Etofenprox	pyrethroids	C25H28O3	80844-07-1	376.203.845	7	GC	etofenprox-D5	0.1
159	Fluvalinate	pyrethroids	C26H22ClF3N2O3	69409-94-5	502.127.105	7.7	GC	Perylene-D12	1
160	Permethrin	Pyrethroids	C21H20Cl2O3	52645-53-1	390.078.951	6.5	LC-ESI pos	BDE99_13C12	0.1
161	Prallethrin	Pyrethroids	C19H24O3	23031-36-9	300.172.545	4.3	LC-ESI pos	PCB101_13C12	1
162	Tefluthrin	Pyrethroids	C17H14ClF7O2	79538-32-2	418.057.054	5.4	LC-ESI pos	Phenanthrene-D10	0.1
163	Transfluthrin	Pyrethroids	C15H12Cl2F4O2	118712-89-3	370.015.048	5	LC-ESI pos	PCB52_13C12	0.1
164	2-Amino-9H-pyrido[2,3-b]indole (AalphaC)	pyridiondole	C11H9N3	26148-68-5	183.079.647	2.6	LC-APCI neg	Imidacloprid-D4	0.2
165	1-Phenylnaphthalin	others	C16H12	605-02-7	204.093.900	4.9	LC-APCI neg	Tonalide-D3	0.2
166	p-Benzyldiphenyl	others	C19H16	613-42-3	244.125.200	5.9	GC	PCB52_13C12	1
167	3,3'-Dichlorobenzidine	others	C12H10Cl2N2	91-94-1	252.022.104	3.5	LC-APCI neg	Mono-isobutylphthalate-D4	0.2
168	Methyltolylsulfone	others	C8H10O2S	3185-99-7	170.040.152	2.2	GC	acenaphthen-D10	10

S9 (see next page)

Table S9: Genotoxic characteristics and environmental persistence of detected chemical compounds in sediment. Information sources and additional remarks can be found at the end of the table.

			Hazard class (1)			Toxtree (2)		VEGA (v. 1.1.5) / modus CONSENSUS v. 1.0.3 (3)		Scarid, [ng/mg OC]				Scrain, [ng/mg OC]				Scarid, [ng/g dw]				Scrain, [ng/g dw]			
#	Substance group	Compound	Mutagenic	Carcinogenic	Reprotoxic	DNA binding alert for	Predicted mutagenic activity	Predicted MN activity	Predicted genotoxic potential in the Ames fluctuation assay	W2	W3	W4	W5	W2	W3	W4	W5	W2	W3	W4	W5	W2	W3	W4	W5
1	acyclin olefin	trans-Stilben	-	-	-	Michael Acceptor	non-mutagenic	not predicted	no	1.217				0.148	1.765	4.464	0.059	7.157				1.236	7.673	17.170	0.217
2	aliphatic halogen	1,1-dichloro-2,2-bis[4-methoxyphenyl]ethan	-	-	-	Michael Acceptor/Acyl Transfer Agent	mutagenic	active	yes								0.008								0.030
3	aromatic amine	1-Aminoanthraquinone	-	-	-	Michael Acceptor/SN1	mutagenic	active	yes		0.007	0.010	0.006	0.015	0.039	0.099	0.067		0.095	0.058	0.069	0.126	0.171	0.381	0.247
4	aromatic amine	2-Aminoanthracene	2	-	-	Michael Acceptor/SN1	mutagenic	active	yes					0.306	0.290							2.552	1.260		
5	aromatic amine	2-Bromaniline	-	-	-	Michael Acceptor/SN1	non-mutagenic	not predicted	no								0.046								0.170
6	aromatic amine	4-Aminopyrene	2	-	-	Michael Acceptor/SN1	mutagenic	not predicted	yes	0.117	0.127	0.110	0.122	0.153	0.225	0.983		0.688	1.810	0.647	1.357	1.275	0.977	3.780	
7	aromatic amine	6-Aminochrysene	-	-	-	Michael Acceptor/SN1	mutagenic	not predicted	yes	0.324	0.090	0.051	0.082	0.298	0.384		0.120	1.906	1.285	0.300	0.909	2.482	1.671		0.445
8	aromatic amine	Dioctyldiphenylamine	-	-	-	Michael Acceptor/SN1	non-mutagenic	inactive	no	0.577	0.024	0.050	0.040	0.147	0.072	0.088	0.055	3.395	0.341	0.292	0.447	1.228	0.311	0.337	0.204
9	azobenzene	Azobenzene	-	-	-	Michael Acceptor/SN1	mutagenic	inactive	yes				0.043			0.058	0.121				0.478			0.223	0.448
10	carbazole	2,7-Dibromocarbazole	-	-	-	Michael Acceptor/SN1	mutagenic	not predicted	yes			0.009	0.011	0.001	0.016	0.019	0.022			0.056	0.125	0.010	0.072	0.072	0.082
11	carbazole	2-Chlorocarbazole	-	-	-	Michael Acceptor/SN1	mutagenic	not predicted	yes	0.024	0.019	0.044	0.050	0.015	0.087	0.136	0.240	0.143	0.267	0.258	0.556	0.128	0.377	0.522	0.889
12	carbazole	3,6-Dibromocarbazole	-	-	-	Michael Acceptor/SN1	mutagenic	not predicted	yes		0.002	0.009	0.011	0.002	0.016	0.018	0.021		0.023	0.054	0.121	0.013	0.068	0.068	0.077
13	carbazole	3,6-Dichlorocarbazole	-	-	-	Michael Acceptor/SN1	mutagenic	not predicted	yes	0.153	0.108	0.246	0.374	0.098	0.420	0.873	1.342	0.898	1.545	1.449	4.161	0.814	1.825	3.358	4.972
14	carbazole	3,6-Diiodocarbazole	-	-	-	Michael Acceptor/SN1	mutagenic	not predicted	yes	0.026	0.025		0.007	0.002			0.006	0.155	0.363		0.076	0.016			0.023
15	carbazole	3-Bromocarbazole	-	-	-	Michael Acceptor/SN1	mutagenic	not predicted	yes		0.002		0.001	0.003	0.008	0.003	0.008		0.022		0.016	0.022	0.035	0.010	0.031
16	carbazole	3-Bromocarbazole	-	-	-	Michael Acceptor/SN1	no data	no data	no	0.005			0.014				0.014	0.031			0.155				0.052
17	carbazole	3-Iodocarbazole	-	-	-	Michael Acceptor/SN1	mutagenic	not predicted	yes	0.050	0.032	0.018	0.014	0.006	0.030	0.035	0.032	0.297	0.458	0.104	0.155	0.051	0.128	0.135	0.117
18	carbazole	Carbazole	2	2	2	Michael Acceptor/SN1	non-mutagenic	not predicted	no	5.815	1.049	2.727	1.486	6.484	4.175	5.404	2.056	34.206	14.979	16.042	16.512	54.034	18.151	20.783	7.616
19	chrorinated hydrocarbons	Pentachloronitrobenzene	-	-	-	SN1	mutagenic	active	yes				0.182	0.220			0.240			1.072	2.443				0.888
20	halogenated PAH	4,4'-DDMU	-	-	-	Michael Acceptor/SN1	non-mutagenic	active	no				0.028				0.017			0.164					0.061
21	indole	2-Phenylindole	-	-	-	Michael Acceptor/SN1	mutagenic	not predicted	yes					0.319	0.310							2.660	1.346		
22	indole	7-Azaindole	-	-	-	no	non-mutagenic	active	no							0.204								0.784	
23	nitroarene	2,4-Dinitroaniline	-	-	-	Michael Acceptor/SN1	mutagenic	active	yes	0.017	0.009	0.022	0.021	0.009	0.035	0.070	0.040	0.099	0.129	0.127	0.228	0.079	0.153	0.269	0.147
24	nitroarene	1-Nitropyrene	2	2	-	Michael Acceptor	mutagenic	active	yes					0.014	0.012	0.025						0.117	0.050	0.097	
25	nitroarene	3-Nitrofluoranthene	2	-	-	Michael Acceptor/SN1	mutagenic	active	yes				0.007		0.015	0.012	0.027				0.039		0.126	0.053	0.105	
26	nitroarene	4-Nitropyrene	-	2	2	Michael Acceptor/SN1	mutagenic	active	yes	0.005	0.009	0.008	0.009	0.010	0.020	0.009	0.017	0.028	0.122	0.048	0.102	0.087	0.087	0.033	0.064
27	nitroarene	6H-Benzo[cd]pyrene-6-one	-	-	-	Michael Acceptor/SN1	non-mutagenic	active	no	1.958	0.561	1.334	0.685	1.400	2.183	24.129	1.276	11.517	8.016	7.844	7.616	11.664	9.491	92.804	4.724
28	nitroarene	6-Nitrobenzo[a]pyrene	-	-	-	Michael Acceptor/SN1	mutagenic	active	yes	0.561	2.021	1.877	0.312					3.301	28.870	11.039	3.472				
29	nitroarene	9-Nitroanthracene	-	-	-	Michael Acceptor/SN1	mutagenic	active	yes	0.004	0.004			0.005	0.006	0.015		0.025	0.061			0.038	0.027	0.058	
30	PAH	1,2-Dihydroxyanthraquinone	-	-	-	Michael Acceptor/Acyl Transfer Agent	mutagenic	active	yes		0.030				0.243	0.418	0.094		0.433				1.057	1.606	0.349
31	PAH	1,3-Dihydroxyanthraquinone	-	-	-	Michael Acceptor	mutagenic	active	yes					0.009	0.372	0.455	0.159					0.079	1.618	1.751	0.590
32	PAH	1,7-Phenanthroline	-	-	-	Michael Acceptor	mutagenic	active	yes					0.020		0.052						0.169		0.201	
33	PAH	1,8-Dihydroxyanthraquinone	-	1B	-	Michael Acceptor	mutagenic	active	yes	0.379	0.029	0.120	0.079					2.231	0.409	0.704	0.880				
34	PAH	1,9-Benz-10-anthrone	-	-	-	Michael Acceptor	mutagenic	active	yes	2.958	0.863	2.716	1.032	8.280	8.553	34.892	1.848	17.397	12.334	15.975	11.469	68.998	37.185	134.200	6.845
35	PAH	10-Azabenzo[a]pyrene	-	-	-	Michael Acceptor	mutagenic	active	yes	5.417	1.972	2.745	1.759	6.738	6.916	13.056	2.783	31.866	28.165	16.148	19.540	56.154	30.072	50.217	10.308
36	PAH	2-Acetylfluorene	-	-	-	Michael Acceptor	mutagenic	active	yes	3.042	2.006	2.475	1.372	2.242	7.260	19.908	4.237	17.894	28.663	14.558	15.241	18.680	31.565	76.568	15.694
37	PAH	2-Acetylphenanthrene	-	-	-	Michael Acceptor/SN1	mutagenic	not predicted	yes	0.084	0.030	0.064	0.058	0.088	0.191	0.896	0.142	0.492	0.422	0.378	0.646	0.730	0.829	3.447	0.524
38	PAH	2-Hydroxyanthraquinone	-	-	-	Michael Acceptor	mutagenic	active	yes	0.089	0.052	0.119	0.054	0.069	0.206	0.501	0.150	0.526	0.740	0.702	0.600	0.579	0.895	1.926	0.555
39	PAH	2-Methylanthraquinone	-	-	-	Michael Acceptor	mutagenic	active	no	2.999	0.843	1.737	0.895	2.797	4.807	15.749	2.192	17.641	12.045	10.219	9.949	23.311	20.900	60.573	8.118
40	PAH	3-Acetylphenanthrene	-	-	-	Michael Acceptor/SN1	mutagenic	not predicted	yes	0.095	0.033	0.058	0.038	0.100	0.194	0.899	0.159	0.559	0.469	0.344	0.427	0.830	0.843	3.457	0.588
41	PAH	4H-Cyclopenta[def]phenanthrene	-	-	-	Michael Acceptor	mutagenic	active	yes	16.695	3.066	8.553	4.336	26.769	55.488	296.560	7.497	98.208	43.802	50.314	48.173	223.072	241.252	1140.614	27.768
42	PAH	9-Acetylanthracene	-	-	-	Michael Acceptor	mutagenic	active	yes	0.019	0.060	0.022	0.019	0.148	0.295	1.212	0.209	0.113	0.857	0.129	0.206	1.237	1.284	4.661	0.773
43	PAH	9-Fluorenone	-	-	-	Michael Acceptor	non-mutagenic	active	no	3.336	1.661	2.901	2.044	3.768	6.267	8.591	3.435	19.625	23.723	17.064	22.712	31.399	27.250	33.041	12.722
44	PAH	9-vinylanthracene	-	-	-	Michael Acceptor	mutagenic	active	yes	0.563	0.096	0.204	0.148	1.109	1.083	6.265	0.257	3.313	1.369	1.201	1.643	9.240	4.708	24.098	0.953
45	PAH	Anthracene	1B	2	-	Michael Acceptor	mutagenic	active	yes	44.607	4.219	10.171	3.736	95.806	33.157	375.258	7.386	262.396	60.266	59.826	41.516	798.380	144.159	1443.300	27.355
46	PAH	Anthraquinone	-	1B	-	Michael Acceptor	mutagenic	active	yes	1.162	0.586	1.007	0.565	0.944	1.282	2.720	0.804	6.833	8.375	5.925	6.275	7.870	5.573	10.463	2.979
47	PAH	Benz[a]anthracene-7,12-dione	-	-	-	Michael Acceptor	mutagenic	active	yes	1.519	0.773	1.033	0.765	1.478	4.167		1.361	8.937	11.037	6.075	8.502	12.313	18.118		5.041
48	PAH	Benz[a]anthracene	1B	-	-	Michael Acceptor/SN1	mutagenic	active	yes	132.148	27.287	39.072	27.813	320.658	181.009	891.139	44.341	777.340	389.938	229.833	309.031	2672.149	786.997	3427.456	164.226
49	PAH	Benzo[b]fluorene	-	-	-	Michael Acceptor	mutagenic	active	yes	23.052	6.229	7.325	4.711	92.906	59.598	474.365	11.038	135.600	88.984	43.090	52.347	774.213	259.121	1824.480	40.881
50	PAH	Benzo[a]fluorene-11-one	-	-	-	Michael Acceptor	mutagenic	active	yes	7.513	2.907	3.930	3.054	10.060	10.442	17.901	4.652	44.194	41.530	23.116	33.938	83.835	45.400	68.851	17.229
51	PAH	Benzo[a]pyrene	1B	1B	1B	Michael Acceptor/SN1	mutagenic	active	yes	113.043	17.908	29.668	18.038	190.748	126.858	858.196	30.456	664.959	255.833	174.521	200.419	1589.568	551.557	3300.754	112.800
52	PAH	Benzo[b]fluoranthene	-	1B	-	Michael Acceptor/SN1	mutagenic	not predicted	yes	210.219	31.461	51.323	41.109	362.575	230.015	1098.913	62.934	1236.582	449.439	301.899	456.768	3021.457	1000.065	4226.590	233.088
53	PAH	Benzo[b]fluorene-11-one	-	-	-	Michael Acceptor	mutagenic		no	8.368	2.634	4.239	2.979	11.016	11.566	31.230	4.571	49.221	37.628	24.935	33.101	91.799	50.288	120.115	16.930
54	PAH	Benzo[e]pyrene	-	1B	-	Michael Acceptor/SN1	mutagenic	active	yes	113.167	11.077	17.076	14.000	106.302	74.532	363.967	18.467	665.687	158.241	100.449	155.558	885.851	324.051	1399.872	68.398
55	PAH	Benzo[ghi]perylene	-	-	-	Michael Acceptor	mutagenic	active	yes	68.159	9.595	18.783	10.459	94.920	86.953	496.784	24.455	400.937	137.076	110.490	116.214	791.002	378.057	1910.706	90.573
56	PAH	Benzo[k]fluoranthene	-	-	-	Michael Acceptor	mutagenic	not predicted	yes	63.425	9.651	16.867	13.080	105.295	70.286	343.054	18.538	373.086	137.869	99.218	145.338	877.457	305.589	1319.439	68.659
57	PAH	Chrysene	2	1B	-	Michael Acceptor/SN1	mutagenic	not predicted	yes	140.291	28.771	41.278	29.450	339.951	192.116	945.504	46.628	825.244	411.020	242.814	327.226	2832.923	835.289	3636.554	172.694
58	PAH	Cyclopenta[cd]pyrene	-	-	-	Michael Acceptor	mutagenic	not predicted	yes	115.976	23.950	34.221	24.188	291.138	166.172	948.056	38.580	682.212	342.137	201.301	268.751	2426.147	722.486	3646.369	142.888
59	PAH	Dibenz[a,h]anthracene	1B	-	-	Michael Acceptor/SN1	mutagenic	active	yes	15.307	2.756	4.185	2.654	31.896	22.073	106.844	6.447	90.042	39.376	24.616	29.489	265.798	95.971	410.938	23.878
60	PAH	Fluoranthene	-	-	-	Michael Acceptor	mutagenic	not predicted	yes	217.839	46.940	347.069	50.870	723.142	2252.685	11958.879	181.604	1281.406	670.566	2041.580	565.219	6026.181	9794.282	45995.689	672.608
61	PAH	Fluorene	-	-	-	Michael Acceptor/SN1	non-mutagenic	inactive	no	65.937	8.944	34.037	9.048	72.086	154.072	327.756	24.279	387.863	127.767	200.219	100.529	600.716	669.879	1260.598	89.924
62	PAH	Indeno[1,2,3-cd]fluoranthene	-	-	-	Michael Acceptor	non-mutagenic	active	no	6.996	0.194	0.553	0.264	2.090	2.026	10.576	0.598	41.151	2.778	3.250	2.937	17.488	8.807	40.678	2.214
63	PAH	Indeno[1,2,3-cd]pyrene	-	2	2	Michael Acceptor	mutagenic	not predicted	yes	75.119	12.196	21.712	12.449	116.043	99.160	575.792	28.843	441.879	174.231	127.719	138.322	967.028	431.129	2214.583	106.826
64	PAH	m-Terphenyl				Michael Acceptor	non-mutagenic	not predicted	no	1.056	0.352	0.548	0.511	2.497	2.292	9.249	0.851	6.214	5.026	3.224	5.680	20.812	9.965	35.574	3.153
65	PAH	O-Terphenyl				Michael Acceptor	non-mutagenic	not predicted	no	0.094	0.018	0.042	0.047	0.197	0.179	0.732	0.109	0.551	0.262	0.249	0.521	1.643	0.777	2.817	0.402
66	PAH	Perylene	-	-	-	Michael Acceptor	mutagenic	active	yes	36.903	63.453	113.520	8.155	55.149	151.709	239.929	23.403	217.074	906.468	667.765	90.607	459.572	659.603	922.803	86.679
67	PAH	Phenanthren-9,10-dione	-	-	-	Michael Acceptor/Schiff base formation	non-mutagenic	not predicted	no	0.951	0.485	0.643	0.407	0.442	0.829	2.233	0.436	5.594	6.933	3.780	4.524	3.685	3.604	8.589	1.614
68	PAH	Phenanthrene	1	-	-	Michael Acceptor/SN1	mutagenic	not predicted	yes	141.833	22.644	69.261	34.904	219.644	326.508	1008.698	46.375	834.310	323.481	407.420	387.828	1830.368	1419.600	3879.608	171.761
69	PAH	Pyrene	-	-	-	Michael Acceptor	mutagenic	inactive	yes	200.403	32.144	78.053	43.319	395.150	460.829	4039.157	59.287	1178.842	459.202	459.138	481.325	3292.920	2003.603	15535.221	219.582
70	PCB	PCB 101	-	-	-	Michael Acceptor	non-mutagenic	not predicted	no	0.346	0.024	0.384	0.059	1.477	0.811	4.727	0.233	2.033	0.340	2.260	0.656	120.620	3.527	18.182	0.864
71	PCB	PCB 118	-	-	-	Michael Acceptor	non-mutagenic	not predicted	no	0.147	-0.020	0.106	0.028	3.061	0.365	1.034	0.112	0.867	-0.282	0.623	0.309	25.509	1.588	3.976	0.416
72	PCB	PCB 138	-	-	-	Michael Acceptor	non-mutagenic	not predicted	no	0.831	0.045	0.814	0.196	25.638	1.312	9.328	0.514	4.886	0.637	4.788	2.178	213.649	5.705	35.876	1.904
73	PCB	PCB 149	-	-	-	Michael Acceptor	non-mutagenic	not predicted	no	0.655	0.030	0.703	0.138	22.814	1.272	9.205	0.442	3.854	0.425	4.136	1.533	190.113	5.530	35.403	1.635
74	PCB	PCB 153	-	-	-	Michael Acceptor	non-mutagenic	not predicted	no	1.123	0.076	1.094	0.232	32.355	1.751	11.957	0.633	6.604	1.089	6.436	2.577	269.626	7.615	45.989	2.346
75	PCB	PCB 170	-	-	-	Michael Acceptor	non-mutagenic	not predicted	no	0.321		0.365	0.059	9.395	0.315	4.395	0.148	1.891		2.147	0.661	78.296	1.369	16.905	0.547
76	PCB	PCB 18	-	-	-	Michael Acceptor	non-mutagenic	not predicted	no	0.061	0.011	0.109	0.063	0.381	0.208	0.544	0.207	0.360	0.161	0.642	0.705	3.173	0.906	2.092	0.766
77	PCB	PCB 180	-	-	-	Michael Acceptor	non-mutagenic	not predicted	no	0.645	0.039	0.689	0.136	17.807	0.711	6.090	0.316	3.797	0.558	4.055	1.516	148.388	3.093	31.115	1.170
78	PCB	PCB 194	-	-	-	Michael Acceptor	non-mutagenic	not predicted	no			0.061		2.897		0.919				0.358		24.145		3.536	
79	PCB	PCB 28/31								0.099	0.023	0.139	0.094	0.293	0.266	0.608	0.315	0.584	0.326	0.819	1.040	2.443	1.158	2.340	1.167
80	PCB	PCB 44	-	-	-	Michael Acceptor	non-mutagenic	not predicted	no	0.085	0.017	0.138	0.062	2.408	0.255	1.182	0.176	0.500	0.246	0.811	0.693	20.063	1.109	4.546	0.650
81	PCB	PCB 52	-	-	-	Michael Acceptor	non-mutagenic	not predicted	no		0.028	0.144	0.041	2.223	0.181	0.511	0.152		0.396	0.849	0.455	18.523	0.789	1.965	0.565
82	phenanthroline	1,10-Phenanthrolin-5,6-dion	-	-	-	Schiff base formation/Michael Acceptor	mutagenic	active	yes								0.116								0.431
83	phenanthroline	1,10-Phenanthroline	-	-	-	Michael Acceptor	non-mutagenic	active	no	0.189	0.058	0.177	0.112	0.070	0.280	0.350	0.327	1.110	0.832	1.038	1.240	0.583	1.218	1.345	1.209
84	polycyclic heteroarene	1H-Benzo[g]indole	-	-	-	Michael Acceptor	mutagenic	not predicted	yes	0.078	0.021		0.033	0.062	0.073		0.046	0.457	0.297		0.364	0.517	0.316		0.172
85	polycyclic heteroarene	Acridone	-	-	-	Michael Acceptor/for SN 1	non-mutagenic	active	no	0.596	0.096	0.501	0.511	0.399	0.973		1.623	3.506	1.376	2.944	5.683	3.327	4.232		6.010
86	polycyclic heteroarene	Anthracene-1,4-dione	-	-	-	Michael Acceptor	mutagenic	active	yes	4.594	0.955	2.837	1.304	4.735	7.120	19.346	2.758	27.022	13.648	16.689	14.484	39.456	30.957	74.409	10.213
87	polycyclic heteroarene	Benz[a]acridine	-	-	-	Michael Acceptor	mutagenic	active	yes	4.081	0.532	0.944	0.969	2.779	4.141	7.800	1.627	24.008	7.603	5.552	10.764	23.160	18.005	30.002	6.024
88	polycyclic heteroarene	Dibenz[a,j]acridine	2	2	-	Michael Acceptor/SN1	mutagenic	active	yes	3.968	1.363	1.731	0.934	5.062	4.909	13.941	1.786	23.344	19.474	10.183	10.377	42.180	21.345	53.620	6.615
89	polycyclic heteroarenes	1-Phenylnaphthalin	-	-	-	Michael Acceptor	non-mutagenic	not predicted	no	2.484	0.491	1.254	0.674	4.284	5.461	31.522	1.084	14.610	7.015	7.378	7.485	35.703	23.741	121.237	4.016
90	polycyclic heteroarenes	p-Benzyldiphenyl	-	-	-	Michael Acceptor	non-mutagenic	active	no					0.092			0.300					0.767			1.110
91	polycyclic heteroarenes	p-Terphenyl	-	-	-	Michael Acceptor	non-mutagenic	not predicted	no	0.736	0.217	0.344	0.378	2.092	1.476	4.980	0.587	4.327	3.107	2.021	4.198	17.171	6.418	19.155	2.174
92	PPP	Dicofol	-	-	-	Michael Acceptor/SN1	non-mutagenic	active	no	0.043	0.010	0.290	0.043	0.048	0.115	0.454	0.254	0.254	0.143	1.706	0.477	0.397	0.498	1.746	0.940
93	PPP	Diphenyl sulfone	-	-	-	Michael Acceptor	non-mutagenic	not predicted	no	0.023		0.071	0.085	0.176	0.406	0.624	0.270	0.137		0.417	0.944	1.471	1.766	2.400	1.001
94	PPP	Xanthone	-	-	-	Michael Acceptor	mutagenic	active	yes	0.136	0.058	0.122	0.080	0.135	0.337	0.745	0.284	0.799	0.831	0.717	0.887	1.127	1.464	2.866	1.053

1: Pubchem® open source. According to the Globally Harmonized System of Classification and Labelling of Chemicals (GSH). Values derived from different databases, e.g., ECHA

2: Toxtree (v. 3.1.0, Ideaconsult Ltd.). Schiff base: Derivate of ketone or aldehyde with imine (R1R2C=NR3) or azomethine (RHC=NR') instead of a carboxyl group (Schiff, 1864). Michael Acceptor: Substituent from the nucleophilic addition of a nucleophile to an electron-withdrawing group (Michael, 1887). SN1: Nucleophilic substitution with unimolecular rate-determining step (slowest step). SN2: Nucleophilic substitution with bi-molecular rate-determining step. Acyl Transfer agent: Agent which transfers an acetyl group.

3: VEGA (v. 1.1.5) / modus CONSENSUS v. 1.0.3

S10

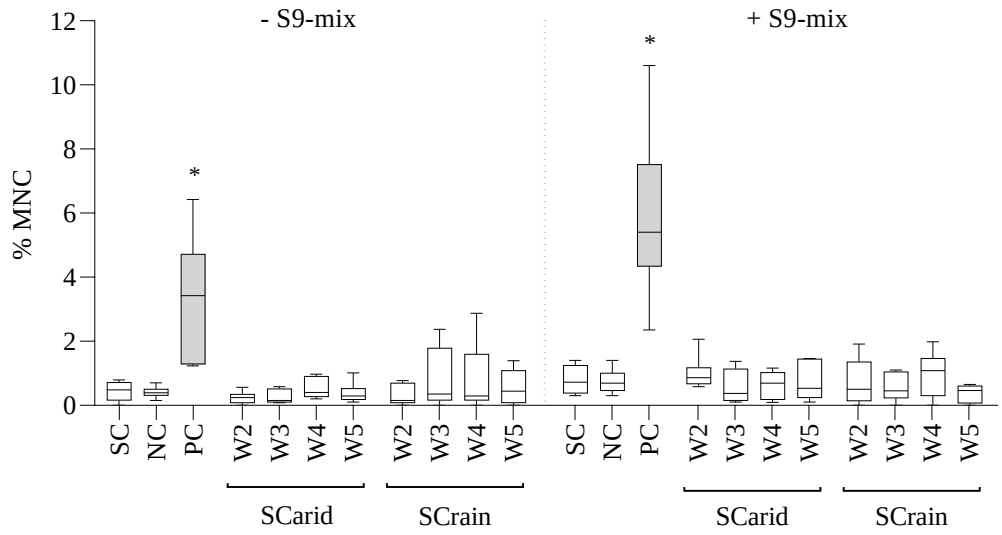

Figure S10: Average micronuclei rate in organic sediment extracts (25 g SEQ dw/mL). Error bars indicate a standard deviation from three independent replicates. Statistically significant differences to the negative control (two-way ANOVA, $p<0.05$) are signed with '*'.

S11

Table S11: Evaluation of the sediment quality class according to the chemical and ecotoxicological analysis. The desirable goal of the chemical sediment quality is class II.

		SCarid				SCrain			
		W2	W3	W4	W5	W2	W3	W4	W5
class Ecotox	Micronucleus assay	I	I	I	I	I	I	I	I
	Ames fluctuations assay	III	III	III	III	IV	IV	IV	IV
class Chemistry	PCBs	II	II	II	II	IV	II	II-III	II
	DDT	-	-	I-II	-	-	-	-	I-II
	EPA-PAHs	II	II	II	II	III	III	VI	I

For quality characterization of sediment samples from the examined sampling sites, the authors applied the sediment quality strategy according to Ahlf et al. (2002) and adjusted to the current study by a combination of the ecotoxicological and chemical target analysis for the substances with established threshold values (EPA-PAHs, PCBs, organochlorine insecticide 1,1,1-trichloro-2,2-*bis*(*p*-chlorophenyl) ethane, DDT). The threshold values for chemical sediment quality classes were updated according to the position paper of the German Federal/State Working Group on Water (*ger*. LAWA) 'Integrated sediment management in river basins' (LAWA, 2019). The sediment quality strategy represents a multifactorial procedure for sediment classification with the desirable goal of reaching the sediment class II or smaller. The ecotoxicological analysis showed more pronounced genotoxic potential within the Ames fluctuation assay during SC_{rain} and rated as a sediment ecotoxicological class IV. Sediments during the SC_{arid} were assigned with the sediment ecotoxicological class III. The negative results in the Micronucleus assay at all sediment samples were rated as the ecotoxicological sediment class I. Based on quantified concentrations of chemicals target compounds, all sediments sampled during dry weather revealed a desirable chemical status of class I-II. In contrast, under fluctuating hydrological conditions in 2018, PAHs exceeded the threshold value of the chemical class II (1-4 µg/g), reaching the worst chemical class of VI (>35 µg/g) at the effluent sampling site W4. PCBs failed the threshold value for the chemical class II of 0.2 µg/g at the sampling site W2 and W4. The concentration of DDT was not of concern, explained by its continuous degradation in the environment (Ricking and Schwarzbauer, 2012).

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ANNEX 2

Title: Extensive rain events have a more substantial impact than advanced effluent treatment on the endocrine-disrupting activity in an effluent-dominated small river

Journal: Science of the Total Environment

Contributing authors: Aliaksandra Shuliakevich, Katja Schroeder, Laura Nagengast, Yvonne Wolf, Ira Brückner, Melis Muz, Peter A. Behnisch, Henner Hollert, Sabrina Schiwy

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Table Annex 2: Author contributions for Annex 2.

Concept and design	Aliaksandra Shuliakevich	50%
	Henner Hollert	20%
	Sabrina Schiwy	30%
Conducting tests and experiments	Aliaksandra Shuliakevich (ERα- and anti-AR-CALUX® assays with sediment extracts from the sampling campaigns SCR 1 and SCR 2_S; ERα- and anti-AR-CALUX® assays with water and particulate matter extracts from the sampling campaign SCR 2_W; analysis of the sediment total organic carbon)	60%
	Katja Schroeder (anti-AR-CALUX® assays with sediment extracts from the sampling campaign SCR_1; analysis of the sediment total organic carbon)	12.5%
	Laura Nagengast (ERα- and anti-AR-CALUX® assays with sediment extracts from the sampling campaign SCR 2_S)	12.5%
	Yvonne Wolf (ERα- and anti-AR-CALUX® assays with water extracts from the sampling campaign SCR 2_W)	10%
	Melis Muz (chemical analysis)	5%
Compilation of data sets and figures	Aliaksandra Shuliakevich (compilation of data sets and figures of all bioassays and supplementary information)	60%
	Katja Schroeder (supportive compilation of data sets from the CALUX® assay with sediment extracts from the sampling campaign SCR 1)	10%
	Laura Nagengast (supportive compilation of data sets from the CALUX® assay with sediment extracts from the sampling campaign SCR 2_S)	10%
	Yvonne Wolf (compilation of data sets from the CALUX® assay with water extracts from the sampling campaign SCR 2_W)	10%
	Melis Muz (compilation of data sets of the chemical analysis)	10%
Analysis and interpretation of data	Aliaksandra Shuliakevich (analysis and interpretation of all data generated in CALUX® assays, supportive analysis, and interpretation of data generated during chemical analysis)	60%
	Katja Schroeder (supportive analysis and interpretation of data generated in CALUX® assays)	2.5%
	Laura Nagengast (supportive analysis and interpretation of data generated in CALUX® assays)	2.5%
	Yvonne Wolf (supportive analysis and interpretation of data generated in CALUX® assays)	2.5%
	Melis Muz (analysis and interpretation of data generated during chemical analysis)	2.5%
	Henner Hollert (interpretation of data)	15%
	Sabrina Schiwy (interpretation of data)	15%

continuation of the table on the next page

Table Annex 2 (continuation): Author contributions for Annex 2

Drafting of manuscript	Aliaksandra Shuliakevich	40%
	Katja Schroeder	5%
	Laura Nagengast	5%
	Yvonne Wolf	5%
	Ira Brückner	5%
	Melis Muz	5%
	Peter A. Behnisch	5%
	Henner Hollert	10%
	Sabrina Schiwy	20%

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Extensive rain events have a more substantial impact than advanced effluent treatment on the endocrine-disrupting activity in an effluent-dominated small river

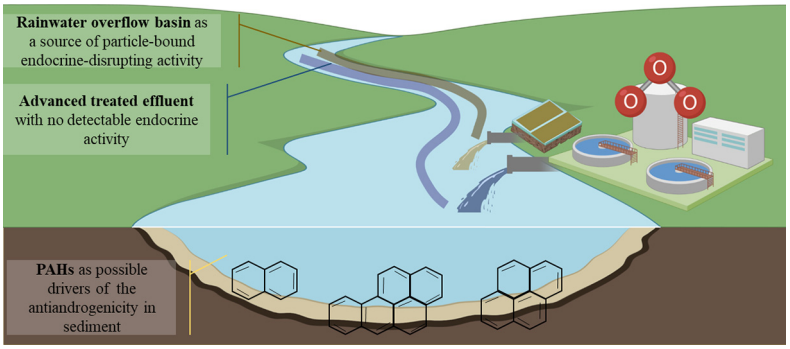
Aliaksandra Shuliakevich [a], Katja Schroeder [b], Laura Nagengast [b], Yvonne Wolf [b], Ira Brückner [c], Melis Muz [d], Peter A. Behnisch [e], Henner Hollert [a,*], Sabrina Schiwy [a]

[a] Goethe University Frankfurt/Main, Department Evolutionary Ecology and Environmental Toxicology, Max-von-Laue-Strasse 13, 60438 Frankfurt/Main, Germany
[b] RWTH Aachen University, Institute of Biology V, Worringerweg 1, 52074 Aachen, Germany
[c] Eifel-Rur Waterboard (WVER), Eisenbahnstr. 5, 52354 Düren, Germany
[d] Helmholtz Centre for Environmental Research UFZ, Department of Effect-Directed Analysis, Permoserstrasse 15, 04318 Leipzig, Germany
[e] BioDetection Systems B.V. (BDS), Science Park 406, 1098 XH Amsterdam, the Netherlands

HIGHLIGHTS

- Rainwater overflow basin is a possible driver for endocrine-disrupting activity in water and in sediment phases
- Ozonation can eliminate the estrogenic and antiandrogenic activity from the effluent measured within the CALUX® assays
- Particle-bound PAHs are possible drivers of the antiandrogenicity in sediment

GRAPHICAL ABSTRACT



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ABSTRACT

Wastewater treatment plants (WWTPs) remain an important primary source of emission for endocrine-disrupting compounds in the environment. As an advanced wastewater treatment process, ozonation is known to reduce endocrine-disrupting activity. However, it remains unclear to which extend improved wastewater treatment may reduce the endocrine-disrupting activity in the receiving water body. The present study investigated possible factors for the endocrine-disrupting activity in a small receiving water body, the Wurm River (North-Rhine Westphalia, Germany), up- and downstream of a local WWTP. The cell-based reporter gene CALUX® assay was applied to identify the endocrine-disrupting activity in the water, sediment, and suspended particulate matter. The water phase and the effluent sampling were primarily driven by applying the full-scale effluent ozonation (sampling campaigns in June 2017 and March 2019). In contrast, the sediment sampling aimed to compare the particle-bound endocrine-disrupting activity during dry (June 2017) and rainy summer (June 2018) seasons. The water phase showed low to moderate estrogenic/antiandrogenic activity. Advanced effluent treatment by ozonation led to a complete reduction of the endocrine-disrupting activity according to

Abbreviations: AA-EQS, average environmental quality standards; BDS, BioDetection Systems b.v.; CALUX, chemical-activated luciferase expression; DCM, dichloromethane; DDT, dichlorodiphenyltrichloroethane; DHT, dihydrotestosterone; DMSO, dimethylsulfoxide; E1, estrone; E2, 17β-estradiol; EBT, effect-based trigger value; EDC, endocrine-disrupting compound; EDP, endocrine-disrupting potential; EE2, 17α-ethinyl estradiol; EU, European Union; Flu, flutamide; HLB, hydrophilic-lipophilic balance sorbent; MeOH, methanol; MTT, 3-(4,5-dimethyltetrazolium-2-yl)-2,5-diphenyltetrazolium bromide; *o,p′*-DDT, dichlorodiphenyltrichloroethane (isomeric impurity of a commercial DDT-mixture); PAH, polycyclic aromatic hydrocarbon; SEQ, sediment equivalent; SPM, suspended particulate matter; SPMEQ, suspended particulate matter equivalent; WWTP, wastewater treatment plant.

* Corresponding author at: Goethe University Frankfurt, Max-von-Laue-Str. 13, 60438 Frankfurt am Main, Germany.
E-mail address: Hollert@bio.uni-frankfurt.de (H. Hollert).

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0048-9697/

Stormwater
CALUX® assay
Rainwater overflow

the limit of detection of the CALUX® assays. The suspended particulate matter originated from the water phase of the second sampling campaign revealed antiandrogenic activity only. Sediments at the sampling sites along the local WWTP revealed higher estrogenic and antiandrogenic activity after extensive rain events and were not affected by the ozonated effluent. Fluctuation patterns of the endocrine-disrupting activity in sediments were in line with fluctuated concentrations of polycyclic aromatic hydrocarbons. Rainwater overflow basin release was suggested as a vector for particle-bound and dissolved endocrine-disrupting activity in the receiving water body. The present study underlined the necessity for monitoring both water and sediment phases to achieve reliable profiling of the endocrine-disrupting activity. The receptor-mediated CALUX® assays were proven to be suitable for investigating the endocrine-disrupting activity distribution in different river compartments and WWTP effluents.

1. Introduction

The European Water Framework Directive requires the Member States to monitor the European water bodies' quality status. Thus, to achieve a good chemical status, Environmental Quality Standards for 53 priority substances should not be exceeded (European Parliament and Council, 2013). Among them are 28 potential endocrine-disrupting compounds (EDCs), mainly represented by plant protection chemicals (Escher et al., 2018; Montes-Grajales and Olivero-Verbel, 2015). Münze et al. (2017) identified wastewater treatment plants (WWTPs) as an important point source for plant protection chemicals in aquatic ecosystems resulting in a significantly higher concentration of plant protective chemicals downstream of the effluent. Furthermore, the three most potent steroidal estrogens (17α-ethinyl estradiol, EE2, 17β-estradiol, E2, and estrone, E1) were included in the Watch List as candidates for prioritization (European Commission, 2018).

The presence of EDCs in freshwater ecosystems and their possible adverse effects on the reproduction of humans and wildlife is one of the most intensively discussed pollution-related hazards within the last two decades (Jobling and Tyler, 2006; Kidd et al., 2007; Trasande et al., 2015). WWTPs are the primary point source of EDCs in the environment worldwide (EEA, 2018; Eggen et al., 2014; Sonavane et al., 2018). The study of Purdom et al. (1994) was the first one, describing alterations in the sex ratio of fish exposed to a WWTP effluent. Subsequently, numerous scientific studies investigated adverse effects of synthetic (plasticizers, pesticides, industrial chemicals, drugs) and natural (phytoestrogens, excreted hormones) EDCs in WWTP effluents (Jarošová et al., 2014; Johnson et al., 2007; Vajda et al., 2008; Vajda et al., 2011; Vethaak et al., 2005). However, effect-based monitoring of WWTP effluents is still not required at the EU level (Wigh et al., 2018). Advanced wastewater treatment by ozonation is increasingly used to eliminate EDCs (Escher et al., 2009; Giebner et al., 2018). Ozone-induced hydroxyl radicals (•OH) oxidize chemical compounds to mostly less toxic, short-chain, and hydrophilic transformation products (Sonntag and von Gunten, 2012).

Due to humic acids' adsorption capability, hydrophilic EDCs (e. g., steroid hormones) might be detected in sediment (Hollert et al., 2005; Liu et al., 2014; Neale et al., 2009; Sangster et al., 2015). However, many EDCs are quite hydrophobic and tend to sorb to particles. For example, ubiquitously present and persistent polycyclic aromatic hydrocarbons (PAHs) resulted from asphalt abrasion, tire wear, incomplete combustion of vehicle exhausts, and other organic materials were shown to be not typical EDCs providing a long-term endocrine-disrupting potential (EDP) in aquatic ecosystems. In this context, Hilscherova et al. (2002) and Hilscherová et al. (2010) identified PAHs as primary compounds contributing to estrogenicity in sediments from the Drevnice River (Czech republic), which is a small water body affected by WWTP effluents. Additionally, the endocrine-disrupting potencies of several PAHs were described as being comparable to those of such xenoestrogens as alkylphenols or bisphenol A. Brinkmann et al. (2014) and Alvarez-Muñoz et al. (2015) showed adverse effects of PAHs on the population level of estuarian clams *Scrobicularia plana* with intersex combined with high antiandrogenic activity in sediment. Furthermore, PAHs were identified as potential EDCs in crude oil, representing one more source of potential EDCs in the environment (Johann et al., 2020). The ubiquitous presence of PAHs in aquatic ecosystems and their high relevance in the toxicology of humans and wildlife (multiple adverse effects) justify the necessity of investigating the potential endocrine activity of environmental PAHs (Brinkmann et al., 2013; Maqbool et al., 2016).

Due to climate change, extensive rain events, and early snowmelts, the discharge frequency of untreated wastewater from rainwater overflow basins of WWTPs into receiving water bodies may increase (EEA, 2019). In addition, an unbalanced concentration ratio of chemical compounds in sediment and water can release hydrophilic and hydrophobic particle-bound EDCs into the water phase (Hollert et al., 2007; Macikova et al., 2014; Parsons et al., 2007). Therefore, based on the previous considerations, monitoring both the water and the particulate matter phase is essential to gain complete information about the chemical burden in aquatic ecosystems.

The cell-based reporter gene CALUX® assays (Chemical-Activated LUciferase eXpression) were multiple times successfully applied to investigate the endocrine-disrupting activity in the aquatic environment due to their precision and reproducibility (Houtman et al., 2007; Kase et al., 2018; Könemann et al., 2018; Kunz et al., 2017; Leusch et al., 2010; Vethaak et al., 2005). Furthermore, as some EDCs such as triclosan may interact with the androgenic receptor (Rostkowski et al., 2011), it is necessary to investigate both agonistic and antagonistic potencies. Effect-based methods, which detect complex mixtures via their responses in biotests (Hollert et al., 2005; Wernersson et al., 2015), have been proposed to monitor chemical pollution in surface waters (Brack et al., 2019; Posthuma et al., 2019) because of the complexity of mixtures and often insufficient chemical detection limits for potent EDCs.

The present study focuses on identifying the endocrine activity in the water, suspended particulate matter, and sediments under different weather and wastewater treatment conditions utilizing the *in vitro* CALUX® assays taking the Wurm River as a case study. This river is a small stream in the west of Germany (federal state North-Rhine Westphalia, Aachen) highly impacted by anthropogenic activities, serving as the receiving water body for eight WWTPs (MKULNV NRW, 2015). These include the Aachen-Soers WWTP, one of Germany's biggest WWTP (max. daily inflow rate: 3000 L/s, annual wastewater treatment capacity: 27 Mio. m^3). The wastewater effluent from the Aachen-Soers WWTP accounts for more than two-thirds of the Wurm River's total water discharge. To reduce the impact of wastewater contaminants on water quality, the Aachen-Soers WWTP received a full-scale ozonation treatment of the effluent in 2018. Researchers and stakeholders accompanied its implementation within the $DemO_3^{AC}$ project (https://demo3ac.wver.de/index.php/en/). The present study was a part of the $DemO_3^{AC}$ project.

Within this study, the authors pursued (i) to describe the distribution of the endocrine-disrupting activity in the receiving water body among different environmental compounds at opposite extreme weather conditions; (ii) to assess a possible contribution of full-scale

ozonation as an advanced treatment step of the WWTP effluent, to reduce the endocrine-disrupting activity in the receiving water body; (iii) to evaluate possible PAHs contribution to the observed endocrine-disrupting activity.

2. Material and methods

2.1. Study area and design

The Wurm River extends over 356 m^2, predominantly shaped by urban and agricultural land use (see Fig. 1).

The current study was carried out in a section in the middle course of the Wurm River up- and downstream of the Aachen-Soers WWTP at four sampling sites (W2, W3, W4, and W5) (see Table 1). The sampling site W4 represented the sampling site directly at the effluent of the WWTP Aachen-Soers. In that case, the sample W4 from SCR 1 and SCR 2_W was the effluent itself, while sediment W4 was sampled close to the effluent of the Aachen-Soers WWTP.

The first sampling campaign took place in June 2017 (SCR 1). It included water and sediment sampling at stable hydrological conditions during a dry summer period before implementing the full-scale effluent ozonation. A sampling of SPM during SCR 1 was not conducted because surface sediments could be used as a proxy for suspended particulate matter (SPM) (Niu et al., 2021). The second sampling campaign for each environmental compartment was carried out at different dates due to the following arguments:

(i) the water sampling (SCR 2_W) was driven by progress in implementing the full-scale ozonation within the Aachen-Soers WWTP, launched early in 2018. To assess the possible impact of the ozonation on the endocrine-disrupting activity in the Wurm River, the second water sampling campaign took place one year after the full-scale effluent ozonation and one and a half years later after the first sampling campaign (March 2019). During the second water sampling, hydrological conditions were characterized by high fluctuations due to the continuous rainy period. In order to investigate possible toxic carrier capacity of SPM under unstable hydrological conditions, an investigation of SPM during SCR 2_W on its endocrine-disrupting activity was conducted;

(ii) the second sediment sampling was conducted precisely one year later. This sampling campaign was performed in dry weather after the rainy period in June 2018 (SCR 2_S).

2.2. Materials and chemicals

See Supplementary information, Table S1.

2.3. Preparation of the water samples

Grab water samples of 2.5 L were taken directly from the middle river stream, transported to the laboratory in isolation boxes with cooling packs, and filtered through a glass fiber filter (0.5 μm, MN GF-2). Before the sampling procedure, each glass fiber filter was scaled and separately stored in single Petri dishes. Solved organic compounds were concentrated using five Oasis® HLB cartridges (0.5 L per cartridge) with a polymeric material to extract polar and non-polar compounds. Before extraction, Oasis® HLB cartridges were preconditioned by a subsequent elution of 6 mL each dichloromethane (DCM), methanol (MeOH), and deionized water. The elution procedure included washing with 6 mL MeOH followed by 6 mL DCM. Glass fiber filters with retained SPM from the sampling campaign of the water phase during rainy weather (SCR 2_W) were freeze-dried and weighed again to quantify the content of SPM in 2.5 L of proceeded water samples.

Water extracts with an additional 200 μL of a solvent keeper (dimethylsulfoxide, DMSO) were reduced under a gentle nitrogen stream and transferred into amber glass vials. The final concentration was adjusted to 2.5 L/mL DMSO.

2.4. Preparation of the particulate matter and sediment for biotesting

Sediment samples were taken from the surface sediment layer (first 10 cm) at each sampling site in the cross and longitudinal profiles and mixed to one representative grab sediment sample. The storage of the

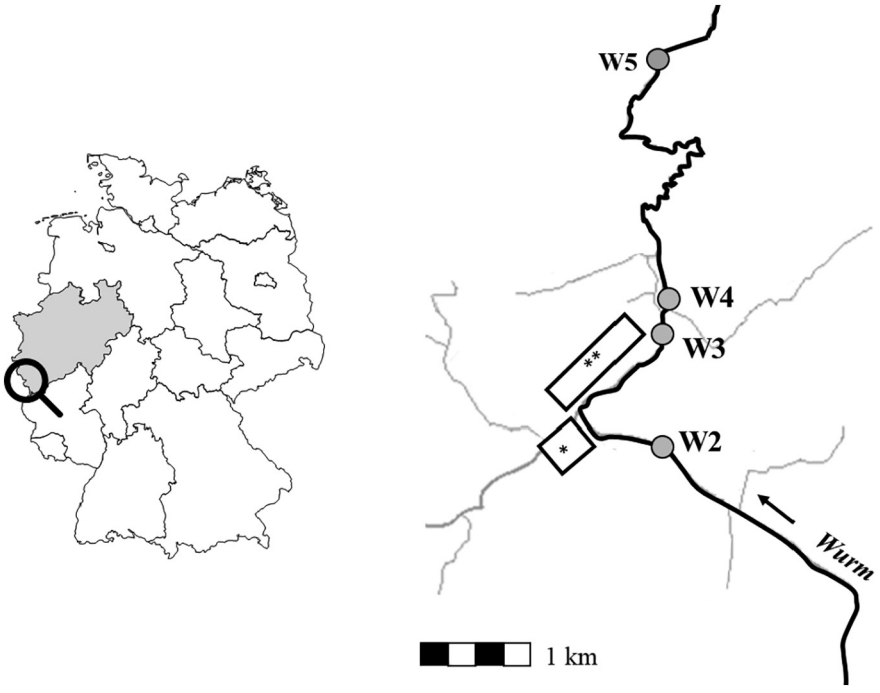

Fig. 1. Location of the sampling sites along the Wurm River (1:36,112). Sampling sites are marked with numbers ascending in the stream direction (2 to 5) preceded by the letter 'W' for Wurm River. *: Rainwater overflow basin. **: Aachen-Soers WWTP.
Source: ELWAS-WEB v. 4.0.0, www.elwasweb.nrw.de, modified by the authors.

Table 1
Overview of the performed samplings.

	Date	Matrix	Specific characteristics		
			Weather	Hydrology	Wastewater treatment mode
SCR 1	June 2017	Water	Dry weather	Stable	Conventional treatment (three treatment steps, no ozonation)
SCR 1		Sediment			
SCR 2_S	June 2018	Sediment	Dry weather after a rainy period	Stable	Advanced treatment by ozonation
SCR 2_W	March 2019	Water SPM	Rainy weather	Instable	Advanced treatment by ozonation

sediment samples occurred in brown-glass bottles at 4 °C. All sediment samples were freeze-dried within 2–3 weeks. Before extraction, freeze-dried sediments were sieved (2 mm) and homogenized. Sediment aliquots of 25 g (dry weight, dw) were extracted using accelerated solvent extraction with MeOH and DCM (first cycle: 100:0, v/v %, 100 °C, max. pressure 100 bar; second cycle: 0:100, v/v %, 100 °C, max. pressure 100 bar) (SpeedExtractor E-914/E-916, BUCHI Corporation). A mixture of MeOH and DCM has been shown to be suitable for extracting the endocrine-active compounds from environmental sediment samples (Creusot et al., 2011). Quartz sand served as a packaging matrix for extraction cells and as a process control sample. During the second sampling campaign, loaded glass fiber filters from water filtration were cut into pieces and extracted in the same way as the sediments.

Particulate matter extracts with an additional 200 μL of DMSO were reduced under a gentle nitrogen stream and transferred into amber glass vials. The final concentration of sediment extracts was adjusted to 25 g SEQ dw/mL DMSO. Organic compounds extracted from SPM were referred to the gravimetrically determined amount on waterless SPM as mg dw SPMEQ/mL DMSO.

2.5. Preparation of the sediment extracts for chemical analysis

For the Gas Chromatography High-Resolution Mass Spectrometry (GC-HRMS) analysis, freeze-dried sediment samples were extracted in the same way as for the biotesting. The amount of extracted sediments was adjusted to 100 mg TOC per sample (see S2). The extracts were evaporated and adjusted to 500 μL MeOH final volume. A clean-up step was performed using flash chromatography to avoid matrix effects caused by co-eluted natural organic matter. Details of the clean-up step are given in S3. The final volume was adjusted to 500 μL in ethyl acetate and spiked with 1 μg/mL internal standard mix in ethyl acetate. Details regarding the internal standards used can be found in S4.

2.6. CALUX® assays

Extracts were tested with a total concentration of 0.1% DMSO in the test medium. Cytotoxic effects by the solvent DMSO and extracts were excluded using the MTT (3-(4,5-dimethyltetrazolium-2-yl)-2,5-diphenyltetrazolium bromide) assay (Mosmann, 1983). Briefly, vital CALUX® cells can metabolize the MTT salt to insoluble product formazan, which can be measured photometrically (492 nm) (Mosmann, 1983).

The agonistic endocrine activity was detected using the CALUX® bioassays with licensed cells (BioDetection Systems B.V., the Netherlands) according to the ISO Guideline 19040-3 (2018). The antagonistic endocrine activity was measured according to the supplier's proposal (Besselink, 2015). Briefly, the CALUX® cells are human osteoblastic osteosarcoma cells with transfected human estrogen α (hERα) or androgen receptor (AR). After binding the ligand to the receptor, this complex moves to the highly sensitive nucleus-internal responsive elements (EREs and AREs, respectively), controlling the reporter gene's expression for the enzyme luciferase. After adding the specific substrate luciferin, the product amount can be measured as the intensity of light. In turn, the intensity of light correlates directly to the ligand amount bound to the receptor. Relative luminescent units (RLU) quantify the light intensity and can be translated into equivalents of the standard substance (17β-estradiol (E2) in the ERα and flutamide (Flu) in the anti-AR CALUX®).

An antagonistic assay is performed by prior exposure of the cells to the EC50 of the agonistic substance dihydrotestosterone (DHT) and decreased luminescence intensity compared to those activated by the EC50 concentration of DHT.

For both agonistic and antagonistic CALUX® bioassays, the limit of the detection (LOD) within each tested plate was calculated as the average of RLU values within the blank of the standard row plus its three-fold standard deviation. The limit of quantification (LOQ) was calculated as the three-fold LOD. Exceedance of effect-based trigger values (EBT) according to van der Oost et al. (2017) were used to prioritize the sampling sites: 0.5 ng E2 eq./L and 25 μg Flu eq./L for the ERα and the anti-AR CALUX®, respectively. Moreover, EBT-values were used for the ranking of sampling sites (Alygizakis et al., 2019) as those with low (0–3-times EBT) and moderate (3–10-times EBT) endocrine-disrupting activity.

Evaluation of statistically significant differences in the endocrine-disrupting activity was performed using an ANOVA test with a post-hoc Tukey test ($p < 0.05$). All statistical procedures were carried out with GraphPad Prism software (v. 7.04 for Windows; GraphPad Software, Inc.).

2.7. GC-HRMS analysis and evaluation

Cleaned extracts were analyzed with GC-HRMS (QExactive, Thermo Fisher Scientific, Germany) in Full Scan mode (res. 60,000). A Trace 1310 GC was linked with a Thermal Desorption Unit (TDU), and a Cooled Injection System (CIS 4) (Gerstel, Mülheim/Ruhr, Germany) was coupled to the QExactive. Aliquots of 2 μL extract were taken with the Gerstel MultiPurpose autosampler into a TDU tube. The desorption temperature of the TDU was set at 300 °C (720 °C/min heating rate) and held for five minutes. The transfer line between the TDU and the CIS system was kept at 320 °C. A baffled, deactivated glass liner was used for refocusing at −10 °C, the temperature increased with a rate of 12 °C/min to 300 °C (10 min holding time), and the analytes were injected in splitless mode (splitless time of 2 min).

Nineteen target PAHs were separated on a DB-5ms ultra inert GC column (30 m × 0.25 mm ID, 0.25 μm film thickness) at an initial column temperature of 60 °C with a hold time of 1 min, which was then increased to 150 °C (heating rate of 30 °C/min). The column temperature was further increased to 186 °C (heating rate of 6 °C/min) and finally to 300 °C (heating rate of 4 °C/min) with a holding time of 11.5 min. The analytes were transferred to the QExactive via the transfer line, which was kept at 280 °C.

The QExactive was operated in the electron ionization (EI) mode at 70 eV in positive full-scan mode (70–810 m/z) and a nominal resolution of 60,000 (FWHM at m/z 200). PAHs were quantified using method matrix-matched calibrations. Twelve calibration solutions (0.2, 0.5, 1, 2, 5, 10, 20, 50, 100, 200, 500 and 1000 ng/mL) of PAHs in DCM and a calibration blank were subjected to the same clean-up as the samples. Data were evaluated using software TraceFinder 5.1. Target PAHs were confirmed by the exact mass of the base peak, two fragments, retention times, and isotopic patterns. The detection limits were taken as the

lowest calibration point where these criteria were fulfilled. The list of analyzed PAHs with respective internal standards can be found in S4.

3. Results

3.1. Endocrine-disrupting activity in the water phase, suspended particulate matter, and sediment

The present study recorded the endocrine-disrupting activity distribution in the Wurm River during two annual sampling campaigns. No cytotoxicity was detected in any of the tested organic extracts. During SCR 1, all organic water extracts showed low endocrine-disrupting activity. The sampling site W2, located upstream of the Aachen-Soers WWTP, revealed 1.3-times EBT in the ERα CALUX® assay (0.5–0.7 ng E2 eq./L) and <1 EBT in the anti-AR CALUX® assay (23.4 μg Flu eq./L) (see Table 2). Similar estrogenic activity of 0.2–0.3 ng E2 eq./L (<1 EBT) and 0.2–0.3 ng E2 eq./L (<1 EBT), respectively, as well as an antiandrogenic activity of 35.6–57.1 μg Flu eq./L (2.3-times EBT) and 28.9–65.5 μg Flu eq./L (2.6-times EBT), respectively, was identified in the organic water extracts from the sampling sites W3 (downstream of the rainwater overflow basin) and W5 (2.5 km downstream of the effluent). No estrogenic activity was quantified at the sampling site W2 during SCR 2_W. A moderate estrogenic activity (5.1-times and 4.9-times EBT) was measured at the sampling sites W3 (2.6 ng E2 eq./L) and W5 (1.8–2.4 ng E2 eq./L). During SCR 2_W, the antiandrogenic activity was the highest at the sampling site W3 (6.4-times EBT, 53.4–159.2 μg Flu eq./L). The sampling sites W5 (5-times EBT, 31.9–123.9 μg Flu eq./L) and W2 (2.4-times EBT, 37.3–60.0 μg Flu eq./L) were characterized as 'moderate' regarding their antiandrogenic activity.

In connection with a significant increase (two-way ANOVA, $p < 0.05$) of the endocrine-disrupting activity in the receiving water in 2019 compared to 2017 (see S5), the role of SPM (>0.5 μm) as a possible carrier for contaminants with endocrine activity between sediment and water was examined. The SPM amount obtained by filtration of the water samples from the SCR 2_W remarkably increased in the flow direction from 27 mg SPM/L at the sampling site W2 (upstream of the WWTP) and up to 76.2 mg SPM/L at the sampling site W5 (2.5 km downstream the effluent). The effluent water sample (W4) did not contain quantifiable SPM. No particle-bound estrogenic activity was observed at all sampling sites, while the antiandrogenic activity was detected at each riverine sampling site without significant differences (2.3 ± 0.9 μg Flu eq./mg dw SPMEQ) (see Fig. 2).

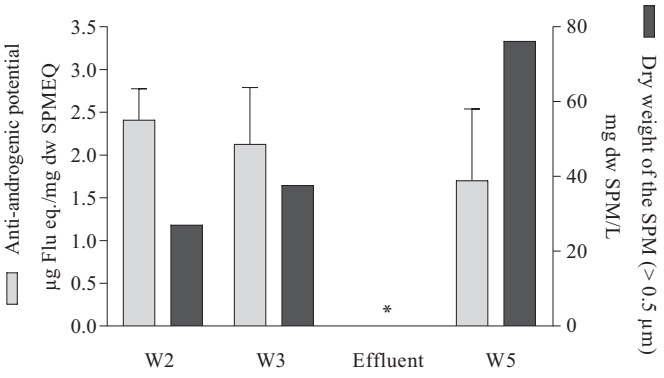

Fig. 2. Antiandrogenic activity detected in the anti-AR CALUX® assay (left x-ax) in SPM and its respective dry weight (right x-ax). The anti-AR CALUX® assay results are expressed as equivalents of the reference standard derived from the dose-response curve within the respective test run. Each bar represents the mean values ± standard deviation of three independent replicates. *: The antiandrogenic activity (because below the LOQ) and the dry weight (no particles >0.5 μm) could not be quantified.

Surface sediments from the Wurm River were sampled in summer in consecutive years 2017 (SCR 1) and 2018 (SCR 2_S). During SCR 1, estrogenic activity in the organic sediment extract from the sampling site upstream of the Aachen-Soers WWTP (W2) was found within the range of 0.05–0.1 ng E2/g dw SEQ. Sediment samples (W3 to W5) showed significantly lower (one-way ANOVA, $p < 0.05$) estrogenic activity. Investigation of the endocrine-disrupting activity in sediments from 2018 provided comparable results for the estrogenic activity at the sampling site W2 in both years (0.05–0.1 ng E2/g dw SEQ vs. 0.06–0.1 ng E2/g dw SEQ). At the sampling sites along the Aachen-Soers WWTP (W3 and W4), the estrogenic activity in sediment increased three-fold compared to 2017 (see Table 3). Sediment samples collected at the sampling site W3 (directly after the rainwater overflow basin) during a prolonged dry period in summer 2017 (SCR 1) recorded significantly lower (one-way ANOVA, $p < 0.05$) antiandrogenic activity in comparison to the sampling site W2 (upstream the Aachen-Soers WWTP) (4.8 ± 1.8 μg Flu eq./g dw SEQ vs. 8.1 ± 3.3 μg Flu eq./g dw SEQ).

Table 2
The whole range on the endocrine-disrupting activity detected in organic water extracts (2.5 L/mL DMSO) using the ERα and anti-AR CALUX® assays. The maximal equivalent concentration was used for the calculation of the factor describing the exceedance of the EBT_{fixed} (0.5 ng E2 eq./L for the ERα CALUX® assay and 25 μg Flu eq./L for the anti-AR CALUX® assay) (van der Oost et al., 2017). The factors are ranked regarding the action plan within the effect-based monitoring of water bodies: Low (0–3-times EBT) and moderate (3–10-times EBT) endocrine-disrupting activity in the Wurm River (Alygizakis et al., 2019). XX[1]: Value was quantified in one from three replicates.

Sample	Min	Max	AV ± SD	LOD (plate)$_{min-max}$	LOQ (plate)$_{min-max}$	Max/EBT_{fixed}
ERα CALUX® [ng E2 eq./L]						
SCR 1_W2	0.5	0.7	0.6 ± 0.1	0.04–0.3	0.1–1.0	1.3
SCR 2_W_W2	LOD - <LOQ			0.11–0.12	0.3–0.4	<1
SCR 1_W3	0.2	0.3	0.2 ± 0.04	0.2–0.4	0.5–1.2	<1
SCR 2_W_W3		2.6[1]		0.05–0.2	0.15–0.6	5.1
SCR 1_W4 (effluent)	0.2	1.0	0.6 ± 0.6	0.1–0.2	0.3–0.7	1.3
SCR 2_W_W4 (effluent)	LOD-<LOQ			0.1–0.2	0.2–0.5	<1
SCR 1_W5	0.2	0.3	0.2 ± 0.03	0.1–0.2	0.3–0.7	<1
SCR 2_W_W5	1.8	2.4	2.2 ± 0.3	0.1–0.2	0.3–0.5	4.9
Anti-AR CALUX® [μg Flu eq./L]						
SCR 1_W2		23.4[1]		15.9–17.5	52.3–57.6	<1
SCR 2_W_W2	37.3	60.0	48.6 ± 16.1	19.2–29.3	63.4–96.6	2.4
SCR 1_W3	35.6	57.1	44.3 ± 11.3	18.2–22.7	59.9–75.0	2.3
SCR 2_W_W3	53.4	159.2	92.0 ± 37.3	17.1–23.0	55.2–74.4	6.4
SCR 1_W4 (effluent)	35.3	38.9	37.1 ± 2.5	12.7–24.9	41.9–82.2	1.5
SCR 2_W_W4 (effluent)	LOD-<LOQ			23.5–25.1	77.6–82.7	<1
SCR 1_W5	28.9	65.5	53.2 ± 21.1	18.6–28.0	61.0–92.4	2.6
SCR 2_W_W5	31.9	123.9	61.7 ± 29.6	8.6–25.3	28.4–83.6	5

Table 3
The whole range of the endocrine-disrupting activity measured in organic sediment extracts (25 g dw SEQ/mL DMSO) using the ERα and anti-AR CALUX® assays. LOD: limit of detection. LOQ: limit of quantification.

Sample	Min	Max	AV ± SD	LOD (plate)$_{min-max}$	LOQ (plate)$_{min-max}$
ERα CALUX® [ng E2 eq./g dw SEQ]					
SCR 1_W2	0.05	0.1	0.07 ± 0.01	0.01–0.2	0.03–0.5
SCR 2_S_W2	0.06	0.1	0.08 ± 0.02	0.05–0.4	0.2–1.1
SCR 1_W3	0.03	0.1	0.05 ± 0.01	0.03–0.1	0.1–0.2
SCR 2_S_W3	0.1	0.2	0.16 ± 0.02	0.18–0.2	0.6–0.7
SCR 1_W4	0.03	0.1	0.06 ± 0.02	0.06–0.2	0.2–0.6
SCR 2_S_W4	0.18	0.2	0.2 ± 0.02	0.02–0.2	0.1–0.7
SCR 1_W5	0.03	0.05	0.04 ± 0.01	0.07–0.1	0.2–0.3
SCR 2_S_W5	0.06	0.1	0.08 ± 0.02	0.1–0.2	0.3–0.7
anti-AR CALUX® [μg Flu eq./g dw SEQ]					
SCR 1_W2	4.5	13.9	8.1 ± 3.3	11.8–14.9	38.9–49.2
SCR 2_S_W2	5.9	7.5	6.4 ± 0.9	10.2–12.7	33.5–42.0
SCR 1_W3	3.3	7.2	4.8 ± 1.8	14.2–29.2	46.7–96.5
SCR 2_S_W3	3.5	39.2	8.7 ± 10.9	6.4–22.0	21.2–72.7
SCR 1_W4	4.5	7.3	5.7 ± 1.1	12.3–23.1	40.7–76.3
SCR 2_S_W4	9.9	22.9	15.9 ± 5.6	9.8–19.3	32.4–63.8
SCR 1_W5	3.0	8.5	4.6 ± 2.0	11.3–34.1	37.4–112.4
SCR 2_S_W5	1.7	8.6	4.8 ± 2.2	12.4–23.1	41.0–76.2

3.2. Impact of the ozonated effluent on the endocrine-disrupting activity in the Wurm River

During dry weather (SCR 1) and before implementing the full-effluent ozonation, the effluent testing showed estrogenic activity, which was up to three-fold higher (0.6 ± 0.6 ng E2 eq./L) than the estrogenic activity in the Wurm River. However, the entrance of the effluent in the Wurm River did not increase the estrogenic activity at the sampling site W5, located 2.5 km downstream (see Fig. 3). The antiandrogenic activity during dry weather was within the range of 28.9–65.5 μg Flu eq./L with a slight increase at the sampling site W5.

Testing of the ozonated effluent in the background of dry weather but after extensive rain events (SCR 2_W) showed no quantified endocrine-disrupting activity. However, the effluent entrance in the Wurm River did not reduce the endocrine-disrupting activity downstream (sampling site W5). The estrogenic and antiandrogenic activities could still be detected downstream of the Aachen-Soers WWTP (2.2 ± 0.3 ng E2 eq./L and 61.7 ± 29.6 μg Flu eq./L, respectively), clearly indicating masking effects by weather conditions on the input of the ozonated effluent.

3.3. Sediment contamination by PAHs

Due to the predominantly anthropogenic impacted catchment area of the Wurm River, high contamination of SPM and the river sediment by PAHs was suspected. Rainfalls increase PAHs' transport into aquatic ecosystems, mainly through urban and agricultural runoffs (Selbig, 2009). Sediment samples contained 18 of 19 analyzed PAHs, while fluoranthene predominated at each sample. Molecular indices (see S6), calculated as a ratio of representative PAHs, confirmed the anthropogenic origin of the detected PAHs spectrum (Baumard et al., 1998; Colombo et al., 1989). The highest molecular index of 9.8 for phenanthrene/anthracene was measured in the sediment samples collected during the rainy season at the sampling site W3 (directly after the rainwater overflow basin), indicating the entrance of urban runoffs and sewage overflow into the Wurm River (see S6). Indeed, during rainy weather, both estrogenic and antiandrogenic potentials followed the tendency of PAHs to increase (see Fig. 4). Thus, up- and 2.5 km downstream the Aachen-Soers WWTP (sampling sites W2 and W5), the sum PAH concentration doubled. Organic sediment extracts from the sampling sites along the WWTP (W3, downstream the rainwater overflow basin, and W4, at the WWTP effluent) showed a 14- and a 26-fold increase

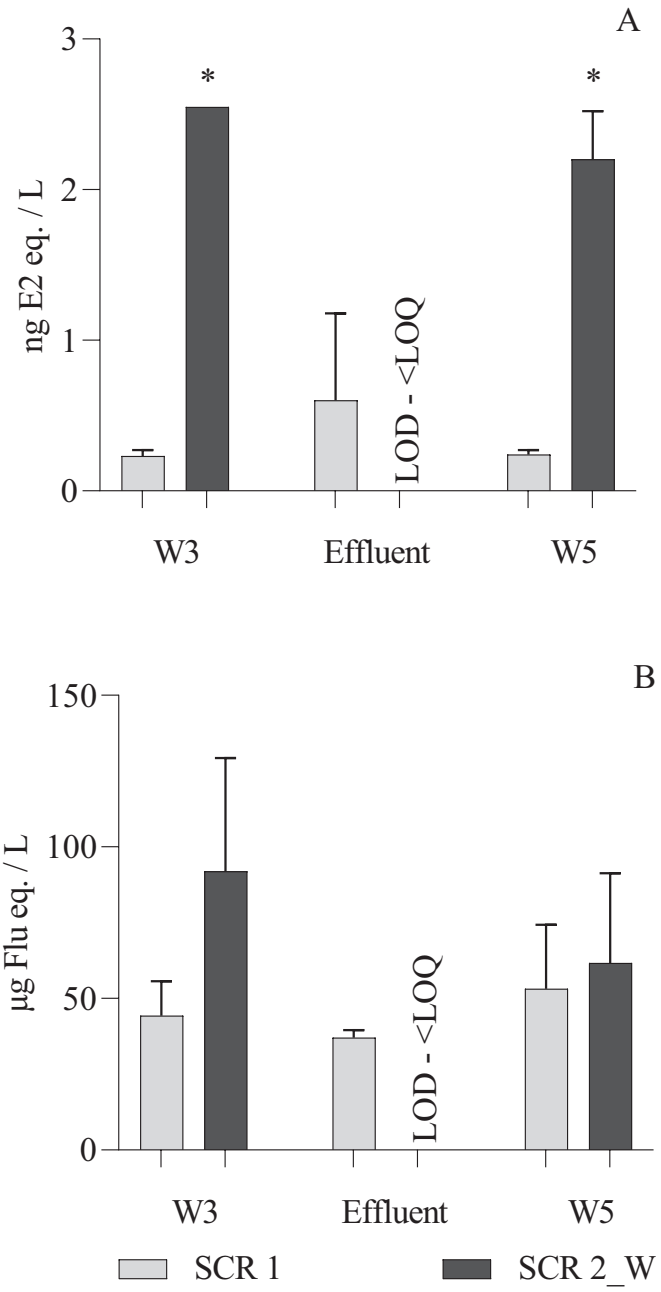

Fig. 3. Estrogenic (A) and antiandrogenic (B) activity of the WWTP effluent under conventional (SCR 1) and advanced effluent treatment by ozonation (SCR 2_W) within the Wurm River's endocrine-disrupting activity background. The statistical evaluation show significance (*) in different endocrine-disrupting activities on a sampling site during different sampling campaigns (one-way ANOVA; *$p < 0.05$).

(4420 ng/mg OC and 23,338 ng/mg OC, respectively) of the sum PAHs during SCR 2_S compared to SCR 1 (see S7).

4. Discussion

4.1. Endocrine-disrupting activity in the water phase

Evaluation of the present study results, according to Alygizakis et al. (2019), showed that after a long dry weather period (SCR 1), upstream of the Aachen-Soers WWTP (sampling site W2), low estrogenic (1.3-times EBT, 0.52–0.65 ng E2 eq./L) and antiandrogenic (<1 EBT, 23.4 μg Flu eq./L) activities occurred. In the further Wurm River course, the

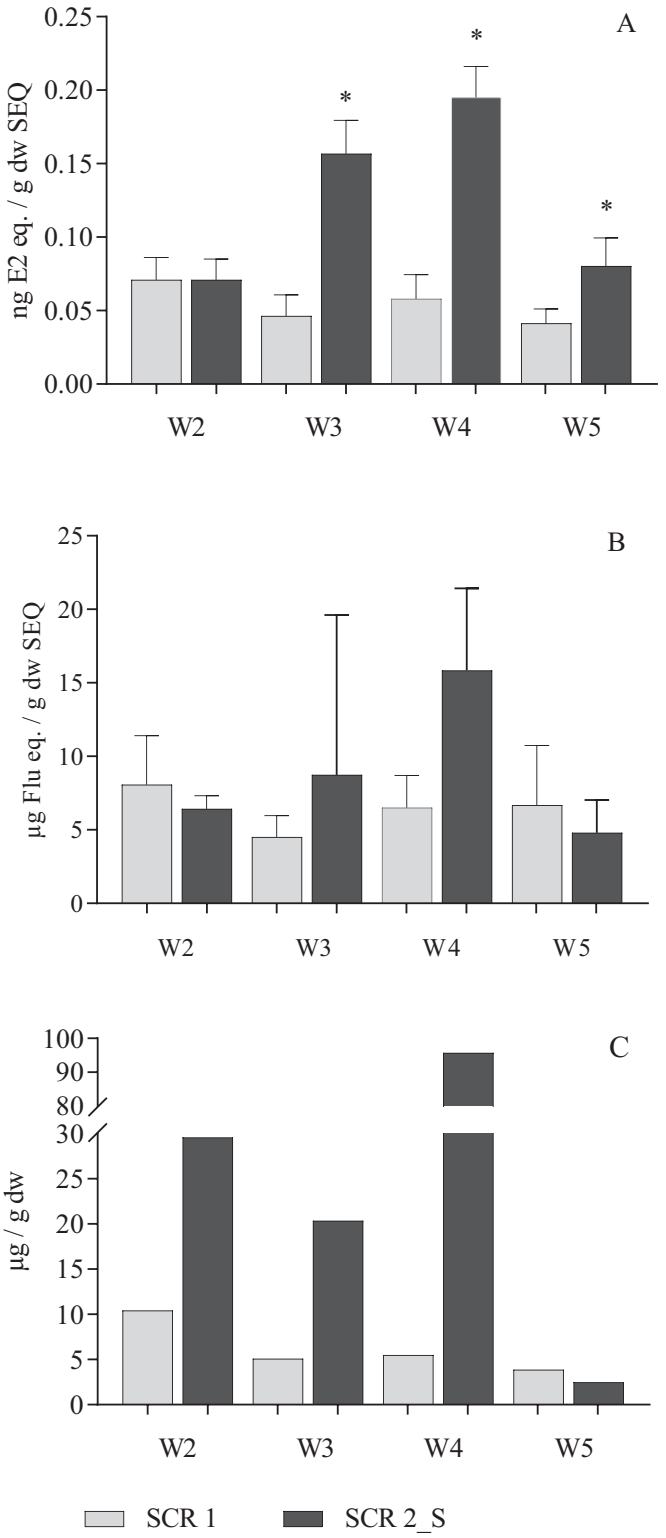

Fig. 4. Estrogenic (A) and antiandrogenic (B) activity (CALUX® assay) in organic sediment extracts from the Wurm River and respective concentrations of 19 PAHs (sum PAHs; C) normalized for the OC content. Results of the CALUX® assays are expressed as equivalents of the reference standard E2 or Flu, derived from the dose-response curve within the respective test run. Each bar represents the mean values ± standard deviation of three independent replicates. Statistically significant differences within one sampling site are represented by asterisks (one-way ANOVA; *$p < 0.05$).

estrogenic activity remained out of concern (<1 EBT). The antiandrogenicity increased to a level of 2.3-times EBT (35.6–57.1 µg Flu eq./L) at the sampling site W3 and 2.6-times EBT (28.9–65.5 µg Flu eq./L) at the sampling site W5. The discharged secondary effluent of the Aachen-Soers WWTP, which predominated the total water flow of the Wurm River during SCR 1, showed both estrogenic (0.6–1.2 ng E2 eq./L) and antiandrogenic (35.6–65.6 µg Flu eq./L) activities at the sampling site W5. Contrarily to SCR 1, extensive rain events potentially contributed to the increase of the endocrine-disrupting activity in the water phase of the Wurm River (SCR 2_W). Thus, moderate estrogenic activity was identified downstream of the rainwater overflow basin (5.1-times EBT, 2.55 ng E2 eq./L, at W3 and 4.9-times EBT, 1.83–2.43 ng E2 eq./L, at W5). In comparison, antiandrogenicity was already detected upstream of the Aachen-Soers WWTP (2.4-times EBT, 37.3–60.0 µg Flu eq./L, at W2) following by the moderate antiandrogenic activity at W3 (6.4-times EBT, 53.4–159.2 µg Flu eq./L).

The endocrine-disrupting activity quantified in the water phase was comparable with the results of the SAFE project (Endocrine disruption in Switzerland: Assessment of fish exposure and effects) as a part of the Swiss national research program on endocrine disruptors (NRP50) (Vermeirssen et al., 2005). Interestingly, the endocrine activity detected in the present study during the first sampling campaign in summer 2017 (SCR 1; 0.21–0.65 ng E2 eq./L) was in the range of winter values in Swiss waters. This result could be partially explained by degrading steroid estrogens in water by photolysis, biodegradation, and sorption (Adeyeye and Laub, 2020). Additionally, the entered WWTP effluent contributed to the input of nutrients and water warmer than in the receiving stream. Such favorable conditions promoted non-pathogenic microorganisms and algae growth, contributing to steroid estrogens' biodegradation (Juergens et al., 2002).

High estrogenic activity from SCR 2_W (1.8–2.6 ng E2 eq./L) was equivalent to the one detected within the SAFE project in the Canton Aargau in northern Switzerland in summer. The authors explained this result with a low river flow due to a long dry period (Vermeirssen et al., 2005). However, the Wurm River water level accounted for 70 cm in summer due to prolonged rain events, whereas the typical water level during dry weather usually lies at 18 cm (ELWAS-WEB, 2021). Neale et al. (2020) recorded increased estrogenic activity in streams connected with rain events and, thereby, initiated sewer overflows.

By comparison of the detected estrogenic activity in the studied conventionally treated effluent (SCR 1_W) with the safe level for municipal WWTP effluents (0.1–0.4 ng E2 eq./L and 0.5–2 ng E2 eq./L for a long-term (>60 days) and short-term (<60 days) fish exposure) postulated by Jarošová et al. (2014), the effluent of the Aachen-Soers WWTP could potentially bear a risk to fish. Thorpe et al. (2009) exposed male fathead minnows *Pimephales promelas* to comparable in the present study values of 0.5–3.0 ng E2 eq./L showing a consequent nine-fold increase of plasma vitellogenin and impaired reproductive activity,

Improvement of wastewater treatment by implementing full-scale ozonation showed a 100% elimination of the estrogenic and antiandrogenic activity in the effluent (CALUX® assays) without reduction of the endocrine-disruptive activity in the water phase downstream.

The range of the antiandrogenic activity in organic water extracts recorded during the SCR 1 (23.4–65.5 µg Flu eq./L) was comparable with the endocrine-disrupting activity detected at the hot spot for antiandrogenicity downstream of a local WWTP in the Holtemme River (Saxony-Anhalt, Germany) (Muschket et al., 2018). Within the mentioned study, organic surface water extracts revealed an antiandrogenic activity of 32–60 µg Flu eq./L in the anti-AR CALUX® assay, which was initially driven by 4-methyl-7-diethylaminocoumarin (C47) and its two derivatives. The parent compound C47 is 3.7-times more potent than the reference compound flutamide and explained 32% of the detected antiandrogenicity in the Holtemme River. Within the European Case Study on WWTP effluents organized by the NORMAN Association members, C47 and its two derivates (7-diethylamino-4-methylcoumarin and 7-(ethylamino)-4-methylcoumarin) were detected in the effluent of the Aachen-Soers WWTP at a total concentration of 16 ng/L (Dulio et al.,

2018; NORMAN, 2017). As this value was one order of magnitude lower than the C47 concentration detected in the Holtemme River (13.7 μg/L), the current study's authors assume other drivers of anti-androgenicity in the Wurm River.

Hill et al. (2010) showed accumulation of antiandrogenic activity in fish bile after the fish was exposed to the WWTP effluent with 161 ± 10 μg Flu eq./L comparable to the current study results (23.4–159.2 μg Flu eq./L in the Wurm River). Bioconcentration of antiandrogenic compounds in fish can lead to demasculinization/feminization of male fish (Hill et al., 2010). Jobling et al. (2009) showed using a statistical model combining modeled concentrations and activities of estrogenic and antiandrogenic chemicals and feminization cases of wild fish observed in U.K. rivers that a mixture of both classes of EDCs could cause sexual disruption in the wild fish population. Detection of estrogenic and antiandrogenic activity in different compartments of the Wurm River match studies scenarios by Jobling et al. (2009) and Hill et al. (2010), indicating the risk to the wild fish population in the studied water body.

4.2. Particle-bound antiandrogenic activity after extensive rain events

Based on the monitoring data provided by the manager of the Aachen-Soers WWTP, the Eifel-Rur Waterboard (WVER), the authors tried to assess the transport of particle-bound contaminants into the receiving water body. During the entire sampling day (02:00–20:00, 03/11/2019) of the SCR 2_W, the rainwater overflow basin of the Aachen-Soers WWTP released over 58,000 m^3 of the untreated rainwater-wastewater mixture into the Wurm River, nearly almost neglecting the upstream water portion. The total suspended solids (TSS) concentration within all rainwater overflow basins on 03/11/2019 was 54 mg/L, from which around 66.7% (36 mg/L) was comprised of particles smaller than 63 μm. This fine particulate fraction is characterized by a high specific surface and high binding affinity to organic pollutants and heavy metals (Salomons and Brils, 2004). As within the present study, the riverine water samples were filtered through glass fiber filters with a mesh size of 0.5 μm, thus a considerable amount of the total TSS from the rainwater overflow basin was not assessed in this study and is suggested to be found at W3.

Eganhouse and Sherblom (2001) showed that the particulate phase of the stormwater is a primary transmitter of hydrophobic pollutants such as EDCs into the receiving stream. Indeed, the highest antiandrogenic activity (up to 39.2 μg Flu eq./g dw SEQ) was observed at the sampling site next to the rainwater overflow basin (W3, 0.3 km distance), suggesting the rainwater overflow basin as a source of particle-bound antiandrogenic contaminants in the receiving water body. Thus, due to the frequent release (40 times per year) of the rainwater overflow basin of the Aachen-Soers WWTP and a high vulnerability of small water bodies toward the entrance of significant water amounts, the aquatic ecosystem of Wurm River experienced regularly hydrological and organic contaminants pressures probably contributing to the failure of the European Water Framework Directive's goals (Launay et al., 2016; Nickel and Fuchs, 2019; Schertzinger et al., 2019). Due to insufficient knowledge about rainwater overflow basins as a potential source of pollution, further investigations are needed.

4.3. Endocrine-disrupting activity in sediments and its possible drivers

During dry weather, sediments from the Aachen-Soers WWTP sampling site upstream (W2) revealed the highest estrogenic and antiandrogenic activity of 0.07 ± 0.01 ng E2 eq./g dw SEQ and 8.1 ± 3.3 μg Flu eq./g dw SEQ. In contrast, the downstream sampling sites (W3, W4, and W5) showed lower endocrine-disrupting activity. Prolonged extensive rain events changed this situation during SCR 2_S by a significant increase of the estrogenic activity at the sampling sites downstream of the rainwater overflow basin: 0.03 ± 0.07 ng E2 eq./g dw SEQ in SCR 1 vs. 0.14 ± 0.20 ng E2 eq./g dw SEQ in SCR 2_S at the sampling site W3 and 0.03 ± 0.07 ng E2 eq./g dw SEQ in SCR 1 vs. 0.18 ± 0.21 ng E2 eq./g dw SEQ in SCR 2_S at the sampling site W4. Hilscherova et al. (2002) observed a significant increase in estrogenic potential in sediments from the Morava River and its tributary Drevnice River (Czech Republic) after flood events. The second sediment sampling campaign (SCR 2_S) was conducted in dry weather but after a rainy period with a consequently release of 75,000 m^3 of the untreated rainwater-wastewater mixture from the rainwater overflow basin of the Aachen-Soers WWTP three days before sampling. As a result, the antiandrogenic activity in studied surface sediments indicated a slight increase. However, W3 and W4 revealed the strongest increase up to 39.2 μg Flu eq./g dw SEQ at the sampling site W3. Identified levels of the antiandrogenic activity in sediments were comparable with the antiandrogenic activity from the moderate impacted Lézarde River in the North of France (Kinani et al., 2010).

Several studies described estrogenic (Johann et al., 2020; Villeneuve et al., 2002; Zhang et al., 2016) and antiandrogenic (Macikova et al., 2014; Vinggaard et al., 2000; Weiss et al., 2009) potential of PAHs. Within the present study, the potency factors for ten parent PAHs within the ERα and anti-AR CALUX® assays (provided by BioDetection Systems B.V.) corresponded to the PAHs from the target analysis were compared. None of the ten PAHs showed estrogenic activity in the ERα-CALUX® assay. This result suggests that compounds other than PAHs are responsible here as drivers of particle-bound estrogenicity.

Seven PAHs (dibenz[*a*,*h*]anthracene, benzo[*a*]pyrene, benzo[*k*]fluoranthene, fluoranthene, indeno[1,2,3-*cd*]pyrene, phenanthrene, and pyrene) revealed antiandrogenic activity in the anti-AR CALUX® assay related to 0.9–1.1 potency factor of the synthetic antiandrogen and standard compound flutamide (see S8). In addition, fluoranthene with the potency factor of 1.0 was identified in the highest concentration among other PAHs in all sediment samples (0.7–46 μg/g dw). The study by Vinggaard et al. (2000) confirmed the strong antiandrogenic activity of fluoranthene in the anti-AR CALUX® assay (IC50 value of 4.6 μM). Later, Weiss et al. (2009) elucidated fluoranthene utilizing effect-directed analysis as a driver for the antiandrogenicity (55 μg Flu eq./g dw SEQ) in surface sediments from the Schijn River close to the city of Antwerp (Belgium).

The concentration of PAHs like dibenzo[*a*,*h*]anthracene, indeno[1,2,3-*cd*]pyrene, anthracene, benzo[*ghi*]perylene, perylene, and benzo[*e*]pyrene identified in sediments from the Wurm River were comparable with those from the demonstrably anthropogenic impacted Neckar River in Germany (Hollert et al., 2002) (see S9). Interestingly, particle-bound PAH concentrations from all sampling sites analyzed during dry weather (SCR 1) and from the sampling site W5 (2.5 km downstream of the effluent) sampled after extensive rain events (SCR 2_S) revealed a remarkable similarity with the PAH concentrations detected in the Danube (Keiter et al., 2008) and the Rhine (Gocht et al., 2001) Rivers. However, freshwater sediments collected within the present study from the sampling sites W2-W4 during SCR 2_S showed much higher (up to 100-times) PAH concentrations. Indeed, the Wurm River section between W2 and W4 was straightened, supporting high sediment perturbation. Contrarily, the 2.5 km Wurm River section between W4 (effluent) and W5 follows a pristine meandering watercourse with natural sediment retention areas, which potentially explained the relatively low sediment PAH concentration even after strong rain events.

The abovementioned comparisons showed that even such industrially unimpacted, small water bodies as the Wurm River, which serve just as recipient water for WWTPs, could be highly polluted by ubiquitous PAHs with the following consequences: PAHs (as well as flutamide (Mollergues et al., 2017)) become more potent after metabolization by the liver enzymes (Lam et al., 2018; Sievers et al., 2013; Wenger et al., 2009). That means that wildlife organisms from the Wurm River and especially the fish population were permanently exposed to highly potent PAHs. Therefore, bound chemical pollutants mask a risk not only during flood events but continuously as particles can be swallowed by fish, deploying direct exposure of the fish organism to contaminants

(Barceló and Petrovic, 2007). Furthermore, due to multiple metabolic pathways of PAHs and their bioaccumulative potential in the organism, severe harmful effects may occur on the organism and population level (Alegbeleye et al., 2017). Thus, fish exposure to PAHs can lead to disruption of vitamin metabolism and signaling (Berntssen et al., 2015), stress response (Reddam et al., 2017), immunotoxicity (Reynaud and Deschaux, 2006), genotoxicity (Nacci et al., 2002), carcinogenicity (Stegeman and Lech, 1991), teratogenicity and embryotoxicity (Kais et al., 2017; Schiwy et al., 2015; Seiler et al., 2014), disruption of vitellogenesis (Nicolas, 1999), or reduction of sex hormones concentration in plasma (Kennedy and Smyth, 2015). Additionally, previous studies showed poor health (Grung et al., 2016), increased cancer incidence and immunosuppression (Rose et al., 2000), and behavioral disruptions (Vignet et al., 2014) in fish populations exposed to PAHs.

The current study shared considerations regarding PAHs in one aquatic ecosystem. Further research on PAHs equivalent concentrations for explaining the potency of a complex mixture (e.g., the 'iceberg modeling', Neale et al. (2018)) and its adjustment for sediment samples is needed. However, considering the presence of thousands of micropollutants in European rivers with an extensive spectrum of possible adverse effects to aquatic organisms (Busch et al., 2016; Malaj et al., 2014), identification of other sediment contaminants and the assessment of the mixture toxicity is an initial goal for further studies. Additionally, the investigation strategy can include exposure studies with fish to conclude the endocrine-disrupting potential outgoing from the effluent.

5. Conclusion

The present study describes strong fluctuations of the endocrine-disrupting activity in a small receiving water body during different weather conditions. As a small water, the Wurm River (Germany) was not resilient towards the hydrological and material impact of massive rain events resulting in a significant endocrine-disrupting activity change. The study of a temporal and spatial sampling of different water and particle matrices (sediment and suspended solids) showed that a stormwater overflow basin of a local wastewater treatment plant might be responsible for the increase of endocrine activity in the water body after emptying into the receiving water body. Sediments also recorded an increase of estrogenic and antiandrogenic activities due to heavy rain events. Furthermore, antiandrogenic activity in the sediment samples showed a positive correlation to highly potent PAHs in sediments, indicating them as possible drivers of the antiandrogenicity. However, further investigations on the endocrine-disrupting activity of single PAHs and mixtures are needed.

Improving wastewater treatment by implementing a full-scale ozonation plant showed a 100% elimination of the estrogenic and antiandrogenic activity using highly sensitive human cell-based CALUX® bioassays. However, endocrine-disrupting activity reduction in the effluent by installing an additional treatment step did not necessarily lead to a fast reduction of the endocrine-disrupting activity in the receiving water body.

The present study emphasized the relevance of complex monitoring of water, sediment, and suspended particulate matter for an adequate risk assessment in aquatic ecosystems. The authors confirmed that the CALUX® reporter-gene assays are suitable for assessing the endocrine-disrupting activity in aquatic ecosystems showing mixture effects by all chemicals in environmental samples of different origins.

For a complex assessment of possible ecotoxicity drivers in the Wurm River, further seasonal and spatial investigations of sediments implementing such strategies as effect-directed analysis are needed. Nevertheless, the present study results (also considering the mixture toxicity) suggest the presence of different contaminants in sediment impacting the ecotoxicological sediment quality, which can significantly have an impact on aquatic organisms and, therefore, should no longer be discriminated by the EU WFD.

CRediT authorship contribution statement

Aliaksandra Shuliakevich: Conceptualization, Methodology, Investigation, Writing - original draft, Data curation, Formal analysis, Visualization.

Katja Schroeder: Writing - review & editing, Methodology, Investigation, Data curation, Formal analysis.

Laura Nagengast: Writing - review & editing, Investigation, Data curation, Formal analysis.

Yvonne Wolf: Writing - review & editing, Methodology, Investigation, Data curation, Formal analysis.

Ira Brückner: Resources, Supervision, Funding acquisition, Writing - review & editing.

Melis Muz: Methodology, Investigation, Writing - original draft, Data curation, Formal analysis.

Peter A. Behnisch: Writing - review & editing.

Henner Hollert: Conceptualization, Resources, Supervision, Project administration, Funding acquisition, Writing - review & editing.

Sabrina Schiwy: Conceptualization, Resources, Supervision, Project administration, Funding acquisition, Data curation, Writing – review & editing.

All authors have approved the final text.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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Supplementary information

Title: Extensive rain events have a more substantial impact than advanced effluent treatment on the endocrine-disrupting activity in an effluent-dominated small river

Contributing authors: Aliaksandra Shuliakevich[a], Katja Schroeder[b], Laura Nagengast[b], Yvonne Wolf[b], Ira Brückner[c], Melis Muz[d], Peter A. Behnische[e], Henner Hollert[a*], Sabrina Schiwy[a]

[a]: Goethe University Frankfurt/Main, Department Evolutionary Ecology and Environmental Toxicology, Max-von-Laue-Strasse 13, 60438 Frankfurt/Main, Germany

[b]: RWTH Aachen University, Institute of Biology V, Worringerweg 1, 52074 Aachen, Germany

[c]: Eifel-Rur Waterboard (WVER), Eisenbahnstr. 5, 52354 Düren, Germany

[d]: Helmholtz Centre for Environmental Research – UFZ, Department of Effect-Directed Analysis, Permoserstrasse 15, 04318 Leipzig, Germany

[e]: BioDetection Systems B.V. (BDS), Science Park 406, 1098 XH Amsterdam, The Netherlands

*Corresponding author: Hollert@bio.uni-frankfurt.de. Goethe University Frankfurt, Max-von-Laue-Str. 13, 60438 Frankfurt am Main, Germany

S1

Table S1: Information on materials and chemicals used within the current study.

Material/chemical	CAS Reg. No.	Supplier
Solvents		
Acetone ≥99.9%, UV/IR grade, for analysis	67-64-1	AppliChem GmbH
Methanol (MeOH) Reag. Ph. Eur. for analysis, ACS, ISO	67-56-1	AppliChem GmbH
Dichloromethane (DCM) Chromasolv®, for HPLC, ≥99.8%	75-09-2	AppliChem GmbH
Dimethylsulfoxide (DMSO) 99.5%, for synthesis	67-68-5	AppliChem GmbH
Ethyl acetate hypergrade for LC-MS LiChrosolv®	141-78-6	Merck KGaA
Water extraction		
Oasis® extraction cartridges with a hydrophilic-lipophilic balance (HLB) sorbent (30 µm, cartridge type: 6 cc, 200 mg)	-	Waters Corporation
Filter paper circles (MN GF-2, filter material: glass fiber paper, particle retention: 0.5 µm, diameter: 90 mm)	-	Macherey-Nagel GmbH & Co. KG
Particulate phase extraction		
Fat-free extraction quartz sand (0.3 - 0.9 mm)	-	BUCHI Corporation
Top cellulose filters for the extraction cell (20 mm)	-	BUCHI Corporation
Bottom cellulose filters for the extraction cell (12.7 mm respectively)	-	BUCHI Corporation
Sediment extracts clean-up		
Chromabond Flash RS4 SiOH, 40-63 µm	-	Macherey-Nagel GmbH & Co. KG
PTFE syringe filter 0.2 µm	-	Macherey-Nagel GmbH & Co. KG
MTT assay		
3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide for biochemistry (MTT)	298-93-1	Merck KGaA
CALUX® assay		
17β-estradiol (E2)	50-28-2	Merck KGaA
Androstan-17β-ol-3-one (dihydrotestosterone, DHT)	521-18-6	Merck KGaA
Flutamide (Flu)	13311-84-7	Merck KGaA

S2

Table S2: Sediment amount containing TOC. Data from the CHN-analyzer (Vario EL III).

Sampling site	SCR 1 mg TOC/mg dw / 100 mg TOC/g dw	SCR 2_S mg TOC/mg dw / 100 mg TOC/g dw
W2	0.006 / 16.7	0.009 / 11.7
W3	0.014 / 7.4	0.004 / 22.7
W4	0.006 / 16.7	0.004 / 26.4
W5	0.011 / 8.9	0.004 / 26.5

S3

Clean-up with flash chromatography was performed with a pre-packed silica gel cartridge with a particle size of 40–63 µm (Chromabond Flash RS4 SiOH, Macherey Nagel) using an Agilent 1200 pump. The cartridge was preconditioned with DCM, and the sediment extract (100 mg TOC eq./500 µL DCM) was loaded on the cartridge using a syringe. DCM was pumped through the cartridge according to the following program: The DCM flow was increased from 0 to 10 mL/min in 0.5 min, followed by a constant flow of 10 mL/min until min 2 and brought back to initial conditions within an additional 0.1 min. The collected eluent was evaporated until 0.5 mL and transferred into new glass tubes by rinsing with ethyl acetate to perform the complete solvent exchange. Under the nitrogen stream, DCM was removed, the volume of the extract in ethyl acetate was reduced up to 0.1 mL and then adjusted to 0.5 mL in EtAc. Glass tubes were then kept at -20°C for 1 h in order to achieve precipitation of lipids. Afterward, the extracts were carefully filtered using a 0.2 µm PTFE syringe filter. 100 µL aliquots were pipetted into a glass vial with an insert, and the internal standards mix was spiked (1 µg/ml in EtAc). All aliquots were kept at -20°C until further chemical analysis.

S4: See the end of this document

S5

Table S5: Statistical testing of the results from the CALUX® assays obtained for water samples as SCR 1 vs. SCR 2_W. Statistical procedure: Two-way ANOVA with a post-hoc Šidák's test. 'ns': No statistically significant difference, $p>0.05$. '*': Statistically significant difference, $p<0.05$. REF: Relative enrichment factor. The REF value of 2.5 refers to 2.5 L of extracted water volume resolved in 1 mL DMSO.

REF	W2		W3		W4 (Effluent)		W5	
	ERα CALUX®	anti-AR CALUX®	ERα CALUX®	anti-AR CALUX®	ERα CALUX®	anti-AR CALUX®	ERα CALUX®	anti-AR CALUX®
0.1	*	ns	ns	ns	ns	ns	ns	ns
0.25	ns	ns	ns	ns	ns	ns	*	ns
0.5	*	ns	*	ns	ns	ns	*	ns
0.75	ns	ns	*	ns	ns	ns	*	ns
1.0	*	ns	*	*	ns	ns	*	ns
1.25	*	ns	*	*	*	ns	*	*
1.5	*	ns	*	*	*	ns	*	ns
2.0	*	ns	*	*	*	ns	*	*
2.5	*	ns	*	*	*	ns	*	ns

S6

Table S6: Molecular indices for the ratio fluoranthene / pyrene and phenanthrene / anthracene to identify the PAHs origin (Sanders et al., 2002).

	SCR 1				SCR 2_S			
	W2	W3	W4	W5	W2	W3	W4	W5
fluoranthene / pyrene	1.1	1.5	4.5	1.2	1.8	4.9	3.0	3.1
phenanthrene / anthracene	3.2	5.3	6.8	9.3	2.3	9.8	2.7	6.3

S7: See the end of this document

S8

Table S8: Relative potency factors (REP) of single PAHs in comparison to the endocrine-activity potency of the standard substance: E2 in the ERα- (REP=1) and Flu in the anti-AR (REP=1) CALUX® assays.

PAH	ERα CALUX®	Anti-AR CALUX®
Naphthalene	not active	not active
Dibenz[a,h]anthracene	no data	1.0
Benzo[a]pyrene	not active	1.0
Benzo[b]fluoranthene	not active	not active
Benzo[k]fluoranthene	not active	1.1
Dibenz[a,h]anthracene	no data	1.0
Fluoranthene	not active	1.0
Indeno[1,2,3-cd]pyrene	not active	0.9
Phenanthrene	not active	1.0
Pyrene	not active	0.9

S9

Table S9: List of the PAH concentrations correlated with detected PAH concentrations in the current study.

	Hollert et al. (2002), Neckar River (Germany), [ng/mg OC]	Gocht et al. (2001), Rhine River, [µg/kg dw]	Keiter et al. (2008), Danube River, [µg/g dw]
Dibenzo[a,h]anthracene	0.5-155.6	76	0.02-0.49
Fluoranthene		733	0.05-4.8
Fluorene			0.04-0.64
Indeno[1,2,3-cd]pyrene	0.9-382.6	321	0.02-1.4
Phenanthrene		195	0.02-4.6
Pyrene		375	0.11-3.4
Anthracene	0.3-232.5		0.01-1
Benz[a]anthracene		369	0.06-2.2
Benzo[a]pyrene		75	0.08-1.7
Benzo[b]fluoranthene		652	
Benzo[ghi]perylene	0.8-402.4	294	0.04-0.97
Benzo[k]fluoranthene		106	0.01-0.24
Chrysen			0.03-0.44
4H-Cyclopenta[def]phenanthrene			
9-vinylanthracene			
Perylene	0.5-180.6	9	
Benzo[e]pyrene	0.5-347.0		
Cyclopenta[cd]pyrene			

S4

Table S4: List of analyzed PAHs, their physico-chemical properties (from the PubChem® open source), names of internal standards used for quantification, and their limits of detection (LOD).

	CAS Reg. No	Formula	Exact mass, g/mol	Log Kow	Internal Standard	LOD, ng/mL
4H-Cyclopenta[def]phenanthrene	203-64-5	C15H10	190.0783	4.4	PCB52_13C12	0.2
9-vinylanthracene	2444-68-0	C16H12	204.0939	5.5	PCB52_13C12	0.2
Anthracene	120-12-7	C14H10	178.0783	4.4	Phenanthrene-D10	1
Benz[a]anthracene	56-55-3	C18H12	228.0939	5.8	Chrysene-D12	2
Benzo[a]pyrene	50-32-8	C20H12	252.0939	6.0	Benzo[a]pyrene-d12	20
Benzo[b]fluoranthene	205-99-2	C20H12	252.0939	6.0	Benzo[a]pyrene-d12	10
Benzo[e]pyrene	192-97-2	C20H12	252.0939	6.4	Benzo[a]pyrene-d12	10
Benzo[ghi]perylene	191-24-2	C22H12	276.0939	6.6	Dibenz[a,h]anthracene D14	2
Benzo[k]fluoranthene	207-08-9	C22H12	252.0939	6.6	Benzo[a]pyrene-d12	2
Chrysene	218-01-9	C18H12	228.0939	5.7	Chrysene-D12	0.2
Cyclopenta[cd]pyrene	27208-37-3	C18H10	226.0783	5.5	Chrysene-D12	20
Dibenz[a,h]anthracene	53-70-3	C22H14	278.1096	6.5	Dibenz[a,h]anthracene D14	0.2
Dibenzo(a,e)pyrene	192-65-4	C24H12	302.1096	7.3	Dibenz[a,h]anthracene D14	10
Fluoranthene	206-44-0	C16H10	202.0783	5.2	Pyrene-D10	10
Fluorene	86-73-7	C13H10	166.0783	4.2	Acenaphthen-D10	20
Indeno[1,2,3-cd]pyrene	193-39-5	C22H12	276.0939	7.0	Dibenz[a,h]anthracene D14	2
Perylene	198-55-0	C20H12	252.0939	5.8	Perylene-D12	2
Phenanthrene	85-01-8	C14H10	178.0783	4.5	Phenanthrene-D10	20
Pyrene	129-00-0	C16H10	202.0783	4.9	Pyrene-D10	2

S7

Table S7: PAHs identified in sediment samples.

PAH	SCR 1, ng/mg OC				SCR 1, µg/g dw				SCR 2_S, ng/mg OC				SCR 2_S, µg/g dw			
	W2	W3	W4	W5	W2	W3	W4	W5	W2	W3	W4	W5	W2	W3	W4	W5
4H-Cyclopenta[def]phenanthrene	16.70	3.07	8.55	4.34	0.098	0.044	0.050	0.048	26.77	55.49	296.56	7.50	0.223	0.241	1.14	0.028
9-vinylanthracene	0.56	0.10	0.20	0.15	0.003	0.001	0.001	0.002	1.11	1.08	6.27	0.26	0.009	0.005	0.024	0.001
Anthracene	44.61	4.22	10.17	3.74	0.262	0.060	0.060	0.042	95.81	33.16	375.26	7.39	0.798	0.144	1.44	0.027
Benz[a]anthracene	132.15	27.29	39.07	27.81	0.777	0.390	0.230	0.309	320.66	181.01	891.14	44.34	2.67	0.787	3.43	0.164
Benzo[a]pyrene	113.04	17.91	29.67	18.04	0.665	0.256	0.175	0.200	190.75	126.86	858.20	30.46	1.59	0.552	3.30	0.113
Benzo[b]fluoranthene	210.22	31.46	51.32	41.11	1.24	0.449	0.302	0.457	362.57	230.01	1098.91	62.93	3.02	1.00	4.23	0.233
Benzo[e]pyrene	113.19	11.10	17.10	14.03	0.666	0.159	0.101	0.156	106.33	74.56	363.99	18.49	0.886	0.324	1.40	0.068
Benzo[ghi]perylene	68.16	9.60	18.78	10.46	0.401	0.137	0.110	0.116	94.92	86.95	496.78	24.45	0.791	0.378	1.91	0.091
Benzo[k]fluoranthene	63.42	9.65	16.87	13.08	0.373	0.138	0.099	0.145	105.29	70.29	343.05	18.54	0.877	0.306	1.32	0.069
Chrysene	140.29	28.77	41.28	29.45	0.825	0.411	0.243	0.327	339.95	192.12	945.50	46.63	2.83	0.835	3.64	0.173
Cyclopenta[cd]pyrene	115.98	23.95	34.22	24.19	0.682	0.342	0.201	0.269	291.14	166.17	948.06	38.58	2.43	0.722	3.65	0.143
Dibenz[a,h]anthracene	15.31	2.76	4.18	2.65	0.090	0.039	0.025	0.029	31.90	22.07	106.84	6.45	0.266	0.096	0.411	0.024
Dibenzo(a,e)pyrene	<LOD															
Fluoranthene	217.81	46.91	347.04	50.84	1.28	0.670	2.04	0.565	723.11	2252.66	11958.85	181.57	6.03	9.79	46.00	0.672
Fluorene	65.94	8.94	34.04	9.05	0.388	0.128	0.200	0.101	72.09	154.07	327.76	24.28	0.601	0.670	1.26	0.090
Indeno[1,2,3-cd]pyrene	75.12	12.20	21.71	12.45	0.442	0.174	0.128	0.138	116.04	99.16	575.79	28.84	0.967	0.431	2.22	0.107
Perylene	36.89	63.44	113.51	8.15	0.217	0.906	0.668	0.091	55.14	151.70	239.92	23.39	0.459	0.660	0.923	0.087
Phenanthrene	141.73	22.54	69.16	34.80	0.834	0.322	0.407	0.387	219.54	326.41	1008.60	46.27	1.83	1.42	3.88	0.171
Pyrene	200.40	32.14	78.05	43.32	1.18	0.459	0.459	0.481	395.15	460.83	4039.16	59.29	3.29	2.00	15.54	0.220
sum PAHs	1771.52	356.04	934.94	347.64	10.42	5.09	5.50	3.86	3548.26	4684.59	24880.63	669.66	29.57	20.37	95.69	2.48

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ANNEX 3

Title: Morphological and behavioral alterations in zebrafish larvae after exposure to river sediments collected in different weather conditions

Contributing authors: Aliaksandra Shuliakevich, Katja Schröder, Laura Nagengast, Melis Muz, Marek Pipal, Ira Brückner, Klara Hilscherova, Werner Brack, Sabrina Schiwy, Henner Hollert

Status: submitted

Table Annex 3: Author contributions for Annex 3.

Concept and design	Aliaksandra Shuliakevich	40%
	Katja Schröder	5%
	Klara Hilscherova	10%
	Werner Brack	5%
	Sabrina Schiwy	20%
	Henner Hollert	20%
Conducting tests and experiments	Aliaksandra Shuliakevich (Sediment contact assay with sediments from the sampling campaigns 2017 and 2018)	40%
	Katja Schröder (Sediment contact assay with sediments from the sampling campaign 2017, Fish embryo toxicity test, Locomotion assay)	40%
	Laura Nagengast (Sediment contact assay with sediments from the sampling campaign 2018)	15%
	Melis Muz (chemical analysis)	5%
Compilation of data sets and figures	Aliaksandra Shuliakevich (compilation of data sets and figures of all bioassays and supplementary information)	40%
	Katja Schröder (compilation of data sets and figures of all bioassays, and supplementary information)	20%
	Laura Nagengast (supportive compilation of data sets from the Sediment contact assay)	5%
	Melis Muz (compilation of data sets of the chemical analysis)	5%
	Sabrina Schiwy (compilation of data sets and figures of all bioassays, chemical analysis, and supplementary information)	15%
	Henner Hollert (compilation of data sets and figures of all bioassays, chemical analysis, and supplementary information)	15%
Analysis and interpretation of data	Aliaksandra Shuliakevich (analysis and interpretation of all data generated in the Sediment contact assay, supportive analysis, and interpretation of data generated during chemical analysis)	40%
	Katja Schröder (analysis and interpretation of all data generated in the Sediment contact assay with sediments from the sampling campaign 2017, Fish embryo toxicity test, Locomotion assay)	20%
	Laura Nagengast (supportive analysis and interpretation of data generated in the Sediment contact assay)	2.5%
	Melis Muz (analysis and interpretation of data generated during chemical analysis)	2.5%
	Marek Pipal (supportive analysis and interpretation of all data generated in the Fish embryo toxicity test and the Locomotion assay)	5%
	Klara Hilscherova (analysis and interpretation of data generated during chemical analysis)	5%
	Sabrina Schiwy (compilation of data sets and figures of all bioassays, chemical analysis, and supplementary information)	10%
	Henner Hollert (compilation of data sets and figures of all bioassays, chemical analysis, and supplementary information)	15%

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Table Annex 3 (continuation): Author contributions for Annex 3.

Drafting of manuscript	Aliaksandra Shuliakevich	35%
	Katja Schröder	20%
	Laura Nagengast	2.5%
	Melis Muz	2.5%
	Marek Pipal	2.5%
	Ira Brückner	2.5%
	Klara Hilscherova	5%
	Werner Brack	5%
	Sabrina Schiwy	10%
	Henner Hollert	15%

Morphological and behavioral alterations in zebrafish larvae after exposure to river sediments collected in different weather conditions

Contributing authors: Aliaksandra Shuliakevich[a], Katja Schröder[b], Laura Nagengast[b], Melis Muz[c], Marek Pipal[e], Ira Brückner[d], Klara Hilscherova[e], Werner Brack[a,c], Sabrina Schiwy[a], Henner Hollert[a]*

a: Goethe University Frankfurt/Main, Department Evolutionary Ecology and Environmental Toxicology, Max-von-Laue-Strasse 13, 60438 Frankfurt/Main, Germany

b: RWTH Aachen University, Institute of Biology V, Worringerweg 1, 52074 Aachen, Germany

c: Helmholtz Centre for Environmental Research – UFZ, Department of Effect-Directed Analysis, Permoserstrasse 15, 04318 Leipzig, Germany

d: Eifel-Rur Waterboard (WVER), Eisenbahnstr. 5, 52354 Düren, Germany

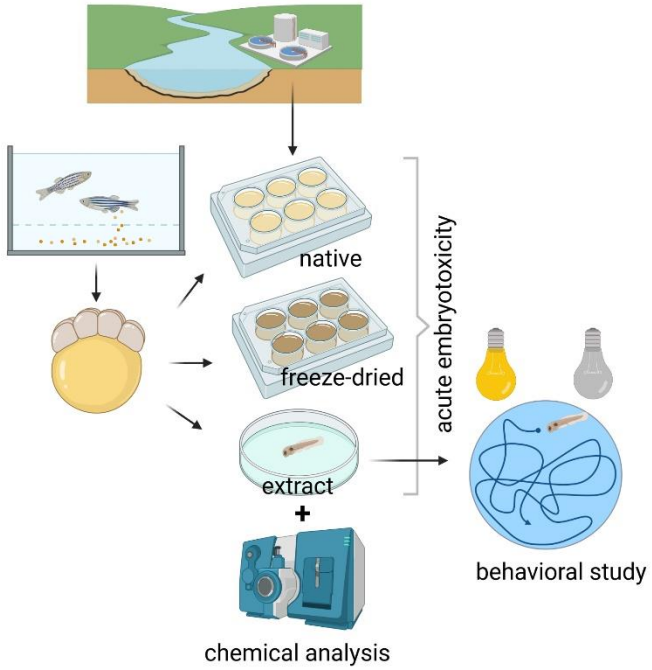
e: RECETOX, Faculty of Science, Masaryk University, Kotlarska 2, Brno, Czech Republic

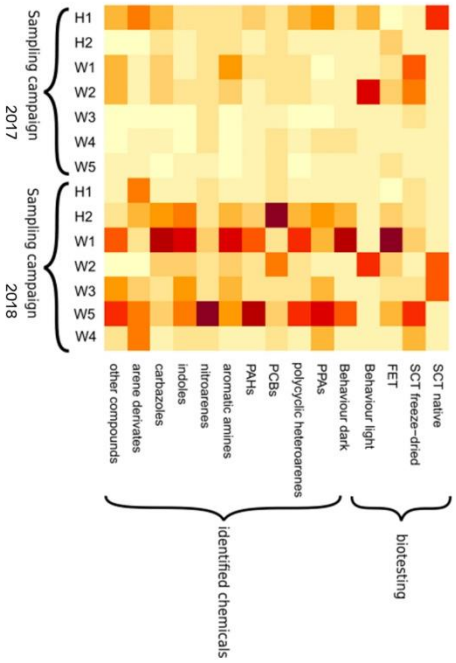
*Corresponding author: Hollert@bio.uni-frankfurt.de. Goethe University Frankfurt, Max-von-Laue-Str. 18 13, 60438 Frankfurt am Main, Germany

Keywords

Behavioral toxicity, embryotoxicity, sediment, zebrafish

Graphical abstract





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Abbreviations

3,4-DCA: 3,4-dichloroaniline

4,4'-DDMU: 1-chloro-4-[2-chloro-1-(4-chlorophenyl)ethenyl]benzene

DDT: Dichlordiphenyltrichlorethane

DMSO: Dimethylsulfoxide

dw: Dry weight

EC: Effect concentration

FET: Fish embryotoxicity test

GC: Gas chromatography

hpf: Hours post fertilisation

HRMS: High-resolution mass spectrometry

LA: Locomotion assay

LC: Liquid chromatography

NOEC: No-observed effect concentration

OC: Organic carbon

PAH: Polycyclic aromatic hydrocarbon

PCB: Polychlorinated biphenyl

ROB: Rainwater overflow basin

SCT: Sediment contact test

SEQ: Sediment-equivalent

WWTP: Wastewater treatment plant

Abstract

Aquatic ecosystems contain a mixture of organic micropollutants, among them neurotoxic ones. Wastewater treatment plants (WWTPs) serve as the primary source of organic micropollutants in aquatic ecosystems. Advanced wastewater treatment by ozonation has been proven to eliminate most organic micropollutants from the effluent. Many micropollutants tend to bind to sediments. Particle-bound micropollutants in aquatic ecosystems remain conserved until remobilization. Bioturbation and flood events remobilize sediments making micropollutants available in the water column again.

Especially during development, the highly complex nervous system of zebrafish larvae is vulnerable to exposure to neurotoxic substances. Furthermore, behavioral changes can be induced at low pollutant concentrations, which do not cause any acute toxicity. Behavioral studies serve as a sensitive indicator for the physiological and functional integrity of the nervous system, building an essential part of neurotoxicity testing in organisms exposed to environmental samples.

The present study characterizes the sampled sediments' toxic potential regarding zebrafish larvae development. While the embryotoxicity test was used to screen for acute toxicity, behavioral studies were applied to elucidate a more subtle impact. The study area includes two river stretches (North-Rhine Westphalia, Germany), which serve as receiving waterbodies for two WWTPs. The effluent of the Eilendorf WWTP with conventional wastewater treatment (mechanical, biological, and chemical) enters the Haarbach River. This is the tributary of the Wurm River receiving the effluent from the Aachen-Soers WWTP with a large-scale ozonation plant as an advanced purification stage. Additionally, the Aachen-Soers WWTP has four rainwater overflow basins released 40 times per year. Seven sampling sites up- and downstream of the mentioned WWTPs were investigated in two subsequent years in different weather conditions.

Exposure to native sediments had no visible adverse effects on the developing larvae, while freeze-dried sediments and organic sediment extracts caused acute sublethal effects. After exposure to sediment extracts, the behavior testing of zebrafish larvae revealed developmental effects below the threshold of visible deformations, emphasizing its high sensitivity. All investigated sediments showed embryotoxic potential. Rainwater overflow basin was identified as a possible source of pollution.

Target chemical analysis highlighted polyaromatic hydrocarbons, polychlorinated biphenyls, and nitroaromatic compounds as possible drivers for observed embryotoxic effects, whereas further toxicity drivers remained unknown. Investigation of mixture toxicity, effect-directed analysis, and further sediment monitoring are needed. Based on the recognized point source of pollution as a rainwater overflow basin, additional hold-back capacities like retention soil filters could help to improve the ecotoxicological profile of the investigated river stretch.

1 Introduction

The European market comprises over 50,000 chemicals (Bond and Garny, 2019). More than 60% of those are hazardous to humans and the environment (Milieu Ltd et al., 2017). Wastewater treatment plant (WWTP) effluents and rainwater overflow still serve as the primary path for organic micropollutants into aquatic ecosystems (Commission of the European Parliament and the Council, 2019). Such organic micropollutants as pharmaceuticals, plant protective agents, flame retardants, polyaromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs) are commonly identified in conventionally treated (mechanical, biological, and chemical treatments) effluents (Alygizakis et al., 2019; Krzeminski et al., 2017). Advanced wastewater treatment by ozone has been proven to be promising for eliminating most organic micropollutants from the effluent (Abegglen et al., 2009; Schneider et al., 2020; Stalter et al., 2010a; Stalter et al., 2010b; Stalter et al., 2020; Triebskorn, 2017; Tuerk et al., 2010).

After release into the receiving waterbody, effluent micropollutants distribute between the water and sediment phase. Some pharmaceuticals, plant protective agents, flame retardants, PAHs, and PCBs tend to bind to sediment particles (Bathi et al., 2012; Birnbaum and Staskal-Wikoff, 2010; Bundschuh et al., 2016; Ding et al., 2010; Martinez et al., 2010; Massei et al., 2019; Yeager et al., 2007). While the water phase in aquatic ecosystems fluctuates, sediments accumulate organic micropollutants over time (Kais et al., 2017; Macikova et al., 2014; Schiwy et al., 2015). Frequent droughts and extreme rain events accelerate soil erosion and run-offs of sediments into aquatic ecosystems (Selbig, 2009). Bioturbation and flood events remobilize sediments making pollutants available in the water column again. Several studies showed ingestion or absorption of particle-bound pollutants through the gill epithelium of fish, consequently leading to a decline of whole fish populations (Bickham et al., 2000; Corredor-Santamaría et al., 2016; Keiter et al., 2006; Theodorakis, 2001).

Several studies identified pharmaceuticals, plant protective agents, flame retardants, and PCBs as human neurotoxic substances (Grandjean and Landrigan, 2006; Herbstman et al., 2010; Maranho et al., 2014). If persistent in the environment (Malaj et al., 2014), these chemicals can act neurotoxic to wildlife, bearing risk for non-target organisms. Adverse effects of neurotoxic substances are mirrored in behavioral changes of the organisms (Ford et al., 2021; Legradi et al., 2018). Especially during

development, the highly complex nervous system is vulnerable to exposure to neurotoxic substances (Moser, 2011). Behavioral changes can be induced at low pollutant concentrations, which do not cause other effects (Melvin and Wilson, 2013). Thus, behavioral studies serve as a sensitive indicator for the physiological and functional integrity of the nervous system (Moser, 2011), building an essential part of neurotoxicity testing in organisms exposed to environmental samples (Ågerstrand et al., 2020; Ford et al., 2021; Legradi et al., 2018).

Currently, aquatic non-target organisms are not included in any regulation of potentially neurotoxic chemical compounds (Ågerstrand et al., 2020; Legradi et al., 2018). However, this situation does not represent the increased sensitivity and precision of laboratory tests with zebrafish as an established standard organism in ecotoxicology (Feiler et al., 2005; Keiter et al., 2010). Furthermore, the development of the nervous (Jurisch-Yaksi et al., 2020; Tegelenbosch et al., 2012; Winberg et al., 1997) and neurotransmitter systems (Haug et al., 2013) of vertebrates is evolutionary conserved, making zebrafish behavioral disturbances relevant for risk assessment in other vertebrates, including mammals. Furthermore, as zebrafish larvae feed on their yolk sack until 120 h post-fertilization (hpf), they can be well used for in vivo testing (Strähle et al., 2012). Recent studies have shown that zebrafish larvae are a suitable animal-alternative behavioral model for testing synthetic opioids (Kirla et al., 2021), ketamine (Félix et al., 2017), antiepileptic drugs (Martinez et al., 2018), phytocannabinoids, and terpenes (Achenbach et al., 2018). However, despite the scientific commitment in modern experimental techniques to act according to the 3R principle (reduction, replacement, and refinement of animal testing) (Russell and Burch, 1959), animal-alternative behavioral models for evaluation of a neurotoxic potential were initially not trusted enough by the scientific community (US EPA, 1998).

The current study is part of the DemO3AC project (Brückner et al., 2018), investigating the effects of a large-scale ozonation plant on the effluent of a wastewater treatment plant (WWTP) and the receiving waterbody in the context of their environment. As a side project, the present study investigated sediments' ecotoxicological and chemical status. In addition, the extent to which the large-scale ozonation plant can further eliminate trace substances and thus also have a positive influence on the sediment in the medium term was examined. The present study focused on the availability of potentially hazardous particle-bound chemicals in freshwater sediments and their

possible organic and somatic (behavioral) adverse effects on zebrafish larvae. Increasing the ecological relevance, zebrafish larvae were directly exposed to native and freeze-dried sediments using the Sediment Contact Test (SCT) to investigate potential organic changes caused by particle-bound chemical compounds (Hollert et al., 2003; Kosmehl et al., 2006). Potential somatic (behavioral) changes occur due to disturbance in the complex interactions of the peripheral and central nervous systems in response to environmental contaminants (National Research Council, 1992). Therefore, zebrafish larvae were exposed to the no-observed effect concentration (NOEC) of the Fish Embryo acute Toxicity test (FET) with sediment extracts, and the zebrafish locomotion assay (LA) was performed. In addition, possible drivers for detected effects were elucidated using highly sensitive analytics methods (Johann et al., 2020; Juan-García et al., 2020; Oliveri et al., 2020).

The present study aimed at: 1) characterizing the toxic potential of the sampled sediments regarding the zebrafish larvae development and behavioral changes; 2) characterizing the sediment chemical burden; 3) identifying possible chemical drivers for detected effects.

2 Material and methods

2.1 Study design

The study objects were the small waterbody Wurm River and its tributary, the Haarbach River (North-Rhine Westphalia, Germany). Sediment samples were collected as described in Shuliakevich et al. (2022b). The first two sampling sites were located in the Haarbach River, up- (H1) and downstream (H2) of the Eilendorf WWTP discharge (4 Mio m3/y; mechanical, biological, and chemical treatment steps, sand filter) (see Figure 1). The effluent of the Eilendorf WWTP made up around 11% of the total water flow of the Wurm River (ELWAS-WEB, 2021a). Before the Haarbach River entered the main waterbody, sediments of the Wurm River were sampled without the tributary's impact (W1). The following sampling site was located after the confluence of the Wurm River and the Haarbach River (W2) and before the Aachen-Soers WWTP (32 Mio m3/y; mechanical, biological, and chemical treatment steps, (ozonation in 2018), sand filter). Sediments in the Wurm River potentially impacted by the rainwater overflow basin (ROB) of the Aachen-Soers WWTP were sampled at the sampling site W3. Furthermore, sediments were sampled directly at the effluent discharging site (W4) and 2.5 km downstream of the effluent in the natural environment (W5).

The current study included two sampling campaigns in 2017 and 2018 (see Table 1). The first sampling campaign in 2017 was characterized by a long arid period, a low water level, stable hydrological conditions, and the dominance of the Aachen-Soers WWTP effluent on the total water flow of the Wurm River (70-95%) (Brückner et al., 2018; ELWAS-WEB, 2018). The second sampling campaign in 2018 was performed exactly one year later, after extensive rain events marked by normal to high water levels, unstable hydrological conditions, and diminished predominance of the Aachen-Soers WWTP effluent on the total water flow of the Wurm River (55%) (Shuliakevich et al., 2022b). Six months before the second sampling campaign, full-stream effluent ozonation was launched at the Aachen-Soers WWTP.

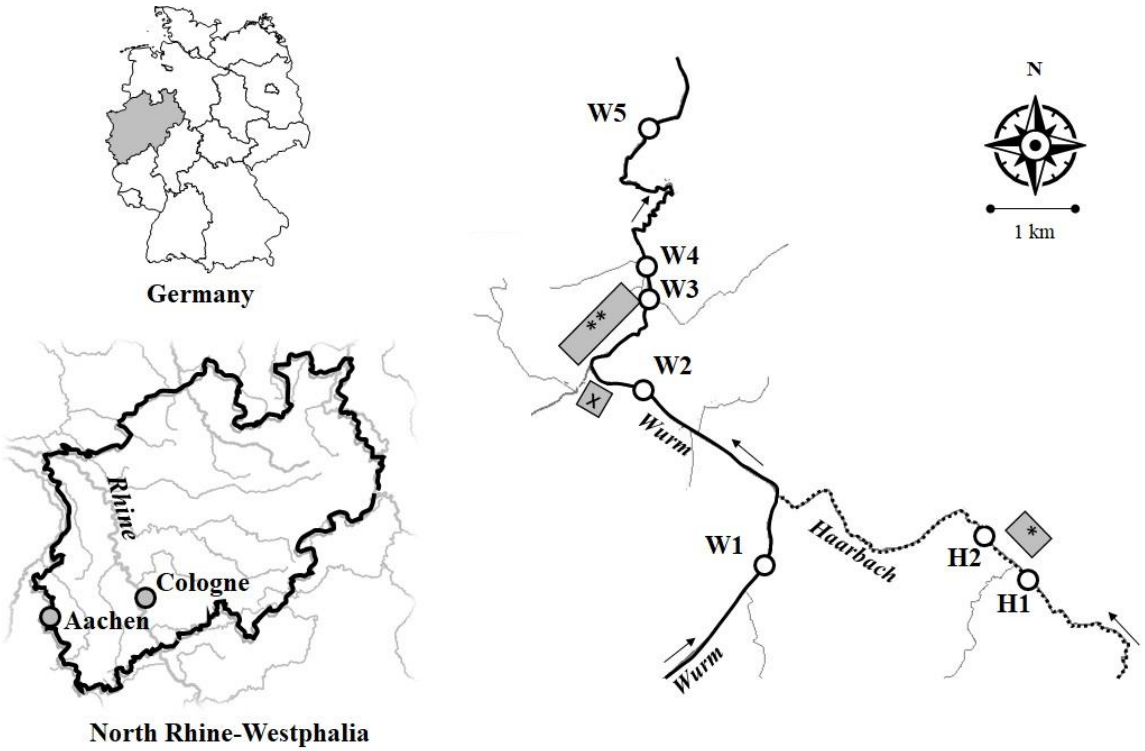

*Figure 1: Sampling sites along the Wurm River and the Haarbach River (1:36,112). *: Eilendorf WWTP (receiving waterbody: Haarbach River). **: Aachen-Soers WWTP (receiving waterbody: Wurm River). X: rain overflow basin of the Aachen-Soers WWTP. Source: ELWAS-WEB v. 4.0.0, www.elwasweb.nrw.de, modified by the authors. Created using Power-user ®.*

Table 1: Study design and sampling sites description. Reference of historical precipitation values: WetterKontor GmbH (2017), WetterKontor GmbH (2018).

River	Sampling Site	Extended and (short) description
Haarbach River	H1	upstream of the Eilendorf WWTP (upstream E-WWTP
	H2	downstream of the Eilendorf WWTP (effluent site E-WWTP)
Wurm River	W1	Wurm River before the confluence with the Haarbach River
	W2	Wurm River after the confluence with the Haarbach River
	W3	downstream of the Aachen-Soers WWTP rainwater overflow basin. Relevant discharges: 10 days before the sampling campaign in June 2017 and 2 days before the sampling campaign in June 2018
	W4	effluent site of the Aachen-Soers WWTP (effluent site AS-WWTP). Treatment steps during the sampling campaign 2017: mechanical, biological, and chemical treatment steps, sand filter. Treatment steps during the sampling campaign 2018: mechanical, biological, and chemical treatment steps, ozonation, sand filter
	W5	2.5 km downstream of W4 (downstream AS-WWTP)
Average precipitation 21 days before each sampling campaign		Sampling campaign 2017: 1 L/m^2 Sampling campaign 2018: 5 L/m^2

2.2 Materials and chemicals

All details can be found in Supplementary Information (SI), S1.

2.3 Sample preparation procedure

Sediment samples were collected from the first 10 cm in the cross and longitudinal profiles and united to one grab sample. For the sediment contact test (SCT), native sediments were tested within two weeks after the sampling. For the SCT with freeze-dried sediments, the same sediments as for extraction were used. Freeze-drying or lyophilization is a broadly applicable procedure for the gentle and immediate preservation of sediments (Duroudier, 2016). After reaching the gas-liquid-solid coexistence (triple point), frozen sediment pore water merges directly into the gas phase. As the freeze-drying procedure does not require heating, thermolabile chemical compounds remain preserved in solid environmental samples (Duroudier, 2016). However, particle-bound chemical compounds become available after removing the water coat from sediment particles. Consequently, freeze-dried sediments can be more toxic than native ones (Kosmehl et al., 2006). The storage of native and freeze-dried sediments was conducted at 4 °C.

For the fish embryo toxicity test (FET) and the locomotion assay (LA), 25 g of freeze-dried sediments (sieved, 2 mm) were extracted using sequential accelerated solvent extraction with methanol and dichloromethane (first cycle: 100:0, v/v, 100°C, max. pressure 100 bar; second cycle: 0:100, v/v, 100°C, max. Pressure 100 bar) (SpeedExtractor E-914/E-916, BUCHI Corporation) according to previous extraction methods (Di Giorgio et al., 2011; Grung et al., 2011; Lübcke-von Varel et al., 2012). After the solvent evaporation (MultivaporTM P-6, BUCHI Corporation), DMSO was added to organic sediment extracts to a final extract concentration of 25 g dry weight sediment-equivalent (dw SEQ)/mL DMSO.

2.4 Chemical analysis

For the chemical analysis, freeze-dried, sieved (2 mm), and homogenized sediments in the amount of 100 mg organic carbon (OC) (see SI, S2) rather than on dry weight (Massei et al., 2018) were extracted using the procedure described in Chapter 2.3. Within the first step, organic sediment extracts were cleaned up using flash chromatography (see SI, S3). Organic sediment extracts for the analysis utilizing Gas Chromatography High-Resolution Mass Spectrometry (GC-HRMS) were adjusted to

500 µL ethyl-acetate (EtAc) and spiked with the internal standard mix (1 µg/mL in EtAc). Organic sediment extracts for the analysis utilizing Liquid Chromatography (LC) were adjusted to 500 µL methanol and spiked with 1 µg/mL internal standard mix in methanol.

Chemical analysis of PAHs, PCBs, pyrethroids, brominated flame retardants, polycyclic heteroarenes was performed with the GC-HRMS (QExactive, Thermo Fisher Scientific, Germany) in Full Scan mode (res. 60,000). Data were evaluated using the software TraceFinder 5.1.

Chemical analysis of the substituted PAHs, quinones, azaarenes, and nitro-compounds was performed with the LC-QExactive Plus with an atmospheric pressure chemical ionization source. Separate runs were conducted in positive and negative ion modes. The chemical analysis of aromatic amines and carbolines was performed with the LC-QExactive Plus with a heated electrospray ionization source in positive mode. Data evaluation was done with TraceFinder version 5.1. Compounds were confirmed by exact mass, isotopic pattern, retention time, and at least one fragment. Details of the method can be found in Muz et al. (2017).

For more details on the target chemical analyses, see Shuliakevich et al. (2022a).

The list of analysed chemical compounds with respective internal standards can be found in SI, S4. This data was partly published in Shuliakevich et al. (2022a).

2.5 Husbandry conditions of the zebrafish Danio rerio

For the SCT, wild-type zebrafish strains of the Institute for Ecosystem Analysis at the RWTH Aachen University (Germany) were used. While for the FET and the LA with sediment extracts, locally purchased wild-type zebrafish strains of the RECETOX at the Masaryk University (Czech Republic) were used. The husbandry conditions of both zebrafish strains were equal and conducted according to OECD (2013). Adult zebrafish were kept in aquariums with a constant water temperature of 26±1 °C and 14 h:10 h light-dark rhythms. Feeding was conducted twice a day: once with commercially available dry flakes mixed with spirulina and dried gammarids and once with live Artemia spec. larvae. On the experiment day, the spawning was induced by turning on the light. Fish eggs collection proceeded into glass dishes covered with mesh. Additional details regarding zebrafish husbandry can be found in Johann et al. (2020) and Pipal et al. (2020).

2.6 Sediment Contact Test (SCT)

Previous studies showed that direct exposure of fish embryos to sediment is prone to producing false-positive results due to gaseous misbalance between the water and the sediment phase (Hollert et al., 2003; Schiwy et al., 2015; Zielke et al., 2011). Therefore, before the zebrafish SCT, all sediment samples (native and freeze-dried) were tested for their possibility of oxygen depletion (oxygen concentration < 0.5 mg/L) within the sediment-artificial water set-up. For this purpose, samples were prepared in the same way as for the SCT but without fish eggs. Oxygen measurement was carried out by a needle-type oxygen microsensor (PreSens NTH-PSt1, PreSens Precision Sensing GmbH, Germany) at room temperature (22±1°C) and without shaking.

The SCT was conducted according to the German Standard DIN EN ISO 15088 (DIN, 2009) with modification regarding the direct exposure to sediment as described by Hollert et al. (2003). Thus, 3 g freeze-dried sediment, the respective amount of native sediment (see SI, S6), or quartz sand (F36; Quarzwerke Frechen, Frechen, Germany) as a reference were placed into each well of a 6-well plate and covered by 4 mL of oxygenated artificial water. The SCT was conducted after a preincubation time of 24 h. Five fertilized fish eggs in 1 mL of artificial water were added to each well of a 6-well plate. For the first 24 h (preincubation) and following 96 h (exposure of fish eggs), 6-well plates were placed on the shaking platform within the incubator (26±1°C, 50 rpm). Each sample was tested in the limit test (highest sediment concentration of 3 g dw SEQ/5 mL) in one technical (three wells per test run) replicate with 15 zebrafish eggs per sample. Every 24 h, fish embryos were collected from the sediment and transferred in the well-internal medium into a 24-well plate to examine possible lethal and sublethal effects using a stereomicroscope. After all examinations, fish embryos were transferred back to the 6-well plate for further incubation.

2.7 Fish embryo toxicity test (FET)

The zebrafish FET with organic sediment extracts was carried out according to the German Standard DIN EN ISO 15088 (DIN, 2009). Briefly, Danio rerio fish eggs in an 8-cell stage were exposed to 0.1% extract in artificial water until 120 hours post-fertilization (hpf) under static conditions (no medium exchange). Each organic sediment extract was tested in five dilution steps (1:2, highest extract concentration 25 mg dw SEQ/mL in the test) with 20 fish eggs per dilution and in three biological replicates (on three different days). Each 24 h fish embryos/larvae were evaluated for

sublethal and lethal effects (see SI, S7). Additionally, next to the negative (artificial water) and positive controls (3,4-dicloroaniline, 3,4-DCA), the solvent control (0.1% DMSO in artificial water) was tested to exclude any effects caused by the solvent. The dose-response relationship was modelled with a non-linear regression in GraphPad Prism 6 to determine the effect concentrations EC50 and EC10. The no-observed effect concentration (NOEC) in the FET was determined using Fisher's exact test.

2.8 Locomotion assay (LA)

Investigation of possible behavioral alterations in zebrafish embryos using the zebrafish LA was conducted according to Pípal et al. (2020) with some modifications. For the LA 5-day old larvae were used after their exposure to the NOEC of the respective organic sediment extract (determined using the FET). 96 larvae (32 per replicate) were used as a negative control, solvent control, or for exposure, respectively. Zebrafish eggs exposure was conducted in 6-well plates containing 10 mL and 20 eggs per well, at 26±1◦C, under the light-dark rhythms of 14 h:10 h. Each 24 h, Danio rerio fish embryos/larvae were inspected for sublethal malformations and lethality. After 96 h, each zebrafish larva was individually transferred in a 96-well plate with one larva per well in 200 µL of liquid (sediment extract solution, negative control, solvent control). Then, 96-well plates were incubated for the next 24 h and checked again for lethal and sublethal effects. The positive control was prepared on the day of measurement 60 min before the experiment by transferring 96 unexposed zebrafish larvae into 1% ethanol solution in artificial water. After that, all zebrafish larvae from the positive control were transferred into one 96 well-plate as described above.

The total distance swam, swimming velocity, and swimming time was assessed using the DanioVision with the EthoVision XT 14 Software and integrated heating unit (26°C). The measurement was started after the adaptation phase of 10 min in the dark. The total test duration was 40 min with light-dark changes every 10 minutes and 30 frames per second acquisition. Results from the three listed endpoints were obtained each minute. In addition, the statistical significance of the differences in locomotion activity of zebrafish larvae after exposure to organic sediment extracts compared to the negative/solvent control was assessed using the Mann-Whitney U test. All statistical calculations and graphs for this test were created using the R 4.1.1 software in RStudio (R Foundation for Statistical Computing, 2021).

2.9 Summary and visualization of results

All results of the bioassays and chemical measurements at the different sampling sites and time points were summarized in a heatmap to simplify and overview the results. The heatmap displays effects/chemical concentrations by scaling colors from bright yellow to dark red to the found values. To retain the meaning of a stronger effect at a darker color, the EC50 values from the FET were inverted and normalized to the highest value first, if not 0. The coloring indicates the distribution of effect intensity or chemical concentration within one test or chemical measurement. As the LA was performed for the NOEC of the respective extract in the FET, the results were not directly comparable.

For this reason, all behavioral effects were normalized to the concentrations they were found at. All other values remained as is. The heatmap was created using the R 4.1.1 software in RStudio (R Foundation for Statistical Computing, 2021).

3 Results

3.1 Effects on zebrafish embryos after exposure to native and freeze-dried sediments

Oxygen levels of the sediment samples were measured to assure that oxygen depletion would not cause any adverse effects. The results showed optimal oxygen conditions with concentrations above 0.5 mg/L 24 h after preparing the sediment-artificial water set-up (see SI, S8 A - S8 B). After contact with native sediments (3 g dw SEQ/5 mL) from the Haarbach and the Wurm Rivers, zebrafish embryos showed inconspicuous sublethal and lethal effect rates below 10% compared to the negative control (see Table 2). Contrarily, exposure of the zebrafish embryos to freeze-dried sediments (3 g dw SEQ/5 mL) showed severe sublethal effects at single sampling sites. No increased lethal effects were observed. Freeze-dried sediment samples collected at dry weather during the sampling campaign 2017 at the sampling site W1 (upstream confluence) led to delayed hatching for 36% of the zebrafish embryos (Figure 2). Also, freeze-dried sediments from the sampling site W2 (confluence site) showed general underdevelopment for 33% of the zebrafish larvae. Freeze-dried sediment samples collected one year later after extensive rain events during the sampling campaign 2018 at the sampling site W4 (effluent site AS-WWTP) caused pericardial edema, blood clogging, and low body pigmentation for 47% of the zebrafish larvae in the stage of 96 hpf (see Table 3). Furthermore, freeze-dried sediment samples from the sampling site W5 (downstream AS-WWTP) caused low pigmentation of eyes and body for 20% of fish larvae at 96 hpf.

The sediment extract gained from sediments upstream of the Eilendorf WWTP (H1) during both sampling campaigns revealed no sublethal effects (NOEC = 25 mg dw SEQ/mL) during the whole test time of 120 h (see Table 2). Organic sediment extracts collected during the sampling campaign 2017 at the sampling sites W3 and W4 (downstream ROB and effluent site AS-WWTP, respectively) required a 1:1 dilution for being non-embryotoxic in the FET (NOEC = 12.5 mg dw SEQ/mL). The remaining organic sediment extracts from the Haarbach (H2) and the Wurm Rivers (W1, W2, W5) showed the NOEC at 6.25 mg dw SEQ/mL. During the sampling campaign 2018, sediments from the sampling site W5 (downstream AS-WWTP) required a 1:1 dilution for reaching the NOEC of 12.5 mg dw SEQ/mL (see Table 3). Organic sediment extracts from the sampling sites W1 (upstream confluence), W3, and W4 revealed lower NOEC

than those collected during the sampling campaign 2017 (1.56, 6.25, and 3.13 mg dw SEQ/mL, respectively). No increased lethal effects during the FET with sediment extracts were observed.

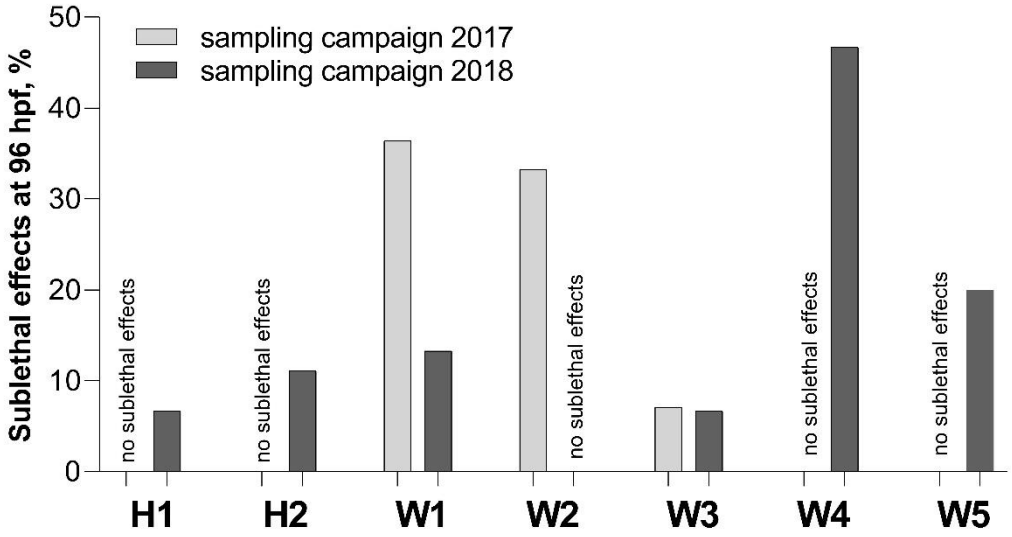

Figure 2: Sublethal effects observed in zebrafish larvae after exposure to freeze-dried sediments (3 g dw SEQ/5 mL).

*Table 2: Summary of endpoints (effects and effect concentrations) from the SCT with native and freeze-dried sediments and the FET with organic sediment extracts. All sediment samples were collected during the sampling campaign 2017. The zebrafish larvae development stage of evaluating the results in the SCT: 96 hpf. The zebrafish larvae development stage of evaluating the results in the FET: 120 hpf. NOEC: no-observed effect concentration. EC: effect concentration. The EC10 and EC50 values were assessed from the dose (sediment concentration) – response (sublethal effect) relationship. 1: maximal tested concentration (25 mg de SEQ/mL in test); * marks extrapolated values.*

Test system	Endpoint (effects / effect concentration)	Sampling campaign 2017 Sampling sites H1	H2	W1	W2	W3	W4	W5
SCT native	sublethal, %	0	0	0	0	0	0	0
	lethal, %	7.7	0	0	0	0	0	0
SCT freeze-dried	sublethal, %	0	0	36.4	33.3	7.1	0	0
	lethal, %	0	0	0	0	0	0	8.3
FET organic extract	NOEC, mg dw SEQ/mL for the LA	25[1]	6.25	6.25	6.25	12.5	12.5	6.25
	EC10, mg dw SEQ/mL	-	6.0	6.3	6.0	10.7	10.8	10.4
	EC50, mg dw SEQ/mL	-	8.0	11.2	8.0	30.3*	25.5*	14.9

*Table 3: Summary of endpoints (effects and effect concentrations) from the SCT with native and freeze-dried sediments and the FET with organic sediment extracts. All sediment samples were collected during the sampling campaign 2018. The zebrafish larvae development stage of evaluating the results in the SCT: 96 hpf. The zebrafish larvae development stage of evaluating the results in the FET: 120 hpf. NOEC: no-observed effect concentration. EC: effect concentration. The EC10 and EC50 values were assessed from the dose (sediment concentration) – response (sublethal effect) relationship. 1: maximal tested concentration (25 mg de SEQ/mL in test); * marks extrapolated values.*

Test system	Endpoint (effects / effect concentration)	Sampling campaign 2018 – Sampling sites						
		H1	H2	W1	W2	W3	W4	W5
SCT native	sublethal, %	0	0	0	6.7	6.7	0	0
	lethal, %	0	0	0	0	0	0	0
SCT freeze-dried	sublethal, %	6.7	0	13.3	0	6.7	46.7	20.0
	lethal, %	0	11.1	0	0	0	0	0
FET organic extract	NOEC, mg dw SEQ/mL for the LA	25[1]	6.25	1.56	6.25	6.25	3.13	12.5
	EC10, mg dw SEQ/mL	-	5.3	1.3	6.2	7.6	4.3	10.1
	EC50, mg dw SEQ/mL	-	8.2	2.3	8.8	12.8	6.7	25.2*

3.2 Zebrafish larvae locomotion behavior assessment

Zebrafish larvae were exposed to the NOEC determined in the fish embryo toxicity (FET) assay with organic sediment extracts to investigate the locomotion behavior. In addition, DMSO as a solvent control was tested in the range implemented in the assay (0.01 %, 0.025 %, 0.1%, and 1 %) to exclude the possible impact on the zebrafish larvae locomotion activity. The results showed a significant deviation (Mann-Whitney U test, $p<0.01$) compared to the negative control at 0.1 % DMSO, which led to the decision to compare all tested samples to the solvent instead of the negative control. Detailed results for the DMSO behavior testing can be found in SI, S9. The locomotion behavior was evaluated based on the parameter distance moved per minute. Results are visualized as fold changes of the solvent control (see Figures 3 and 4). For more details, see SI, S10.

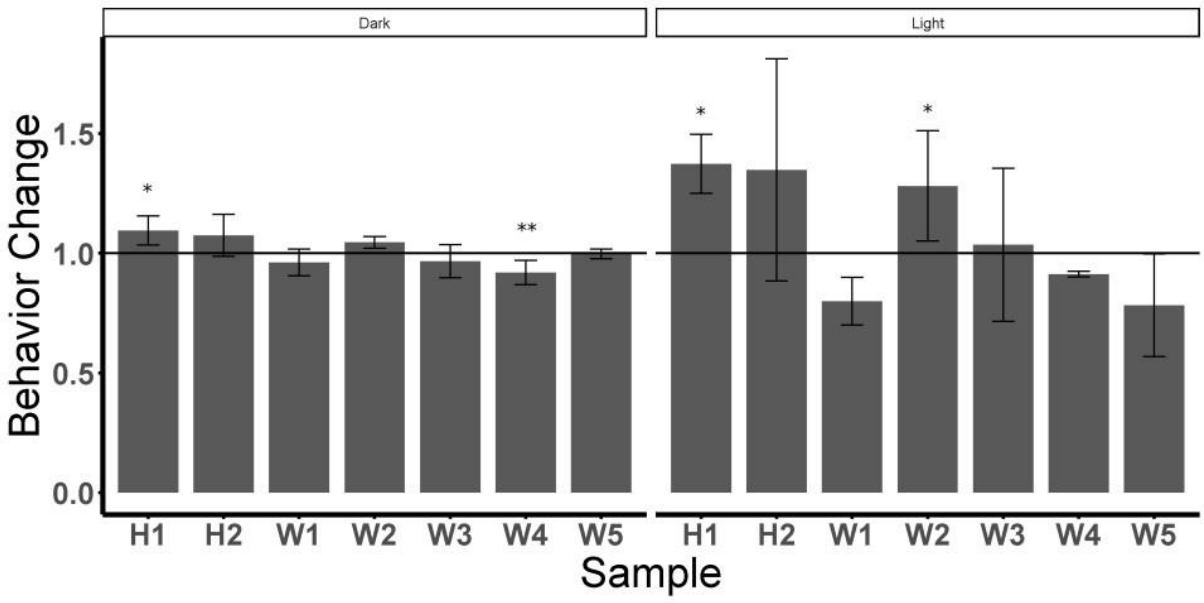

*Figure 3: Behavioral activity in zebrafish larvae (~ 120 hpf) in the LA after exposure to organic sediment extracts collected during the sampling campaign in 2017. The exposure concentration corresponds to the NOEC identified in the FET. Behavior change displayed as Fold Change (FC) compared to the solvent control (FC=1). Statistically significant differences were evaluated using the Mann-Whitney U test and are marked with * p<0.05 and ** p<0.01.*

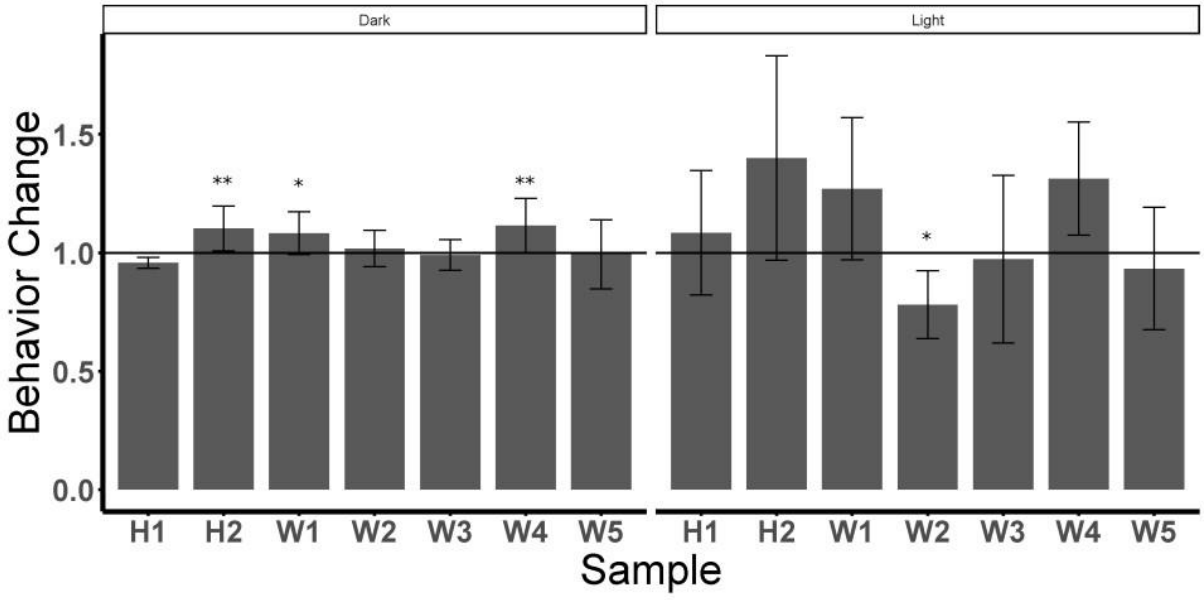

*Figure 4: Behavioral activity in zebrafish larvae (120 hpf) in the LA after exposure to organic sediment extracts collected during the sampling campaign in 2018. The exposure concentration referred to the NOEC identified in the FET. Behavior change displayed as Fold Change (FC) compared to the solvent control (FC=1). Statistically significant differences were evaluated using the Mann-Whitney U test and are marked with * p<0.05 and ** p<0.01.*

Exposure of zebrafish larvae to the NOEC of organic sediment extracts collected during the sampling campaign 2018 at the sampling sites H2 (effluent site E-WWTP; 6.25 mg dw SEQ/mL) and W1 (upstream confluence; 1.56 mg dw SEQ/mL) caused statistically significant (Mann-Whitney U test, $p<0.05$) change of locomotion activity in zebrafish larvae (↑10.2 ± 9.5 % at H2 and ↑8.3 ± 9.0 % at W1) for all endpoints in the dark phase of the experiment. Exposure to the referred organic sediment extracts collected one year before with NOECs of 6.25 mg dw SEQ/mL revealed no differences compared to the solvent control. Interestingly, sediments from the sampling site W2 (confluence site) during the sampling campaign 2018 caused significantly lower locomotion activity during the light phase (6.25 mg dw SEQ/mL, ↓21.9 ± 14.3 %). Contrarily, after exposure to the organic sediment extracts W2 (confluence site; 6.25 mg dw SEQ/mL) collected during the sampling campaign 2017, significantly higher locomotion activity in zebrafish larvae (↑28.0 ± 23.0%) was observed. Sediment samples from the sampling sites W3 (downstream ROB) and W5 (downstream AS-WWTP) showed no significant differences in zebrafish larvae behavior during dark and light phases. However, zebrafish larvae reacted to the low NOEC of 3.13 mg dw SEQ/mL of the organic sediment extract from the sampling site W4 (effluent site AS-WWTP) collected during the sampling campaign 2018 with statistically significant higher movement during the dark phase (↑11.5 ±11.4 %). Contrarily to this result, organic extracts from sediments at W4 (effluent site AS-WWTP) the year before showed significantly lower zebrafish larvae locomotion activity in the dark phase at the NOEC of 12.5 mg dw SEQ/mL (↓8.1 ± 5.0). Sediment samples from the sampling site H1 (upstream E-WWTP) were tested in the dark-light transition test in the highest concentration of 25 mg dw SEQ/mL, causing significantly higher locomotion activity without (↑9.5 ±6.0 %) and with (↑37.2 ±12.3 %) light for samples collected in 2017.

3.3 Sediments chemical pollution

Sediment samples from the Haarbach and the Wurm Rivers were analysed for 168 target substances (data partly published in Shuliakevich et al. (2022a); see SI, S4). Ninety-four target compounds from the groups of aromatic amines, carbazoles, nitroarenes, PAHs, PCBs, phenanthrolines, polycyclic heteroarenes, plant protective agents (dicofol, diphenyl sulfone, xanthone, 1-chloro-4-[2-chloro-1-(4-chlorophenyl) ethenyl]benzene (4,4'-DDMU)), and 2.4-dinitroaniline were quantified (see Table 4

and SI, S5). The sampling site H1, located upstream of the Eilendorf WWTP, revealed prominent concentrations of chemical compounds in June 2017. Furthermore, all chemical groups were found in concentrations higher (increase by 72%) than during the next sampling campaign in 2018. Interestingly, nitroarenes and the group comprising other compounds, not similar in structure or application area, were identified in higher concentrations during the sampling campaign 2017 (increase by 43% and 70%, respectively). The main driver in the other compounds group, whose concentration was lower during the sampling campaign 2017, was tributylamine.

Additionally, sampling site W3 (downstream ROB) showed higher concentrations of nitroarenes (+14%). Contrarily to the mentioned results, all sampling sites, except for the sampling site H1 (upstream E-WWTP), showed a higher chemical burden during the sampling campaign 2018. The most prominent group among all identified chemical compounds were PAHs that occurred in concentrations of µg/mg OC (see Figure 5).

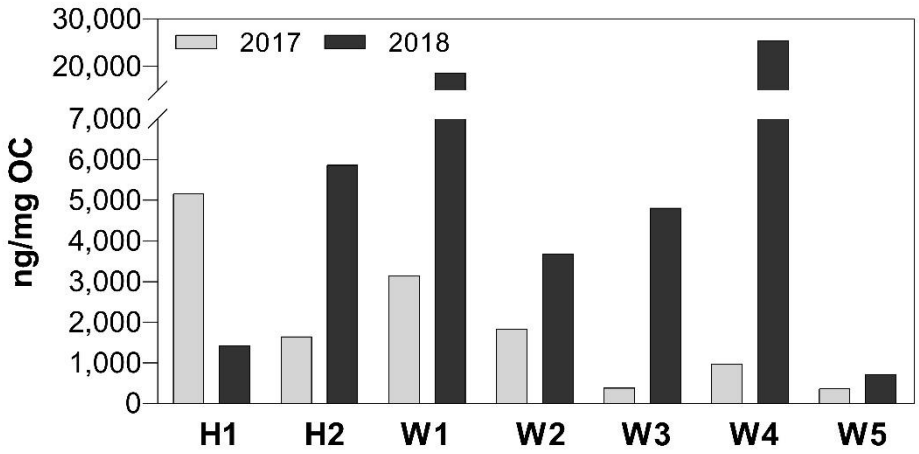

Figure 5: Sediment chemical burden of PAHs depending on the sampling site and weather conditions: 2017 (dry weather), 2018 (after extensive rain events).

*Table 4: Sediment chemical burden comprised of identified particle-bound target compounds. Other compounds: Methyltolysulfone, 3,3'-dichlorobenzidine, 1,1-dichloro-2,2-bis(4-methoxyphenyl)ethan, 4-chloroaniline, Michler's ketone, pentachloronitrobenzene, N-phenyl-1-naphthylamine, 2-nitrotoluene, trans-stilbene, tributylamine. * published in Shuliakevich et al. (2022a).*

Chemical group	ng/mg OC													
	H1		H2		W1		W2*		W3*		W4*		W5*	
	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
aromatic amines	0.9	0.6	0.9	1.3	1.4	2.4	1.0	1.0	0.3	1.3	0.3	1.5	0.4	0.5
arene derivates	0.4	0.4	0.1	0.3	0.1	0.2	0.1	0.1	0.0	0.2	0.1	0.4	0.1	0.4
cabazoles	7.3	2.5	4.6	9.3	5.6	16.8	6.1	6.6	1.2	4.8	3.1	6.5	2.0	3.7
indoles	0.3	0.2	0.2	0.8	0.1	1.2	0.3	0.4	0.1	0.7	0.1	0.8	0.2	0.3
nitroarenes	2.7	2.3	2.0	2.6	1.2	3.7	2.5	1.5	2.6	2.2	3.2	24.2	1.0	1.3
PAHs	5166.5	1434.0	1652.3	5870.1	3150.7	18692.3	1842.0	3694.8	378.5	4813.5	968.2	25527.4	369.0	711.2
PCBs	4.8	2.9	2.5	287.9	2.3	9.2	4.3	133.8	0.3	7.5	4.8	52.5	1.1	3.3
polycyclic heteroarenes	41.4	11.2	14.8	36.6	21.4	76.7	16.5	19.5	3.7	24.2	7.6	77.6	4.8	9.8
plant protective agents	1.0	0.4	0.3	1.1	0.1	0.8	0.2	0.4	0.1	0.9	0.5	1.8	0.2	0.8
phenanthrolines	-	-	-	-	-	-	-	-	-	-	-	-	0.0	1.2
other compounds	6.3	3.6	2.6	4.0	5.9	9.2	6.2	1.9	2.1	6.8	2.5	10.2	3.1	4.0
TOTAL	5231.7	1458.2	1680.3	6214.1	3188.8	18812.5	1879.4	3859.8	388.9	4862.0	990.4	25702.9	381.8	735.6

3.4 Correlation of the biological and chemical results

Combining the bioassays with the chemical analysis into a heat map allowed a comparison of impacts and chemical loading among sampling sites. The visualization of all data gained during the sampling campaign 2018 generally highlighted stronger effects than in sediments from the sampling campaign in 2017. In particularly, sediments from the sampling site W4 (effluent site AS-WWTP) showed embryotoxic (pericardial edema, blood clogging, and low body pigmentation) and behavioral (significantly higher movement during the dark phase) effects in zebrafish larvae and the highest PCB concentration of 26 µg/mg OC among all sampling sites (see Figure 6). Furthermore, sediment samples collected at the sampling site W2, after the confluence of the Wurm and the Haarbach Rivers, were conspicuous in the LA during both sampling campaigns. The upstream located sampling site W1 showed the second-highest total chemical concentration of 19 µg/mg OC among all sampling sites during the 2018 sampling combined with the most substantial effects in the FET and LA during the dark phase.

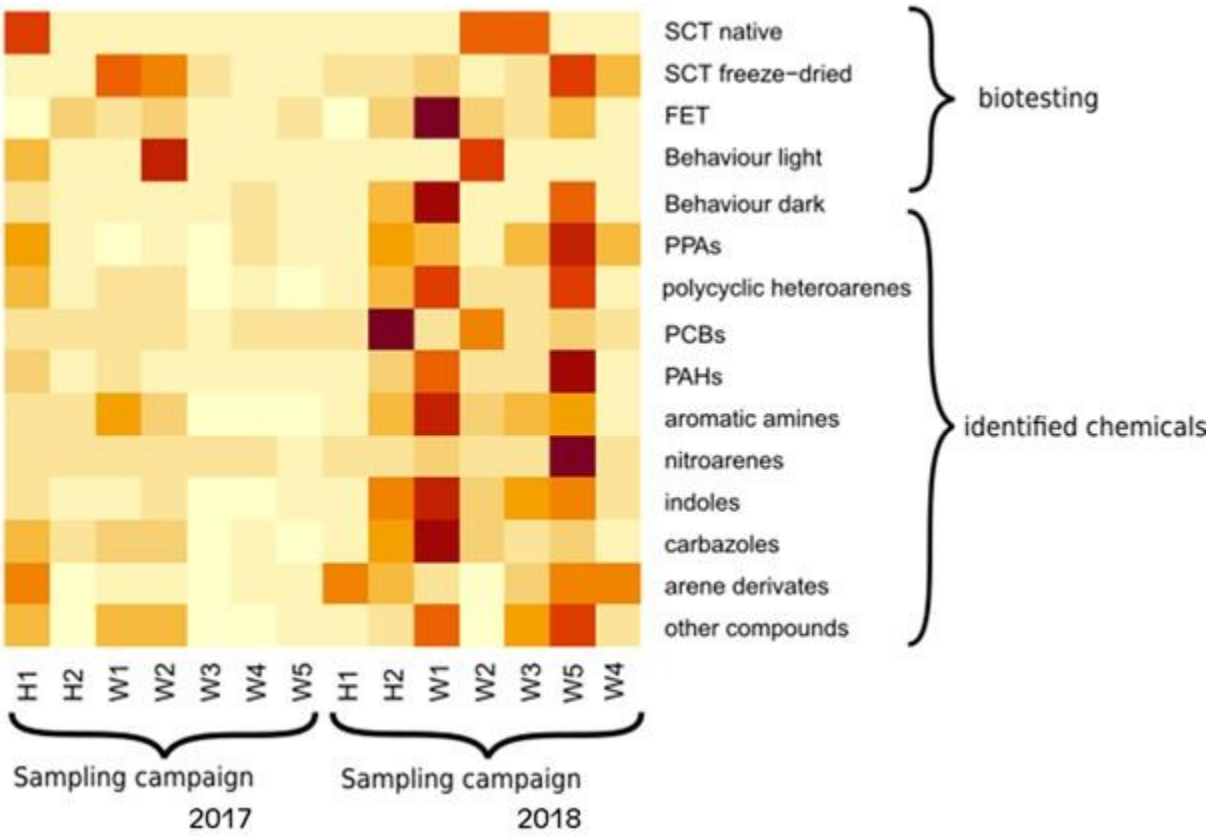

Figure 6: The heatmap shows the relative intensity of effects in bioassays or the concentration levels from chemical analyses at the different sites and sampling campaigns. Bright yellow refers to lower, dark red to higher values. Note that the comparability is given only between columns and not between rows.

4 Discussion

The present study investigated sublethal and lethal effects in zebrafish embryos/larvae during contact with native and freeze-dried sediments. Additionally, the zebrafish embryos/larvae were exposed to organic sediment extracts to investigate potential behavioral changes. Especially in the larval stage, the nervous system is vulnerable to the exposure of chemical compounds (Moser, 2011), even at concentrations below the effect concentration of acute toxicity (Melvin and Wilson, 2013). Larval behavior is a sensitive indicator of the physiological and functional integrity of the fish nervous system (Moser, 2011). Gained results were combined with identified particle-bound chemicals predicting potential drivers of toxicity.

Study sediments were collected from two small receiving waterbodies in North-Rhine Westphalia (Germany), the Haarbach and the Wurm Rivers. The first sediment sampling campaign took place in June 2017 during a dry summer with a low precipitation rate (1 L/m2 within 21 days before sampling). The second sediment sampling took place exactly one year later, in June 2018. This summer was characterized by prolonged rain periods (see Table 1), allowing observation of changes in sediment toxicity depending on weather conditions.

4.1 Sediment contact acute toxicity

Direct exposure of zebrafish embryos/larvae to sediments represents exposure to bioavailable contaminants as the most ecologically realistic scenario (Kosmehl et al., 2006). Exposure to freeze-dried sediments has the advantage of mimicking the ecotoxicological status of sediments during flood events (sediment remobilization) (Crawford et al., 2022). Native sediment samples from the Haarbach and the Wurm Rivers caused no lethal or sublethal effects on zebrafish embryos/larvae. Contrarily, sediment samples collected and freeze-dried during the dry summer in 2017 from the sampling sites W1 (upstream confluence) and W2 (confluence site) caused delayed hatching in 36% of the embryos (W1) and general underdevelopment in 33% of the zebrafish larvae (W2).

Hatching is one of the critical events after 72 hpf of zebrafish development, accompanied by elevated activity of different enzymes (Yamagami, 1988). Perrichon et al. (2014) described similar effects after exposure of zebrafish embryos (96 hpf) to artificial sediment (sand, 5% kaolin clay, 0% peat) containing 6.3 µg/g dw

fluoranthene. However, sediment samples from the sampling sites W1 and W2 contained 0.6 µg/g dw and 0.4 µg/g dw fluoranthene, respectively, indicating other chemical compounds contributing to delayed hatching and general development retardation. Heavy metals are well-studied inhibitors of hatching enzymes (Dave and Xiu, 1991). Analysis of suspended matter showed exceedance of quality concentrations for heavy metals at all studied stretches of the Haarbach and the Wurm Rivers (ELWAS-WEB, 2021b). Furthermore, some pharmaceuticals such as ibuprofen, diclofenac (Xia et al., 2017), and veterinary drug ivermectin (Oliveira et al., 2016) can significantly reduce the hatching rate of zebrafish embryos. Additionally, PCBs are well-known embryotoxic compounds and hatching inhibitors. Observed hatching delay and general underdevelopment might be caused by exposure to a mixture of particle-bound contaminants.

4.2 Rainwater overflow basin as a possible source of pollution

During the exposure to freeze-dried sediments collected after extensive rain events (2018), the strongest embryotoxic effects such as pericardial edema, blood clogging, and low body pigmentation were observed at the effluent site of the Aachen-Soers WWTP, W4, in 47% of the zebrafish larvae in the stage of 96 hpf. Furthermore, freeze-dried sediment samples from the sampling site W5 (downstream AS-WWTP) caused low eyes and body pigmentation (20% of fish larvae at 96 hpf). Thellmann et al. (2015) observed several endpoints of embryotoxicity, including mortality, malformations, reduced hatching rate, and heart rate after exposure of zebrafish embryos to sediments collected in the rivers of Southern Germany downstream of WWTPs.

During the sampling campaign in 2018, two key events took place standing in a possible connection to the observed embryotoxicity. At the time of sediment sampling in summer 2018, the ozonation plant of the Aachen-Soers WWTP was launched within half a year before. The effectiveness of the advanced effluent treatment of the Aachen-Soers WWTP was shown in reducing the endocrine-disrupting activity (Wolf et al., 2022), genotoxic and embryotoxic potentials in the effluent (not published data). The effluent water portion in the Wurm River after rain events comprised about 50% (Shuliakevich et al., 2022b).

Previous investigations have already identified the rainwater overflow basin (ROB) of the Aachen-Soers WWTP as a significant source for endocrine-disrupting (Shuliakevich et al., 2022b) and genotoxic (Shuliakevich et al., 2022a) particle-bound

pollutants. The content of the ROB is represented by a mixture of waste- and rainwater with a high concentration of suspended particles (Shuliakevich et al., 2022b), which serve as a primary transmitter of hydrophobic pollutants into the receiving stream (Eganhouse and Sherblom, 2001). Each ROB release acts as a flash flood with subsequent sediment perturbation bearing the risk of releasing particle-bound chemicals (Schertzinger et al., 2019; Shuliakevich et al., 2022a; Shuliakevich et al., 2022b). Vincze et al. (2014) reported stronger teratogenic effects in fish embryos during flood events in the Neckar River (Germany) in combination with such particle-bound pollutants as PAHs (Wölz et al., 2008).

PAHs are well-known embryotoxic compounds causing various organ dysfunction in zebrafish embryos like edemas and cardio-vascular disorders (Cunha et al., 2020; Zhai et al., 2020). Seiler et al. (2014) comprehensively described the embryotoxicity of different PAHs, grouping them into effective (naphthalene, acenaphthene, phenanthrene, fluoranthene, fluorene, pyrene, and chrysene) and non-effective (benz[a]anthracene, benzo(a)pyrene, and anthracene) compounds. Indeed, fluoranthene as one of the effective PAHs was identified in sediments from the sampling site W4 after extensive rain events in a concentration of 12 µg/mg OC. Compared with the total PAHs concentration at the sampling site W4 (26 µg/mg OC), fluoranthene made up 47% of all PAHs, suggesting that it is potentially responsible for the observed embryotoxicity. However, while PAHs occur in aquatic ecosystems in a mixture, non-effective PAHs and micropollutants can contribute to general toxicity (Seiler et al., 2014).

Furthermore, sampling site W4 revealed the highest concentration of nitroaromatic compounds (24 ng/mg OC) among all sampling sites. Koske et al. (2019) showed nitroarenes as drivers for low pigmentation in zebrafish embryos. Thus, nitroarenes could be potentially responsible for the observed low pigmented zebrafish larvae. Compared to sampling site W4, the downstream located sampling site W5 contained lower concentrations of PAHs and nitroaromatic compounds: 0.7 µg/mg OC PAHs and 1.3 ng/mg OC nitroaromatic compounds. This result indicated other drivers of the described embryotoxicity than PAHs and nitroaromatic compounds.

The results from the FET with sediment extracts strengthened the hypothesis of the ROB as a possible source of pollution. The EC50 values from the FET after exposure to sediment extracts from the sampling sites W3 and W4 in 2018 were three to four-fold

lower than those from 2017: 30.3 mg dw SEQ/mL in 2017 vs. 12.8 mg dw SEQ/mL in 2018 at the sampling site W3; 25.5 mg dw SEQ/mL in 2017 vs. 6.7 mg dw SEQ/mL in 2018 at the sampling site W4. According to the extraction procedure with polar solvent methanol and nonpolar solvent dichloromethane, organic sediment extracts contained hydrophilic and hydrophobic organic contaminants (Creusot et al., 2011). Therefore, artificial water spiked with organic sediment extracts was expected to represent a worst-case scenario uniting a parallel exposure to nearly all organic chemicals bound to sediment particles and transferred into the water phase, considering their mixture toxicity (Hollert et al., 2003).

4.3 Behavioral changes in the zebrafish larvae depending on weather conditions

The investigation of behavioral changes in zebrafish larvae after exposure to the concentrations of organic sediment extracts that did not cause any visible lethal or sublethal effects pointed to three sampling sites with statistically different behavioral patterns than solvent control. These were sediment samples H1 (upstream E-WWTP), W2 (confluence site), and W4 (effluent site AS-WWTP) collected during the sampling campaign 2017. Exposure of zebrafish larvae to the NOEC of organic sediment extracts collected during the sampling campaign 2018 at the sampling sites H2 (effluent site-WWTP), W1 (upstream confluence), and W4 caused significantly higher locomotion activity alteration in zebrafish larvae for all endpoints in the dark phase of the LA testing. Contrarily, exposure to the organic sediment extracts from the sampling site W2 caused significantly lower locomotion activity during the light phase.

The studied section of the Haarbach and the Wurm Rivers is located in an area with pronounced agricultural activity. Indeed, multiple studies showed adverse effects of pesticides on zebrafish development and behavior. The study by Ren et al. (2021) described malformations and behavioral changes in zebrafish larvae after exposure to organophosphorus pesticides (pyrethroids). Equally to the mode of action in insects on inhibition of acetylcholine esterase (Cui et al., 2020), pyrethroids can impair signal transduction in zebrafish larvae leading to spasms, short-term hyperactivity, and sustained hypoactivity (see SI, S11). However, of the 22 pesticides investigated in this study (including potentially toxic chemical compounds such as 4,4'-DDMU and dicofol (metabolites of dichlorodiphenyltrichloroethane, DDT), the herbicides clopyrade and nitrofen, the fungicide iprodione, the pesticide procymidone, and 15 pyrethroids), none

was quantified in the sediment samples tested. This result suggested other chemical compounds being potentially responsible for detected behavioral changes, e. g, PAHs and PCBs, which were present in studied sediments at high concentrations. Vignet et al. (2014) exposed zebrafish eggs to sediment spiked with a mixture of such PAHs as pyrene, phenanthrene, and benzo[a]pyrene with a total concentration of 4 µg/g dw, observing anxiety and lethargy in zebrafish larvae. The sampling sites H1, H2, W1, and W4, revealed even higher concentrations of pyrene, phenanthrene, and benzo[a]pyrene than 4 µg/g dw. However, exposure to such lipophilic persistent pollutants as pyrene, phenanthrene, and benzo[a]pyrene can have other lasting effects on the whole fish population (Hamilton et al., 2021). The concentration of PAHs in sediments from the Haarbach and Wurm Rivers were comparable with these from the demonstrably anthropogenic impacted Neckar River (Hollert et al., 2002) and in key European river water basins such as the Danube (Keiter et al., 2008) and the Rhine (Gocht et al., 2001) Rivers as well.

Investigation of behavioral alterations after the exposure to the NOEC (FET) of organic sediment extracts identified two sampling sites with no detected effects in the LA assay: W3 and W5. While the sampling site W5 is located in natural surroundings, no behavioral changes after exposure to sediments from the sampling site W3 were unexpected. The sampling site W3 is regularly faced with enormous water flow velocity during the release of the rainwater overflow basin of the Aachen-Soers WWTP. This is a plausible reason for the sediment transport to the downstream located sampling site W4 with already described pronounced effects on zebrafish embryos and high concentrations of targeted chemical compounds. However, the sampling site W5 follows a pristine meandering watercourse naturally retaining transported sediment particles.

Conclusions

The present study reflects the complexity of testing complex environmental samples and sediment toxicity depending on the weather conditions. A biotest battery was performed on zebrafish eggs with sediment matrices of various bioavailability, and 168 different chemicals were quantified. Exposure to native sediments as the most realistic scenario resulted in no sublethal/lethal effects, while freeze-dried sediments and organic sediment extracts caused different sublethal effects. After exposure to sediment extracts, the behavior testing of zebrafish larvae revealed developmental effects below the threshold of visible deformations, emphasizing its high sensitivity and relevance for environmental risk assessment. The precautionary principle must apply in environmental risk assessment, in which the major adverse effect drivers are identified. Challenges like mixture toxicities and correct modelling of exposure scenarios, and extrapolation from biotests to the real world make this a challenging task. Effect-directed analysis and further sediment monitoring in high temporal resolution could help answer questions that remained after the present study. In general, an explicit recommendation of this study is to reduce rainwater overflow basin spills by increasing hold-back capacities, as toxicity in the sediments increased after heavy rain events in the river downstream of the two WWTPs.

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Supplementary Information

Title: Morphological and behavioral alterations in zebrafish larvae after exposure to river sediments collected in different weather conditions

Contributing authors: Aliaksandra Shuliakevich[a], Katja Schröder[b], Laura Nagengast[b], Melis Muz[c], Marek Pipal[e], Ira Brückner[d], Klara Hilscherova[e], Werner Brack[a,c], Sabrina Schiwy[a], Henner Hollert[a]

a: Goethe University Frankfurt/Main, Department Evolutionary Ecology and Environmental Toxicology, Max-von-Laue-Strasse 13, 60438 Frankfurt/Main, Germany

b: RWTH Aachen University, Institute of Biology V, Worringerweg 1, 52074 Aachen, Germany

c: Helmholtz Centre for Environmental Research – UFZ, Department of Effect-Directed Analysis, Permoserstrasse 15, 04318 Leipzig, Germany

d: Eifel-Rur Waterboard (WVER), Eisenbahnstr. 5, 52354 Düren, Germany

e: RECETOX, Faculty of Science, Masaryk University, Kotlarska 2, Brno, Czech Republic

*Corresponding author: Hollert@bio.uni-frankfurt.de. Goethe University Frankfurt, Max-von-Laue-Str. 18 13, 60438 Frankfurt am Main, Germany

S1

Table S1: Chemicals and materials.

Material/chemical	CAS Reg. No.	Supplier
Solvents		
Acetone, ≥99.9%, UV/IR grade, for analysis	67-64-1	AppliChem GmbH
Methanol, Reag. Ph. Eur. for analysis, ACS, ISO	67-56-1	AppliChem GmbH
Dichloromethane Chromasolv®, ≥99.8%, for HPLC	75-09-2	AppliChem GmbH
Dimethylsulfoxide, 99.5%, for synthesis	67-68-5	AppliChem GmbH
Ethyl acetate hypergrade for LC-MS LiChrosolv®	141-78-6	Merck KGaA
Particulate phase extraction		
Fat-free extraction quartz sand (0.3-0.9 mm)	-	BUCHI Corporation
Top cellulose filters for the extraction cell (20 mm)	-	BUCHI Corporation
Bottom cellulose filters for the extraction cell (12.7 mm respectively)	-	BUCHI Corporation
Sediment extracts clean-up		
Chromabond Flash RS4 SiOH, 40-63 µm	-	Macherey-Nagel GmbH & Co. KG
PTFE syringe filter 0.2 µm	-	Macherey-Nagel GmbH & Co. KG
Fish Embryo Toxicity test, Sediment Contact Test		
Quartz sand (F36)	-	Quarzwerke Frechen, Germany
3,4-dichloranilin, 98%	95-76-1	Merck KGaA
4-Amino-benzoic acid ethyl ester, 98%	94-09-7	Merck KGaA

S2

Table S2: Sediment amount (g dw) containing 100 mg total organic carbon (TOC). Data from the CHN-analyzer (Vario EL III). * published in Shuliakevich et al. (2022a).

Sampling site	Sampling campaign 2017	Sampling campaign 2018
H1	42.1	93.2
H2	10.8	29.4
W1	6.6	33.2
W2*	16.7	11.7
W3*	7.4	22.7
W4*	16.7	26.4
W5*	8.9	26.5

S3

The clean-up procedure of organic sediment extracts was performed using flash chromatography with a pre-packed silica gel cartridge (particle size: 40–63 µm; Chromabond Flash RS4 SiOH, Macherey Nagel) and an Agilent 1200 pump. Silica gel cartridges were preconditioned with dichloromethane (DCM), and the sediment extract (100 mg TOC equivalents/500 µL DCM) was loaded on the cartridge using a syringe. DCM was pumped through the cartridge according to the following program: The DCM flow was increased from 0 to 10 mL/min in 0.5 min, followed by a constant flow of 10 mL/min until min 2 and brought back to initial conditions within an additional 0.1 min. Finally, the collected eluent was evaporated up to 0.5 mL and transferred into a glass tube by rinsing with ethyl acetate (EtAc) to perform the complete solvent exchange.

For the chemical analysis using Gas Chromatography High-Resolution Mass Spectrometry (GC-HRMS), DCM was removed under a nitrogen stream. The volume of the organic sediment extract in EtAc was adjusted to 0.5 mL in EtAc. For the chemical analysis using Liquid Chromatography (LC), DCM and EtAc were removed under a nitrogen stream. The volume of the organic sediment extract was adjusted to 0.5 mL in methanol.

All glass tubes were then kept at -20°C for 1 h to achieve lipids precipitation. Afterward, organic sediment extracts were filtered using a 0.2 µm PTFE syringe filter. Next, 100 µL of extract aliquots were pipetted into a glass vial with an insert and spiked with the internal standard mix. For the chemical analysis using GC-HRMS, 100 µL organic sediment extract aliquot in EtAc was spiked with 1 µg/mL internal standard mix in EtAc. For the chemical analysis using LC, 100 µL organic sediment extract aliquot in methanol was spiked with 1 µg/mL internal standard mix in methanol. All aliquots were kept at -20°C until chemical analysis.

S4

Table S4: List of analysed chemical compounds. Information on substance groups, molecular formulas, CAS-numbers, exact masses, and the log kow values were derived from the PubChem® open-source, Merck© KGaA internet platform, Thermo Fischer Schientific Inc.© internet platform, Exact Mass Calculator of the Scientific Instrument Services© internet platform by Adaptas Solutions and Wikipedia. This information was published in Shuliakevich et al. 2022a (10.1016/j.watres.2021.117921).

#	Compound	Substance group	Molecular formula	CAS	Exact mass, g/mol	log kow	Analytic procedure	Internal standard	LOD, ng/mL
1	trans-Stilben (trans-/trans-Methyl-Stilben)	acyclin olefin (stilbenoid)	C14H12	103-30-0	180.093.900	4,8	LC-ESI pos	Hexachlorobenzene-13C6	2
2	1,1-dichloro-2,2-bis(4-methoxyphenyl)ethan	aliphatic halogen	C16H16Cl2O2	7388-31-0	310.052.736	4,5	LC-APCI neg	Carbendazim-D4	0,2
3	4-Chloroaniline	aniline	C6H6ClN	106-47-8	127.018.877	1,9	LC-APCI pos	Carbendazim-D4	5
4	1-Aminoanthraquinone	aromatic amine	C14H9NO2	82-45-1	223.063.329	3,2	LC-APCI neg	Bezafibrate-D4	0,2
5	2-Aminoanthracene	aromatic amine	C14H11N	613-13-8	193.089.149	3,5	LC-APCI pos	Benzophenone-3-D5	10
6	2-Bromoaniline	aromatic amine	C6H6BrN	615-36-1	170.968.360	2,1	LC-APCI neg	Imidacloprid-D4	10
7	4-Aminopyrene	aromatic amine	C16H11N	17075-03-5	217.089.149	4,3	LC-APCI pos	Diclofenac_d4	10
8	6-Aminochrysene	aromatic amine	C18H13N	2642-98-0	243.104.799	5	LC-APCI pos	Tri-n-butylphosphate-D27	0,2
9	Dioctyldiphenylamine	aromatic amine	C28H43N	101-67-7	393.339.549	11,6	GC	Perylene-D12	1
10	1,3-Diaminopyrene	aromatic amine	C16H12N2	92821-64-2	232.100.048	3,6	LC-APCI neg	Tri-n-butylphosphate-D27	5
11	1,6-Diaminopyrene	aromatic amine	C16H12N2	14923-84-3	232.100.048	3,6	LC-APCI neg	Tri-n-butylphosphate-D27	5
12	1-Amino-4-bromoanthraquinone	aromatic amine	C14H8BrNO2	81-62-9	300.973.840	3,4	LC-APCI neg	Diclofenac_d4	0,5
13	1-Methylaminoanthraquinone	aromatic amine	C15H11NO2	82-38-2	237.078.979	3,9	LC-APCI neg	Diclofenac_d4	0,2
14	1-Naphthylamine	aromatic amine	C10H9N	134-32-7	143.073.499	2,2	LC-APCI neg	Sulfamethoxazole-D4	5
15	2,4-Dichloroaniline	aromatic amine	C6H5Cl2N	554-00-7	160.979.905	2,9	LC-APCI pos	Carbamazepine-D10	1
16	2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)	aromatic amine	C13H12N4	05650-23-	224.106.196	2,2	LC-APCI neg	p-Toluene-sulfonamide-D4	1
17	2-Amino-3,8-dimethylimidazo-[4,5-f]quinoxaline (MeIQx)	aromatic amine	C11H11N5	77500-04-0	213.101.445	1	LC-APCI neg	Diglyme-D6	0,2
18	2-Aminoanthraquinone	aromatic amine	C14H9NO2	117-79-3	223.063.329	2,7	LC-APCI neg	Bezafibrate-D4	1
19	2-Aminobiphenyl	aromatic amine	C12H11N	90-41-5	169.089.149	2,8	LC-APCI neg	Carbamazepine-D10	0,2
20	2-Aminofluorene	aromatic amine	C13H11N	153-78-6	181.089.149	3,1	LC-APCI pos	Tebuconazole-D9	5
21	3-Aminoacetophenon	aromatic amine	C8H9NO	99-03-6	135.068.414	1,2	LC-APCI pos	Atenolol-D7	10
22	3-Aminofluoranthene	aromatic amine	C16H11N	2693-46-1	217.089.149	4,2	LC-APCI neg	Diclofenac_d4	5
23	4-Isopropylaniline	aromatic amine	C9H13N	99-88-7	135.104.799	2,2	LC-APCI pos	p-Toluene-sulfonamide-D4	5
24	Benzidine	aromatic amine	C12H12N2	92-87-5	184.100.048	1,3	GC	Cotinine-D3	1
25	Phenoxazine	aromatic amine	C12H9NO	135-67-1	183.068.414	3,9	LC-ESI pos	Mecoprop-D3	1
26	Michler's ketone	aromatic dialkylamine	C17H20N2O	90-94-8	268.157.563	3,9	GC	Benzophenone-3-D5	1
27	Phenazine	azaarene	C12H8N2	92-82-0	180.068.748	2,8	LC-ESI pos	Atrazine-13C3	0,5
28	Azobenzene	azobenzene	C12H10N2	103-33-3	182.084.398	3,8	GC	Tri-n-butylphosphate-D27	1
29	Aniline Yellow	azobenzene	C12H11N3	60-09-3	197.095.297	3,4	LC-APCI pos	DEET-D7	0,2
30	2,7-Dibromocarbazole	carbazole	C12H7Br2N	36630-39-	322.894.521	4,8	LC-APCI neg	Laurylsulfate-D25	0,2
31	2-Chlorocarbazole	carbazole	C12H8ClN	10537-08-3	304.970.151	4,5	LC-APCI neg	Diclofenac_d4	0,2
32	3,6-Dibromocarbazole	carbazole	C12H7Br2N	6825-20-3	322.894.521	4,8	LC-APCI neg	Laurylsulfate-D25	0,2
33	3,6-Dichlorocarbazole	carbazole	C12H7Cl2N	5599-71-3	234.995.555	5,4	LC-APCI neg	Laurylsulfate-D25	0,2
34	3,6-Diiodocarbazole	carbazole	C12H7I2N	57103-02-3	418.866.803	4,7	LC-APCI neg	Laurylsulfate-D25	0,2
35	3-Bromocarbazole	carbazole	C12H8BrN	1592-95-6	244.984.010	4,4	LC-APCI neg	Diclofenac_d4	0,2
36	3-Bromocarbazole	carbazole	C12H8BrN	1592-95-6	244.984.010	4,4	LC-APCI pos	PCB118_13C12	0,1
37	3-Iodocarbazole	carbazole	C12H8IN	16807-13-9	292.970.151	4,4	LC-APCI pos	Diclofenac_d4	0,2
38	Carbazole	carbazole	C12H9N	86-74-8	167.073.499	3,7	GC	Clarithromycin-D3	5
39	1,3,6,8-Tetrabromocarbazole	carbazole	C12H5Br4N	55119-09-0	478.715.543	6,2	LC-APCI neg	Laurylsulfate-D25	5
40	2-Hydroxycarbazole	carbazole	C12H9NO	86-79-3	183.068.414	3,5	LC-APCI neg	Carbamazepine-D10	5
41	Pentachloronitrobenzene	chrorinated hydrocarbons	C6Cl5NO2	82-68-8	292.837.169	4,2	LC-ESI pos	Laurylsulfate-D25	20
42	BDE-153	flameretardant	C12H4Br6O	58631-49-2	637.536.230	7,6	GC	13C12 Decachlorobiphenyl	2
43	BDE-154	flameretardant	C12H4Br6O	07122-15-	637.536.230	7,6	GC	13C12 Decachlorobiphenyl	5
44	BDE-47	flameretardant	C12H6Br4O	5436-43-1	481.715.208	6,2	GC	PCB180_13C12	0,1
45	BDE-99	flameretardant	C12H5Br5O	60348-60-9	559.625.720	6,9	GC	BDE99_13C12	1
46	Hexabromobenzene	flameretardant	C6Br6	87-82-1	545.510.016	6,1	GC	Chrysene-D12	5
47	4,4'-DDMU	halogenated PAH / PPP	C14H9Cl3	1022-22-6	281.976.984	6,1	LC-APCI pos	PCB101-13C12	0,2
48	1,10-Phenanthrolin-5,6-dion	heterocyclic PAH	C12H6N2O2	27318-90-7	210.042.928	0.5	LC-APCI neg	Diclofenac_d4	5
49	1,10-Phenanthroline	heterocyclic PAH	C12H8N2	66-71-7	18.006.878	1,8	LC-APCI neg	DEET-D7	0,5
50	2-Phenylindole	indole	C14H11N	948-65-2	193.089.149	3,8	LC-APCI neg	Benzophenone-3-D5	20
51	7-Azaindole	indole	C7H6N2	271-63-6	118.053.098	1,8	LC-APCI pos	Imidacloprid-D4	5
52	Harmine	indole alkaloid compound	C13H12N2O	442-51-3	212.094.963	3,6	GC	Imidacloprid-D4	0,2
53	Harman	indole alkaloid ompound	C12H10N2	486-84-0	182.084.398	3,6	GC	Benzotriazole-D4	0,2
54	N-Phenyl-1-naphthylamine	naphthalene	C16H13N	90-30-2	219.104.799	4,4	GC	Progesterone-D9	0,2
55	2,4-Dinitroaniline	nitro aromatic amine	C6H5N3O4	97-02-9	183.028.007	1	LC-APCI neg	Bentazone-D6	0,5
56	4-Nitrothiophenol	nitroamine	C6H5NO2S	1849-36-1	155.004.101	2	LC-APCI pos	Bezafibrate-D4	0,2
57	1-Nitropyrene	nitroarene	C16H9NO2	5522-43-0	247.063.329	5	LC-APCI neg	Laurylsulfate-D25	0,5
58	3-Nitrofluoranthene	nitroarene	C16H9NO2	892-21-7	247.063.329	4,7	LC-APCI neg	Laurylsulfate-D25	0,5
59	4-Nitropyrene	nitroarene	C16H9NO2	57835-92-4	247.063.329	4,9	LC-APCI pos	Laurylsulfate-D25	0,2
60	6H-Benzo(cd)pyrene-6-one	nitroarene	C20H12O6	3074-00-8	348.063.390	4,9	LC-APCI pos	Triclosan-D3	0,5
61	6-Nitrobenzo[a]pyrene	nitroarene	C20H11NO2	63041-90-7	297.078.979	6,6	LC-APCI neg	Laurylsulfate-D25	1
62	9-Nitroanthracene	nitroarene	C14H9NO2	602-60-8	223.063.329	4,8	LC-APCI pos	Diclofenac_d4	0,2
63	1,3-Dinitropyrene	nitroarene	C16H8N2O4	75321-20-9	292.048.408	4,7	LC-APCI neg	Laurylsulfate-D25	5
64	1,6-Dinitropyrene	nitroarene	C16H8N2O4	42397-64-8	292.048.408	4,7	LC-APCI neg	Triclosan-D3	0,5
65	2-Nitroanthracene	nitroarene	C14H9NO2	3586-69-4	223.063.329	4,5	LC-APCI neg	Diclofenac_d4	0,2
66	2-Nitrofluorene	nitroarene	C13H9NO2	607-57-8	211.063.329	3,7	LC-APCI neg	Diclofenac_d4	0,5
67	3-Nitrobenzanthrone	nitroarene	C17H9NO3	17117-34-9	275.058.244	4,1	LC-APCI pos	Diclofenac_d4	1
68	6-Nitrochrysene	nitroarene	C18H11NO2	########	273.078.979	5,5	LC-APCI pos	Laurylsulfate-D25	0,2
69	7-Nitrobenz[a]anthracene	nitroarene	C18H11NO2	20268-51-3	273.078.979	6	LC-APCI pos	Laurylsulfate-D25	0,2
70	2-Nitrotoluene	nitrotoluene	C7H7NO2	88-72-2	137.047.679	2,3	LC-APCI neg	Diclofenac_d4	1
71	1,2-Dihydroxyanthraquinone	PAH	C14H8O4	72-48-0	240.042.260	3,2	LC-APCI neg	Diclofenac_d4	1
72	1,3-Dihydroxyanthraquinone	PAH	C14H8O4	518-83-2	240.042.260	3,2	LC-APCI neg	Diclofenac_d4	0,5
73	1,7-Phenanthroline	PAH	C12H8N2	230-46-6	180.068.748	2,5	LC-APCI neg	Imidacloprid-D4	1
74	1,8-Dihydroxyanthraquinone	PAH	C14H8O4	117-10-2	240.042.260	3,2	LC-APCI neg	Diclofenac_d4	0,5
75	1,9-Benz-10-anthrone	PAH	C17H10O	82-05-3	230.073.165	4,3	LC-APCI neg	Diclofenac_d4	0,2
76	10-Azabenzo(a)pyrene	PAH	C19H11N	189-92-4	253.089.149	5,3	LC-APCI neg	Tri-n-butylphosphate-D27	0,5
77	2-Acetylfluorene	PAH	C15H12O	781-73-7	208.088.815	3,5	LC-APCI neg	Metolachlor-D6	5
78	2-Acetylphenanthrene	PAH	C16H12O	5960-69-0	220.088.815	4,1	LC-APCI neg	Diclofenac_d4	0,5
79	2-Hydroxyanthraquinone	PAH	C14H8O3	605-32-3	224.047.345	3	LC-APCI neg	Bezafibrate-D4	0,5
80	2-Methylanthraquinone	PAH	C15H10O2	84-54-8	222.068.080	3,9	LC-APCI neg	Diclofenac_d4	0,2

Table S4: Continuation. Line 167 is marked in bold font, which has been revised in the present table, compared to the submitted manuscript.

81	3-Acetylphenanthrene	PAH	C16H12O	2039-76-1	220.088.815	4,1	LC-APCI neg	Diclofenac_d4	0,5
82	4H-Cyclopenta[def]phenanthrene	PAH	C5H10	203-64-5	70.078.250	4,4	LC-APCI pos	PCB52_13C12	0,2
83	9-Acetylanthracene	PAH	C16H12O	784-04-3	220.088.815	4,2	LC-APCI pos	Benzophenone-3-D5	0,5
84	9-Fluorenone	PAH	C13H8O	486-25-9	180.057.515	3,6	LC-APCI pos	Diclofenac_d4	5
85	9-vinylanthracene	PAH	C16H12	2444-68-0	204.093.900	5,5	LC-APCI pos	PCB52_13C12	0,2
86	Anthracene	PAH	C14H10	120-12-7	178.078.250	4,4	LC-APCI pos	Phenanthrene-D10	1
87	Anthraquinone	PAH	C14H8O2	84-65-1	208.052.430	3,4	GC	Bezafibrate-D4	5
88	Benz(a)anthracene-7,12-dione	PAH	C18H10O2	2498-66-0	258.068.080	4,4	GC	Laurylsulfate-D25	0,2
89	Benz[a]anthracene	PAH	C18H12	56-55-3	228.093.900	5,8	GC	Chrysene-D12	2
90	Benzo(b)fluorene	PAH	C17H12	243-17-4	216.093.900	5,8	GC	PCB118_13C12	2
91	Benzo[a]fluorene-11-one	PAH	C17H12	238-84-6	216.093.900	5,2	GC	Triclosan-D3	0,2
92	Benzo[a]pyrene	PAH	C20H12	50-32-8	252.093.900	6	GC	benzo[a]pyrene-d12	20
93	Benzo[b]fluoranthene	PAH	C20H12	205-99-2	252.093.900	6	GC	benzo[a]pyrene-d12	10
94	Benzo[b]fluorene-11-one	PAH	C17H12		216.093.900		GC	Diclofenac_d4	0,2
95	Benzo[e]pyrene	PAH	C20H12	192-97-2	252.093.900	6,4	GC	benzo[a]pyrene-d12	10
96	Benzo[ghi]perylene	PAH	C22H12	191-24-2	276.093.900	6,6	GC	Dibenz[a,h]anthracene D14	2
97	Benzo[k]fluoranthene	PAH	C22H12	207-08-9	276.093.900	6,6	GC	benzo[a]pyrene-d12	2
98	Chrysene	PAH	C18H12	218-01-9	228.093.900	5,7	GC	Chrysene-D12	0,2
99	Cyclopenta[cd]pyrene	PAH	C18H10	27208-37-3	226.078.250	5,5	GC	Chrysene-D12	20
100	Dibenz[a,h]anthracene	PAH	C22H14	53-70-3	278.109.550	6,5	GC	Dibenz[a,h]anthracene D14	0,2
101	Fluoranthene	PAH	C16H10	206-44-0	202.078.250	5,2	GC	Pyrene-D10	10
102	Fluorene	PAH	C13H10	86-73-7	166.078.250	4,2	GC	Acenaphthene-D10	20
103	Indeno(1,2,3-cd)fluoranthene	PAH	C22H12	193-43-1	276.093.900	2,7	GC	Dibenz[a,h]anthracene D14	0,2
104	Indeno[1,2,3-cd]pyrene	PAH	C22H12	193-39-5	276.093.900	7	GC	Dibenz[a,h]anthracene D14	2
105	m-Terphenyl	PAH	C18H14	92-06-8	230.109.550	5,6	GC	Pyrene_D10	0,2
106	O-Terphenyl	PAH	C18H14	84-15-1	230.109.550	6	GC	PCB28_13C12	0,2
107	Perylene	PAH	C20H12	198-55-0	252.093.900	5,8	LC-ESI pos	Perylene-D12	2
108	Phenanthren-9,10-dione	PAH	C14H8O2	84-11-7	208.052.430	2,5	LC-ESI pos	Atrazine-13C3	10
109	Phenanthrene	PAH	C14H10	85-01-8	178.078.250	4,5	LC-ESI pos	Phenanthrene-D10	20
110	p-Terphenyl	PAH	C18H14	92-94-4	230.109.550	5,6	LC-ESI pos	Pyrene_D10	0,2
111	Pyrene	PAH	C16H10	129-00-0	202.078.250	4,9	LC-ESI pos	Pyrene_D10	2
112	1,4-Dihydroxyanthraquinone	PAH	C14H8O4	81-64-1	240.042.260	3,7	LC-APCI neg	Diclofenac_d4	0,2
113	1,5-Dihydroxyanthraquinone	PAH	C14H8O4	117-12-4	240.042.260	3,7	LC-APCI neg	Diclofenac_d4	0,2
114	1,8-Dichloroanthraquinone	PAH	C14H6Cl2O2	82-43-9	275.974.486	4,1	LC-APCI neg	Diclofenac_d4	0,5
115	2,6-Dihydroxyanthraquinone	PAH	C14H8O4	84-60-6	240.042.260	2,2	LC-APCI neg	Bezafibrate-D4	0,2
116	5-Carboline	PAH	C11H8N2	244-69-9	168.068.748	2,3	LC-APCI pos	Diglyme-D6	0,2
117	9-Acetylphenanthrene	PAH	C16H12O	2039-77-2	220.088.815	4,1	LC-APCI pos	Benzophenone-3-D5	0,5
118	Carboline	PAH	C11H8N2	244-76-8	168.068.748	2,9	GC	Verapamil-D6	0,2
119	Dibenzo(a,e)pyrene	PAH	C24H12	192-65-4	300.093.900	7,3	GC	Dibenz[a,h]anthracene D14	10
120	Norharmane (β-Carboline)	PAH	C11H8N2	244-63-3	168.068.748	3,2	GC	Desisopropylatrazine-D5	0,5
121	PCB 101	PCB	C12H5Cl5	37680-73-2	323.883.390	6,5	GC	PCB101_13C12	1
122	PCB 118	PCB	C12H5Cl5	31508-00-6	323.883.390	7,1	GC	PCB118_13C12	0,2
123	PCB 138	PCB	C12H4Cl6	35065-28-2	357.844.418	7,2	GC	PCB138_13C12	2
124	PCB 149	PCB	C12H4Cl6	38380-04-0	357.844.418	7,1	GC	PCB118_13C12	2
125	PCB 153	PCB	C12H4Cl6	35065-27-1	357.844.418	7,2	GC	PCB138_13C12	0,2
126	PCB 170	PCB	C12H3Cl7	35065-30-6	391.805.446	7,9	GC	PCB180_13C12	5
127	PCB 18	PCB	C12H7Cl3	37680-65-2	255.961.334	5,6	GC	PCB28_13C12	1
128	PCB 180	PCB	C12H3Cl7	35065-29-3	391.805.446	7,9	GC	PCB180_13C12	1
129	PCB 194	PCB	C12H2Cl8	35694-08-7	425.766.474	8,6	GC	BDE99_13C12	5
130	PCB 28/31	PCB	C12H7Cl3	7-5 / 1660	255.961.334	5.60 / 5.80	GC	PCB28_13C12	0,5
131	PCB 44	PCB	C12H6Cl4	41464-39-5	289.922.362	5,8	LC-ESI pos	PCB52_13C12	0,2
132	PCB 52	PCB	C12H6Cl4	35693-99-3	289.922.362	6,1	LC-ESI pos	PCB52_13C12	2
133	PCB 209	PCB	C12Cl10	2051-24-3	493.688.530	8,3	GC	PCB209_13C12	20
134	4,7-Phenanthroline	phenanthroline	C12H8N2	230-07-9	180.068.748	2,1	LC-APCI pos	Desisopropylatrazine-D5	20
135	1H-Benzo[g]indole	polycyclic heteroarene	C12H9N	233-34-1	167.073.499	3,3	LC-APCI neg	Clarithromycin-D3	5
136	Acridone	polycyclic heteroarene	C13H9NO	578-95-0	195.068.414	3	LC-APCI pos	Mecoprop-D3	0,2
137	Anthracene-1,4-dione	polycyclic heteroarene	C14H8O2	635-12-1	208.052.430	3	LC-APCI pos	Diclofenac_d4	0,5
138	Benz[a]acridine	polycyclic heteroarene	C17H11N	225-11-6	229.089.149	4,6	GC	Carbamazepine-D10	5
139	Dibenz[a,j]acridine	polycyclic heteroarene	C21H13N	224-42-0	279.104.799	6	GC	Tri-n-butylphosphate-D27	0,2
140	Benzo[h]quinoline	polycyclic heteroarene	C13H9N	230-27-3	179.073.499	3,4	GC	Diazinon-D10	5
141	beta-Naphthoflavone	polycyclic heteroarene	C19H12O2	6051-87-2	272.083.730	4,4	GC	Tri-n-butylphosphate-D27	0,2
142	Dicofol	plant protective agent	C14H9Cl5O	115-32-2	367.909.605	4,3	GC	PCB52_13C12	0,2
143	Diphenyl sulfone	plant protective agent	C12H10O2S	127-63-9	218.040.152	2,4	GC	PCB52_13C12	20
144	Xanthone	plant protective agent	C13H8O2	90-47-1	196.052.430	3,4	LC-ESI pos	Clarithromycin-D3	1
145	Clopyralid	plant protective agent	C6H3Cl2NO2	1702-17-6	190.954.085	1	GC	Hydrochlorothiazide-13C6	50
146	Hexabromocyclododecane	plant protective agent	C12H18Br6	34237-50-	635.650.866	7,1	GC	Laurylsulfate-D25	50
147	Iprodione	plant protective agent	C13H13Cl2N3O3	36734-19-7	329.033.398	3	GC	Diclofenac_d4	10
148	Nitrofen	plant protective agent	C12H7Cl2NO3	1836-75-5	282.980.300	4,3	GC	Triclosan-D3	0,5
149	Procymidone	plant protective agent	C13H11Cl2NO2	32809-16-8	283.016.685	3	LC-ESI pos	Diclofenac_d4	5
150	Acrinathrin	pyrethroids	C26H21F6NO5	01007-06-	541.132.392	6,8	LC-APCI pos	PCB180_13C12	0,5
151	Allethrin	pyrethroids	C19H26O3	584-79-2	302.188.195	4,8	LC-APCI pos	PCB101_13C12	20
152	Bifenthrin	pyrethroids	C23H22ClF3O2	82657-04-3	422.126.042	6	GC	Chrysene-D12	0,2
153	Chlorpyrifos	pyrethroids	C9H11Cl3NO3PS	2921-88-2	348.926.288	5,3	GC	PCB52_13C12	0,1
154	Cyfluthrin	pyrethroids	C22H18Cl2FNO3	68359-37-5	433.064.778	6,2	GC	BDE99_13C12	2
155	Cyhalothrin	pyrethroids	C23H19ClF3NO3	68085-85-8	449.100.556	6,1	GC	PCB180_13C12	0,2
156	Cypermethrin	pyrethroids	C22H19Cl2NO3	52315-07-8	415.074.200	6	GC	benzo[a]pyrene-d12	2
157	Deltamethrin	pyrethroids	C22H19Br2NO3	52918-63-5	502.973.166	6,2	GC	Dibenz[a,h]anthracene D14	5
158	Esfenvalerate	pyrethroids	C25H22ClNO3	66230-04-4	419.128.822	6,2	GC	Perylene-D12	1
159	Etofenprox	pyrethroids	C25H28O3	80844-07-1	376.203.845	7	GC	etofenprox-D5	0,1
160	Fluvalinate	pyrethroids	C26H22ClF3N2O3	59409-94-5	502.127.105	7,7	GC	Perylene-D12	1
161	Permethrin	Pyrethroids	C21H20Cl2O3	52645-53-1	390.078.951	6,5	LC-ESI pos	BDE99_13C12	0,1
162	Prallethrin	Pyrethroids	C19H24O3	23031-36-9	300.172.545	4,3	LC-ESI pos	PCB101_13C12	1
163	Tefluthrin	Pyrethroids	C17H14ClF7O2	79538-32-2	418.057.054	5,4	LC-ESI pos	Phenanthrene-D10	0,1
164	Transfluthrin	Pyrethroids	C15H12Cl2F4O2	18712-89-	370.015.048	5	LC-ESI pos	PCB52_13C12	0,1
165	2-Amino-9H-pyrido[2,3-b]indole (AaIphaC)	pyridiondole	C11H9N3	26148-68-5	183.079.647	2,6	LC-APCI neg	Imidacloprid-D4	0,2
166	1-Phenylnaphthalin	others	C16H12	605-02-7	204.093.900	4,9	LC-APCI neg	Tonalide-D3	0,2
167	**Tributylamine**	**others**	**C12H27N**	**102-82-9**	**185.214.349**	**4**	**LC-ESI pos**	**Imidacloprid-D4**	**0.2**
168	p-Benzyldiphenyl	others	C19H16	613-42-3	244.125.200	5,9	GC	PCB52_13C12	1
169	3,3'-Dichlorobenzidine	others	C12H10Cl2N2	91-94-1	252.022.104	3,5	LC-APCI neg	Mono-isobutylphthalate-D4	0,2
170	Methyltolylsulfone	others	C8H10O2S	3185-99-7	170.040.152	2,2	GC	acenaphthen-D10	10

S5

Table S5: List of analyzed chemical compounds and detected environmental concentrations normalized by blank, [ng/mg OC]. * published in Shuliakevich et al. 2022a (10.1016/j.watres.2021.117921). Column 1 was revised (adjusted) in the present table, compared to the submitted manuscript.

		Sampling campaign 2017							Sampling campaign 2018						
#	Chemical compound	H1	H2	W1	W2*	W3*	W4*	W5*	H1	H2	W1	W2*	W3*	W4*	W5*
1	1,3-Diaminopyrene														
2	1,6-Diaminopyrene														
3	1-Amino-4-bromoanthraquinone														
4	1-Aminoanthraquinone		2,40E-02			6,67E-03	9,91E-03	6,19E-03	3,82E-02	3,99E-02		1,51E-02	3,93E-02	9,90E-02	6,66E-02
5	1-Methylaminoanthraquinone														
6	1-Naphthylamine	5,69E-02	8,64E-02					8,13E-02	1,81E-02	1,17E-01	2,05E-01	4,48E-02	2,07E-01	2,26E-01	1,59E-01
7	2,4-Dichloroaniline														
8	2,4-Dinitroaniline	3,53E-02	1,71E-02	1,15E-02	1,69E-02	9,02E-03	2,17E-02	2,05E-02	4,66E-02	7,11E-02	2,20E-02	9,47E-03	3,52E-02	6,99E-02	3,97E-02
9	2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)														
10	2-Amino-3,8-dimethylimidazo-[4,5-f]quinoxaline (MeIQx)														
11	2-Aminoanthracene		2,80E-01	2,10E-01						3,28E-01	7,43E-01	3,06E-01	2,90E-01		
12	2-Aminoanthraquinone														
13	2-Aminobiphenyl	1,31E-02	1,11E-02	4,70E-03	8,49E-03	6,89E-03	1,01E-02	9,06E-03	1,23E-02	1,69E-02	1,41E-02	7,29E-03	2,56E-02	3,86E-02	4,02E-02
14	2-Aminofluorene														
15	2-Bromoaniline			3,43E-02						1,74E-02	3,94E-02				4,60E-02
16	3-Aminoacetophenon														
17	3-Aminofluoranthene														
18	4-Aminopyrene	1,34E-01	2,19E-01	1,34E-01	1,17E-01	1,27E-01	1,10E-01	1,22E-01	1,20E-01	1,60E-01	3,05E-01	1,53E-01	2,25E-01	9,83E-01	
19	4-Isopropylaniline														
20	6-Aminochrysen	4,27E-01	2,16E-01	2,89E-01	3,24E-01	9,00E-02	5,09E-02	8,19E-02	1,85E-01	4,89E-01	5,30E-01	2,98E-01	3,84E-01		1,20E-01
21	Benzidine														
22	Dioctyldiphenylamine	2,15E-01	1,78E-02	7,08E-01	5,77E-01	2,39E-02	4,97E-02	4,02E-02	1,35E-01	6,33E-02	5,12E-01	1,47E-01	7,16E-02	8,76E-02	5,50E-02
23	Phenoxazine														
24	Phenazine	3,17E-01	5,25E-02	8,43E-02	1,45E-01	4,76E-02	1,43E-01	9,81E-02	2,89E-01	2,31E-01	1,57E-01	5,74E-02	2,12E-01	3,28E-01	3,00E-01
25	Aniline Yellow		3,84E-03					2,70E-03	2,86E-03	4,54E-03	2,59E-03	2,58E-03			3,13E-03
26	Azobenzene	1,10E-01	1,19E-02	2,75E-02				4,31E-02	1,09E-01	6,89E-02				5,79E-02	1,21E-01
27	1,3,6,8-Tetrabromocarbazole														
28	2,7-Dibromocarbazole	3,63E-02	6,97E-03				9,45E-03	1,13E-02	9,35E-02	1,74E-02		1,17E-03	1,65E-02	1,88E-02	2,21E-02
29	2-Chlorocarbazole	7,95E-02	5,40E-02	1,12E-02	2,43E-02	1,87E-02	4,39E-02	5,00E-02	9,34E-02	1,47E-01	3,90E-02	1,54E-02	8,67E-02	1,36E-01	2,40E-01
30	2-Hydroxycarbazole														
31	3,6-Dibromocarbazole	3,39E-02	6,91E-03			1,63E-03	9,20E-03	1,09E-02	8,68E-02	1,65E-02		1,58E-03	1,57E-02	1,78E-02	2,09E-02
32	3,6-Dichlorocarbazole	5,22E-01	3,38E-01	8,46E-02	1,53E-01	1,08E-01	2,46E-01	3,74E-01	7,99E-01	9,02E-01	1,87E-01	9,77E-02	4,20E-01	8,73E-01	1,34E+00
33	3,6-Diiodocarbazole	1,22E-01	4,80E-03	2,56E-03	2,64E-02	2,54E-02		6,82E-03	1,41E-02	6,73E-03		1,88E-03			6,32E-03
34	3-Bromocarbazole		1,37E-03		1,03E-03	1,55E-03	5,30E-04	1,44E-03	1,16E-03	1,60E-03	1,55E-03	2,58E-03	8,03E-03	2,53E-03	8,42E-03
35	3-Bromocarbazole	1,63E-02			5,22E-03			1,40E-02							1,40E-02
36	3-Iodocarbazole	1,22E-01	1,45E-02	8,19E-03	5,05E-02	3,21E-02	1,76E-02	1,40E-02	2,75E-02	3,33E-02	1,65E-02	6,15E-03	2,95E-02	3,50E-02	3,16E-02
37	Carbazole	6,41E+00	4,20E+00	5,48E+00	5,82E+00	1,05E+00	2,73E+00	1,49E+00	1,43E+00	8,22E+00	1,66E+01	6,48E+00	4,17E+00	5,40E+00	2,06E+00
38	BDE-153														
39	BDE-154														
40	BDE-47														
41	BDE-99														
42	Hexabromobenzene														
43	2-Amino-9H-pyrido[2,3-b]indole (AalphaC)													4,32E-03	4,53E-03
44	2-Phenylindole									3,33E-01	6,75E-01	3,19E-01	3,10E-01		
45	7-Azaindole													2,04E-01	
46	Harman	3,21E-01	2,27E-01	1,32E-01	3,23E-01	6,96E-02	6,66E-02	1,43E-01	1,65E-01	4,03E-01	5,06E-01	8,47E-02	3,43E-01	5,77E-01	2,85E-01
47	Harmine		1,99E-02			5,76E-03	4,18E-03	1,34E-02	1,24E-02	5,40E-02	1,25E-02	8,02E-03	1,77E-02	1,49E-02	3,72E-02
48	4-Nitrothiophenol														
49	1,3-Dinitropyrene														
50	1,6-Dinitropyrene														
51	1-Nitropyrene								1,96E-02		1,01E-02	1,40E-02	1,16E-02	2,51E-02	
52	2-Nitroanthracene														
53	2-Nitrofluorene														
54	3-Nitrobenzanthrone		1,58E-02												
55	3-Nitrofluoranthene						6,60E-03		2,13E-02	8,83E-03	1,11E-02	1,51E-02	1,22E-02	2,74E-02	
56	4-Nitropyrene	8,52E-03	8,21E-03	9,16E-03	1,09E-02	8,57E-03	6,91E-03	1,04E-02	2,00E-02	8,53E-03	1,73E-02	1,68E-02	1,51E-02	3,84E-02	7,64E-03
57	6H-Benzo(cd)pyrene-6-one	2,65E+00	1,95E+00	1,17E+00	1,96E+00	5,61E-01	1,33E+00	6,85E-01	1,14E+00	2,53E+00	3,67E+00	1,40E+00	2,18E+00	2,41E+01	1,28E+00
58	6-Nitrobenzo[a]pyrene				5,61E-01	2,02E+00	1,88E+00	3,12E-01	1,13E+00						
59	6-Nitrochrysen														
60	7-Nitrobenz[a]anthracene														
61	9-Nitroanthracene		4,77E-03	6,59E-03	4,24E-03	4,24E-03			3,03E-03	6,13E-03	2,44E-02	4,51E-03	6,21E-03	1,51E-02	3,74E-03
62	Methyltolylsulfone														
63	3,3'-Dichlorobenzidine														
64	1,1-dichloro-2,2-bis(4-methoxyphenyl)ethan														8,15E-03
65	4-Chloroaniline									1,28E-01			9,89E-02	1,36E-01	2,86E-01
66	Michler's ketone	2,27E-02				1,05E-02		9,79E-03	9,40E-03	1,31E-02	1,13E-02	9,38E-03	3,59E-02	7,25E-02	2,53E-02
67	Pentachloronitrobenzene	2,37E-01	1,82E-01				1,82E-01	2,20E-01	3,82E-01	2,16E-01	2,06E-01				2,40E-01
68	N-Phenyl-1-naphthylamine	1,38E-01	9,94E-02	5,95E-02	1,57E-01	3,57E-02	6,64E-02	7,78E-02	7,03E-02	1,24E-01	2,09E-01	1,09E-01	3,05E-01	4,88E-01	2,78E-01
69	2-Nitrotoluene														
70	trans-Stilben (trans-/trans-Methyl-Stilben)	2,46E+00		1,31E+00	1,22E+00				5,03E-01	7,22E-01	1,53E+00	1,48E-01	1,76E+00	4,46E+00	5,87E-02
71	Tributylamine	3,46	2,31	4,55	4,83	2,01	2,22	2,76	2,67	2,82	7,30	1,61	4,63	5,01	3,09
72	1,10-Phenanthroline	3,33E-01	6,72E-02	1,08E-01	1,89E-01	5,82E-02	1,77E-01	1,12E-01	3,64E-01	2,38E-01	1,99E-01	7,00E-02	2,80E-01	3,50E-01	3,27E-01
73	1,2-Dihydroxyanthraquinone					3,03E-02							2,43E-01	4,18E-01	9,43E-02
74	1,3-Dihydroxyanthraquinone											9,42E-03	3,72E-01	4,55E-01	1,59E-01
75	1,4-Dihydroxyanthraquinone														
76	1,5-Dihydroxyanthraquinone														
77	1,7-Phenanthroline									9,99E-02	2,25E-01	2,03E-02		5,22E-02	
78	1,8-Dichloroanthraquinone														
79	1,8-Dihydroxyanthraquinone	2,20E-01	5,29E-02	6,90E-02	3,79E-01	2,86E-02	1,20E-01	7,92E-02	4,67E-02	8,28E-02					
80	1,9-Benz-10-anthrone	4,12E+00	2,89E+00	2,94E+00	2,96E+00	8,63E-01	2,72E+00	1,03E+00	1,73E+00	5,31E+00	7,19E+00	8,28E+00	8,55E+00	3,49E+01	1,85E+00

Table S5: Continuation.

81	10-Azabenzo(a)pyrene	9,71E+00	6,18E+00	5,59E+00	5,42E+00	1,97E+00	2,75E+00	1,76E+00	3,07E+00	8,79E+00	1,23E+01	6,74E+00	6,92E+00	1,31E+01	2,78E+00
82	2,6-Dihydroxyanthraquinone														
83	2-Acetylfluorene	1,06E+01	2,45E+00	2,92E+00	3,04E+00	2,01E+00	2,47E+00	1,37E+00	2,35E+00	6,03E+00	7,78E+00	2,24E+00	7,26E+00	1,99E+01	4,24E+00
84	2-Acetylphenanthrene	1,20E-01	5,76E-02	8,51E-02	8,36E-02	2,96E-02	6,43E-02	5,82E-02	6,16E-02	1,13E-01	3,52E-01	8,76E-02	1,91E-01	8,96E-01	1,42E-01
85	2-Hydroxyanthraquinone	2,02E-01	7,93E-02	1,24E-01	8,94E-02	5,18E-02	1,19E-01	5,40E-02	9,16E-02	1,42E-01	3,25E-01	6,95E-02	2,06E-01	5,01E-01	1,50E-01
86	2-Methylanthraquinone	7,44E+00	2,46E+00	3,15E+00	3,00E+00	8,43E-01	1,74E+00	8,95E-01	1,82E+00	6,23E+00	1,00E+01	2,80E+00	4,81E+00	1,57E+01	2,19E+00
87	3-Acetylphenanthrene	1,37E-01	6,52E-02	9,67E-02	9,50E-02	3,29E-02	5,84E-02	3,84E-02	6,98E-02	1,29E-01	4,01E-01	9,97E-02	1,94E-01	8,99E-01	1,59E-01
88	4H-Cyclopenta[def]phenanthrene	5,36E+01	1,68E+01	4,17E+01	1,67E+01	3,07E+00	8,55E+00	4,34E+00	2,79E+01	8,05E+01	1,96E+02	2,68E+01	5,55E+01	2,97E+02	7,50E+00
89	5-Carboline	4,38E-01	1,80E-01	5,48E-02	1,47E-01	9,91E-02	1,19E-02	8,07E-02	1,38E-01	4,51E-01	8,62E-01	1,52E-01	2,80E-01	0,00E+00	3,77E-01
90	9-Acetylanthracene	1,80E-01	1,41E-01	1,75E-02	1,92E-02	6,00E-02	2,19E-02	1,86E-02	1,51E-01	3,52E-01	1,69E-02	1,48E-01	2,95E-01	1,21E+00	2,09E-01
91	9-Acetylphenanthrene														
92	9-Fluorenone	7,40E+00	4,11E+00	4,18E+00	3,34E+00	1,66E+00	2,90E+00	2,04E+00	2,81E+00	6,87E+00	9,01E+00	3,77E+00	6,27E+00	8,59E+00	3,44E+00
93	9-vinylanthracene	1,07E+00	3,53E-01	8,76E-01	5,63E-01	9,58E-02	2,04E-01	1,48E-01	5,05E-01	1,68E+00	6,52E+00	1,11E+00	1,08E+00	6,27E+00	2,57E-01
94	Anthracene	1,27E+02	1,31E+01	6,32E+01	4,46E+01	4,22E+00	1,02E+01	3,74E+00	3,31E+01	8,84E+01	3,62E+02	9,58E+01	3,32E+01	3,75E+02	7,39E+00
95	Anthraquinone	1,53E+00	9,16E-01	1,03E+00	1,16E+00	5,86E-01	1,01E+00	5,65E-01	9,20E-01	8,99E-01	1,53E+00	9,44E-01	1,28E+00	2,72E+00	8,04E-01
96	Benz(a)anthracene-7,12-dione	5,25E+00	2,10E+00	1,49E+00	1,52E+00	7,73E-01	1,03E+00	7,65E-01	1,50E+00	3,16E+00		1,48E+00	4,17E+00		1,36E+00
97	Benz[a]anthracene	2,61E+02	1,26E+02	1,91E+02	1,32E+02	2,73E+01	3,91E+01	2,78E+01	7,69E+01	3,30E+02	9,05E+02	3,21E+02	1,81E+02	8,91E+02	4,43E+01
98	Benzo(b)fluorene	3,38E+01	2,64E+01	2,93E+01	2,31E+01	6,23E+00	7,33E+00	4,71E+00	1,90E+01	8,20E+01	1,51E+02	9,29E+01	5,96E+01	4,74E+02	1,10E+01
99	Benzo[a]fluorene-11-one	1,32E+01	7,36E+00	1,09E+01	7,51E+00	2,91E+00	3,93E+00	3,05E+00	5,09E+00	1,15E+01	2,24E+01	1,01E+01	1,04E+01	1,79E+01	4,65E+00
100	Benzo[a]pyrene	1,98E+02	7,77E+01	1,09E+02	1,13E+02	1,79E+01	2,97E+01	1,80E+01	5,80E+01	2,37E+02	6,21E+02	1,91E+02	1,27E+02	8,58E+02	3,05E+01
101	Benzo[b]fluoranthene	3,21E+02	1,59E+02	2,03E+02	2,10E+02	3,15E+01	5,13E+01	4,11E+01	1,44E+02	4,20E+02	1,19E+03	3,63E+02	2,30E+02	1,10E+03	6,29E+01
102	Benzo[b]fluorene-11-one	1,43E+01	7,40E+00	1,06E+01	8,37E+00	2,63E+00	4,24E+00	2,98E+00	5,43E+00	1,33E+01	2,54E+01	1,10E+01	1,16E+01	3,12E+01	4,57E+00
103	Benzo[e]pyrene	1,04E+02	5,54E+01	6,13E+01	1,13E+02	1,12E+01	1,72E+01	1,41E+01	5,56E+01	1,35E+02	3,48E+02	1,06E+02	7,46E+01	3,64E+02	1,86E+01
104	Benzo[ghi]perylene	7,10E+01	3,75E+01	4,18E+01	6,82E+01	9,60E+00	1,88E+01	1,05E+01	2,83E+01	1,19E+02	3,39E+02	9,49E+01	8,70E+01	4,97E+02	2,45E+01
105	Benzo[k]fluoranthene	9,17E+01	4,55E+01	6,45E+01	6,34E+01	9,65E+00	1,69E+01	1,31E+01	3,72E+01	1,21E+02	3,43E+02	1,05E+02	7,03E+01	3,43E+02	1,85E+01
106	Carboline	7,84E-02	3,11E-02	2,46E-02	2,95E-02	7,96E-03	2,41E-02	1,22E-02	2,46E-02	6,28E-02	1,45E-01	2,16E-02	5,37E-02	7,75E-02	3,90E-02
107	Chrysen	2,77E+02	1,34E+02	2,02E+02	1,40E+02	2,88E+01	4,13E+01	2,95E+01	8,14E+01	3,50E+02	9,60E+02	3,40E+02	1,92E+02	9,46E+02	4,66E+01
108	Cyclopenta[cd]pyrene	2,35E+02	1,14E+02	1,81E+02	1,16E+02	2,39E+01	3,42E+01	2,42E+01	7,24E+01	3,11E+02	8,44E+02	2,91E+02	1,66E+02	9,48E+02	3,86E+01
109	Dibenzo(a,e)pyrene														
110	Dibenzo[a,h]anthracene	1,60E+01	1,03E+01	1,08E+01	1,53E+01	2,76E+00	4,18E+00	2,65E+00	6,97E+00	3,01E+01	7,84E+01	3,19E+01	2,21E+01	1,07E+02	6,45E+00
111	Fluoranthene	1,36E+03	3,52E+02	8,39E+02	2,18E+02	4,69E+01	3,47E+02	5,09E+01	2,95E+02	1,43E+03	6,46E+03	7,23E+02	2,25E+03	1,20E+04	1,82E+02
112	Fluorene	2,07E+02	2,06E+01	7,82E+01	6,59E+01	8,94E+00	3,40E+01	9,05E+00	5,22E+01	2,27E+02	4,09E+02	7,21E+01	1,54E+02	3,28E+02	2,43E+01
113	Indeno(1,2,3-cd)fluoranthene	8,28E+00	7,16E-01	5,39E+00	7,00E+00	1,94E-01	5,53E-01	2,64E-01	4,45E-01	2,43E+00	6,10E+00	2,10E+00	2,03E+00	1,06E+01	5,98E-01
114	Indeno[1,2,3-cd]pyrene	9,05E+01	4,85E+01	5,01E+01	7,51E+01	1,22E+01	2,17E+01	1,24E+01	3,18E+01	1,38E+02	3,87E+02	1,16E+02	9,92E+01	5,76E+02	2,88E+01
115	m-Terphenyl	2,91E+00	1,00E+00	1,92E+00	1,06E+00	3,57E-01	5,53E-01	5,17E-01	1,52E+00	2,84E+00	8,81E+00	2,50E+00	2,30E+00	9,25E+00	8,57E-01
116	Norharmane (β-Carboline)	8,28E-01	9,11E-01	4,41E-01	7,16E-01	3,39E-01	5,89E-01	2,61E-01	5,79E-01	7,97E-01	5,33E-01	1,63E-01	4,39E-01	4,90E-01	7,81E-01
117	O-Terphenyl	2,44E-01	5,00E-02	1,14E-01	9,36E-02	1,83E-02	4,23E-02	4,69E-02	1,27E-01	1,89E-01	5,19E-01	1,97E-01	1,79E-01	7,32E-01	1,09E-01
118	Perylene	5,15E+01	2,19E+01	3,00E+01	3,69E+01	6,35E+01	1,14E+02	8,15E+00	1,61E+01	6,89E+01	1,63E+02	5,51E+01	1,52E+02	2,40E+02	2,34E+01
119	Phenanthren-9,10-dione	1,52E+00	6,19E-01	8,98E-01	9,51E-01	4,85E-01	6,43E-01	4,07E-01	5,71E-01	4,68E-01	7,69E-01	4,42E-01	8,29E-01	2,23E+00	4,36E-01
120	Phenanthrene	8,56E+02	1,21E+02	4,00E+02	1,42E+02	2,26E+01	6,93E+01	3,49E+01	1,62E+02	6,63E+02	1,88E+03	2,20E+02	3,27E+02	1,01E+03	4,64E+01
121	Pyrene	7,24E+02	2,32E+02	5,03E+02	2,00E+02	3,21E+01	7,81E+01	4,33E+01	2,07E+02	9,62E+02	2,93E+03	3,95E+02	4,61E+02	4,04E+03	5,93E+01
122	PCB 101	3,64E-01	1,80E-01	1,15E-01	3,46E-01	2,38E-02	3,84E-01	5,90E-02	2,00E-01	3,32E+01	9,76E-01	1,45E+01	8,11E-01	4,73E+00	2,33E-01
123	PCB 118	2,64E-01	9,60E-02	7,76E-02	1,77E-01	9,68E-03	1,35E-01	5,72E-02	1,11E-01	6,67E+00	3,93E-01	3,09E+00	3,95E-01	1,06E+00	1,42E-01
124	PCB 138	8,72E-01	4,89E-01	3,66E-01	8,31E-01	4,46E-02	8,14E-01	1,96E-01	4,82E-01	5,01E+01	1,47E+00	2,56E+01	1,31E+00	9,33E+00	5,14E-01
125	PCB 149	6,63E-01	4,16E-01	3,48E-01	6,55E-01	2,98E-02	7,03E-01	1,38E-01	3,96E-01	4,97E+01	1,45E+00	2,28E+01	1,27E+00	9,20E+00	4,42E-01
126	PCB 153	1,02E+00	6,19E-01	5,83E-01	1,12E+00	7,62E-02	1,09E+00	2,32E-01	6,04E-01	6,73E+01	2,44E+00	3,24E+01	1,75E+00	1,20E+01	6,33E-01
127	PCB 170	2,75E-01	2,01E-01	1,67E-01	3,21E-01		3,65E-01	5,95E-02	1,34E-01	2,24E+01	2,36E-01	9,40E+00	3,15E-01	4,40E+00	1,48E-01
128	PCB 18	1,81E-01	2,02E-02	2,87E-02	6,13E-02	1,13E-02	1,09E-01	6,35E-02	1,96E-01	3,51E-01	1,61E-01	3,81E-01	2,08E-01	5,44E-01	2,07E-01
129	PCB 180	7,59E-01	3,59E-01	4,02E-01	6,45E-01	3,91E-02	6,89E-01	1,36E-01	3,38E-01	4,11E+01	1,01E+00	1,78E+01	7,11E-01	8,09E+00	3,16E-01
130	PCB 194						6,08E-02			7,97E+00		2,90E+00		9,19E-01	
131	PCB 209														
132	PCB 28/31	2,19E-01	4,68E-02	5,05E-02	9,93E-02	2,28E-02	1,39E-01	9,36E-02	2,23E-01	3,45E-01	1,94E-01	2,93E-01	2,66E-01	6,08E-01	3,15E-01
133	PCB 44	1,49E-01	5,02E-02	6,29E-02	8,51E-02	1,72E-02	1,38E-01	6,24E-02	1,33E-01	4,63E+00	4,73E-01	2,41E+00	2,55E-01	1,18E+00	1,76E-01
134	PCB 52	6,69E-02	6,43E-02	6,18E-02		2,77E-02	1,44E-01	4,10E-02	1,15E-01	4,10E+00	4,14E-01	2,22E+00	1,81E-01	5,11E-01	1,52E-01
135	1,10-Phenanthrolin-5,6-dion														1,16E-01
136	4,7-Phenanthroline														
137	1H-Benzo[g]indole	3,43E-01	1,27E-01	1,20E-01	7,77E-02	2,08E-02		3,28E-02	2,40E-01	1,57E-01	1,03E+00	6,21E-02	7,26E-02		4,64E-02
138	1-Phenylnaphthalin	1,27E+01	2,71E+00	7,33E+00	2,48E+00	4,91E-01	1,25E+00	6,74E-01	3,77E+00	9,44E+00	2,51E+01	4,28E+00	5,46E+00	3,15E+01	1,08E+00
139	Acridone	1,08E+00	6,68E-01	4,06E-01	5,96E-01	9,64E-02	5,01E-01	5,11E-01	3,21E-01	1,81E+00	1,91E+00	3,99E-01	9,73E-01		1,62E+00
140	Anthracene-1,4-dione	1,42E+01	4,20E+00	6,60E+00	4,59E+00	9,55E-01	2,84E+00	1,30E+00	2,62E+00	1,19E+01	2,35E+01	4,73E+00	7,12E+00	1,93E+01	2,76E+00
141	Benz[a]acridine	5,24E+00	2,50E+00	2,42E+00	4,08E+00	5,32E-01	9,44E-01	9,69E-01	1,74E+00	5,68E+00	1,03E+01	2,78E+00	4,14E+00	7,80E+00	1,63E+00
142	Benzo[h]quinoline														
143	beta-Naphthoflavone														
144	Dibenz[a,j]acridine	6,21E+00	3,95E+00	3,36E+00	3,97E+00	1,36E+00	1,73E+00	9,34E-01	1,72E+00	5,43E+00	8,32E+00	5,06E+00	4,91E+00	1,39E+01	1,79E+00
145	p-Benzyldiphenyl		1,57E-02	4,18E-02						5,81E-02	2,79E-01	9,21E-02			3,00E-01
146	p-Terphenyl	1,60E+00	6,73E-01	1,11E+00	7,46E-01	2,28E-01	3,54E-01	3,89E-01	7,99E-01	2,13E+00	6,25E+00	2,07E+00	1,49E+00	4,99E+00	5,98E-01
147	4,4'-DDMU	2,57E-01	7,41E-03				2,78E-02								1,66E-02
148	Clopyralid														
149	Dicofol	1,07E-01	7,47E-02	1,76E-02	4,32E-02	1,00E-02	2,90E-01	4,30E-02	6,23E-02	2,64E-01	7,91E-02	4,76E-02	1,15E-01	4,54E-01	2,54E-01
150	Diphenyl sulfone	2,18E-02			2,33E-02		7,09E-02	8,50E-02	7,69E-02	4,17E-01	2,52E-01	1,76E-01	4,06E-01	6,24E-01	2,70E-01
151	Hexabromocyclododecane														
152	Iprodione														
153	Nitrofen														
154	Procymidone														
155	Xanthone	6,60E-01	1,85E-01	1,09E-01	1,36E-01	5,82E-02	1,22E-01	7,99E-02	2,28E-01	4,47E-01	4,89E-01	1,35E-01	3,37E-01	7,45E-01	2,84E-01
156	Acrinathrin														
157	Allethrin														
158	Bifenthrin														
159	Chlorpyrifos														
160	Cyfluthrin														
161	Cyhalothrin														
162	Cypermethrin														
163	Deltamethrin														
164	Esfenvalerate														
165	Etofenprox														
166	Fluvalinate														
167	Permethrin														
168	Prallethrin														
169	Tefluthrin														
170	Transfluthrin														

S6

Table S6: Water amount in native sediment samples after the freeze-drying, and the amount of native sediment referred to 3 g dw. * published in Shuliakevich et al. (2022a).

	Sampling campaign 2017		Sampling campaign 2018	
	Water amount, %	Amount native sediment referred to 3 g dw freeze-dried sediment	Water amount, %	Amount native sediment referred to 3 g dw freeze-dried sediment
H1	24.0%	3.7 g	18.5%	3.6 g
H2	18.9%	3.6 g	25.5%	3.8 g
W1	25.1%	3.8 g	20.4%	3.6 g
W2*	35.5%	4.1 g	27.1%	3.8 g
W3*	22.0%	3.7 g	22.0%	3.7 g
W4*	25.8%	3.8 g	18.9%	3.6 g
W5*	23.1%	3.7 g	22.9%	3.7 g

S7

Table S7: Lethal and sublethal effects for effect evaluation in the FET at different time points. hpf: hours post fertilisation.

Sublethal effects	Time point, [hpf]	Lethal effects	Time point, [hpf]
Slow spontaneous movements	24	Coagulation	24
Chorion deformation	24 / 48 / 72 / 96 / 120	Epibolie	24
No blood circulation	48 / 72 / 96 / 120	No somites	24
Impaired blood circulation	48 / 72 / 96 / 120	Tail not detached	24
Blood congestion	48 / 72 / 96 / 120	No spontaneous movements	24
Abnormal heart beat rate	48 / 72 / 96 / 120	No heart beat	24 / 48 / 72 / 96 / 120
Tube heart	48 / 72 / 96 / 120	No hatching	
Edema (yolk sack, pericardium)	48 / 72 / 96 / 120	Death	120
No body pigmentation	48 / 72 / 96 / 120		24 / 48 / 72 / 96 / 120
No eye pigmentation	48 / 72 / 96 / 120		
Spine deformation	48 / 72 / 96 / 120		
Impaired swim behaviour	72 / 96 / 120		
No hatching	72 / 96		

S8

S8 A

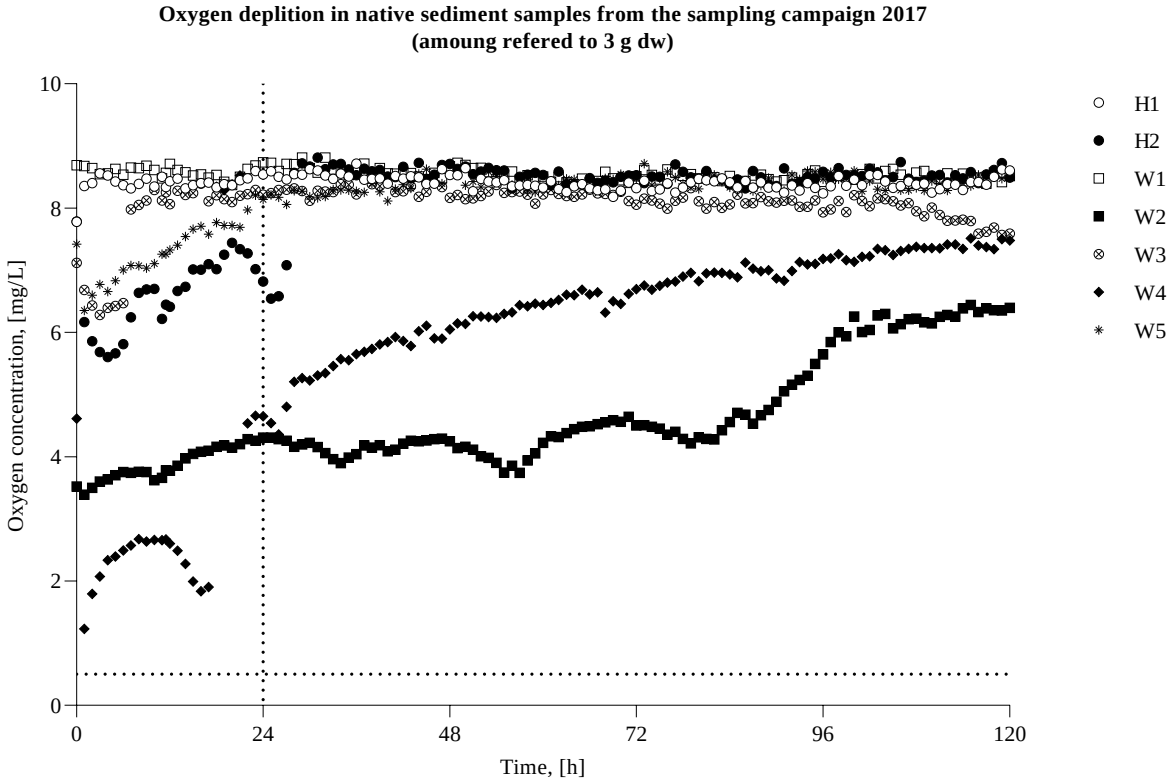

Figure S8 A: Oxygen depletion in native sediment samples collected during the sampling campaign 2017 (dry weather). The native sediment amount is referred to 3 g dw (cf. SI, S5).

S8 B

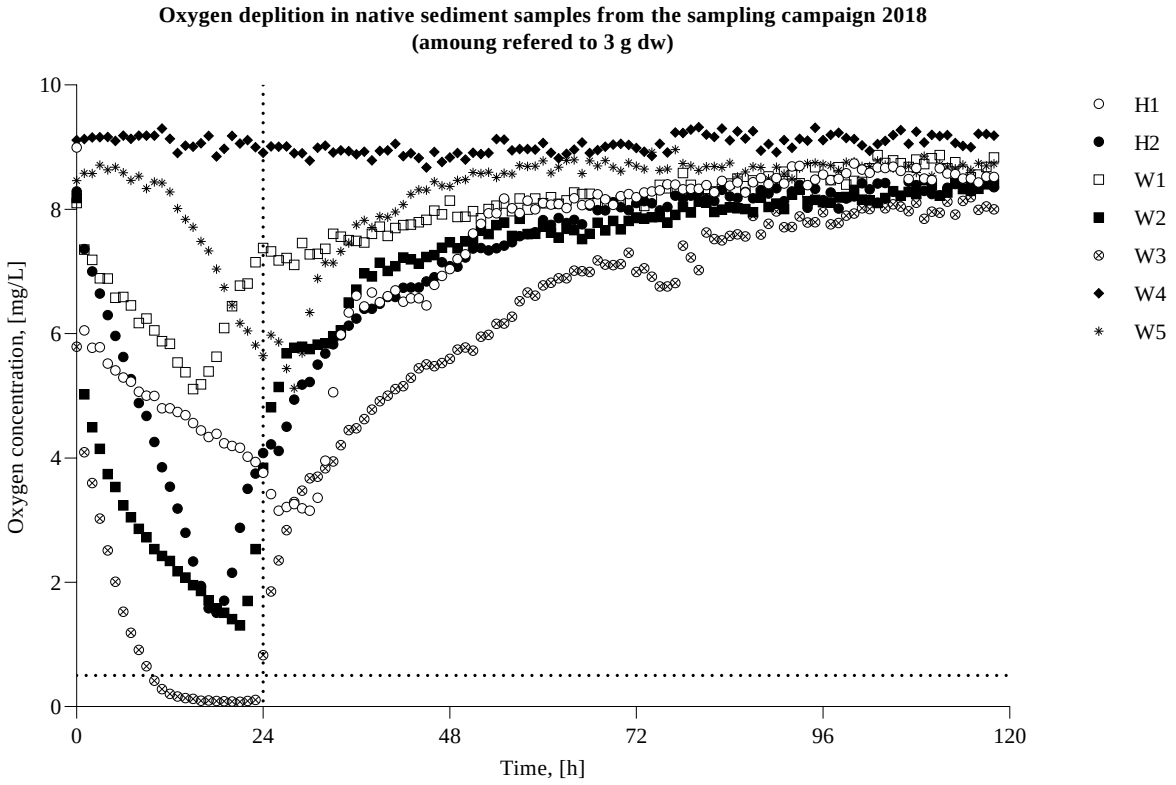

Figure S8 B: Oxygen depletion in native sediment samples collected during the sampling campaign 2018 (after extended rain events). The native sediment amount is referred to 3 g dw (cf. SI, S5).

S9

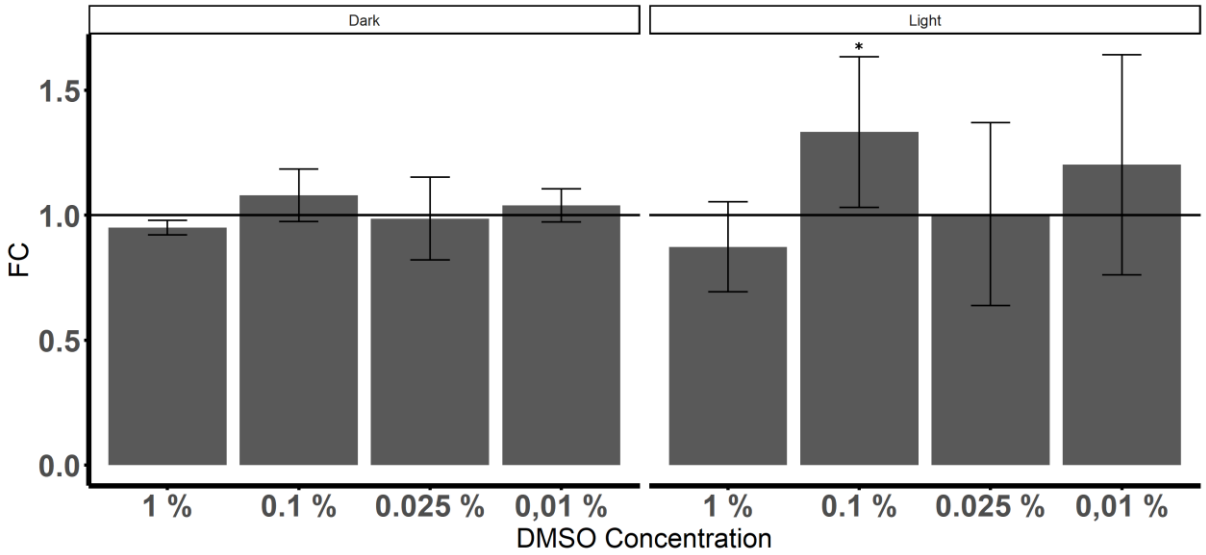

Figure S9: Behavioral activity in zebrafish larvae (~ 120 hpf) in the LA after exposure to different DMSO-concentrations. Behavior change displayed as Fold Change (FC) compared to the solvent control (FC=1). Statistically significant differences were evaluated using the Mann-Whitney U test and are marked with * $p<0.05$ and ** $p<0.01$.

S10

Table S10: Behavioral activity in zebrafish larvae (~ 120 hpf) in the LA after exposure to the NOEC from the FET with sediment extracts. Behavior changes during the light and the dark phase are displayed as Fold Change (FC) compared to the solvent control (FC = 1). n = 96. SD: Standard deviation. Statistically significant differences were evaluated using the Mann-Whitney U test and are marked with * $p<0.05$ and ** $p<0.01$.

Sampling site	Sampling campaign 2017					Sampling campaign 2018				
	Extract concentration, g/mL	light		dark		Extract concentration, g/mL	light		dark	
		Mean	SD	Mean	SD		Mean	SD	Mean	SD
H1	25	1.37*	0.12	1.09*	0.06	25	1.08	0.26	0.96	0.02
H2	6.25	1.35	0.46	1.07	0.09	6.25	1.40	0.43	1.10**	0.09
W1	6.25	0.80	0.10	0.96	0.06	1.5625	1.27	0.30	1.08*	0.09
W2	6.25	1.28*	0.23	1.05	0.02	6.25	0.78*	0.14	1.02	0.08
W3	12.5	1.04	0.32	0.97	0.07	6.25	0.97	0.35	0.99	0.06
W4	12.5	0.91	0.01	0.92*	0.05	3.125	1.31	0.24	1.12*	0.11
W5	6.25	0.78	0.21	1.00	0.02	12.5	0.93	0.26	0.99	0.15

S11

Table S11: Literature summary of behavioral alterations caused by several pyrethroids.

Pyrethroid	Behavioral change	Reference
Bifenthrin	Hypoactivity	Jin et al. (2009)
Chlorpyrifos	Hypoactivity, but adaptability	Hu et al. (2020)
Chorpyrifos + deltamethrin	Hypoactivity	Hu et al. (2020)
Cypermethrin	Hyperactivity	Xu et al. (2018)
Deltamethrin	Hypoactivity / Spasms	Kung et al. (2015) / DeMicco et al. (2010)
Esfenvalerate	Hypoactivity	Wang et al. (2020)
Permethrin	Spasms	DeMicco et al. (2010)

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ANNEX 4

Additional (not published results) results

Title: Hazard evaluation of WWTPs effluents by a comprehensive target-screening: Valuable insights but also a big toxicological data gap

Contributing authors: Aliaksandra Shuliakevich[a], Saskia Finckh[c], Ira Brückner[d], Volker Linnemann[e], Regina Dolny[e], Jörg Ahlheim[c], Martin Krauss[c], Tobias Schulze[c], Sabrina Schiwy[a], Werner Brack[a,c], Henner Hollert[a]

a: Goethe University Frankfurt/Main, Department Evolutionary Ecology and Environmental Toxicology, Max-von-Laue-Strasse 13, 60438 Frankfurt/Main, Germany

b: RWTH Aachen University, Institute of Biology V, Worringerweg 1, 52074 Aachen, Germany

c: Helmholtz Centre for Environmental Research – UFZ, Department of Effect-Directed Analysis, Permoserstrasse 15, 04318 Leipzig, Germany

d: Eifel-Rur Waterboard (WVER), Eisenbahnstr. 5, 52354 Düren, Germany

e: RWTH Aachen University, Institute of Environmental Engineering, Krefelder Str. 299, 52070 Aachen

Abbreviations

EC: Effect Concentration

EU WFD: European Water Framework Directive

LC: Lethal concentration

LC-HRMS: Liquid Chromatography-High Resolution Mass Spectrometry

LOEC: The lowest observed effect concentration

LVSPE: Large-volume solid phase extraction

NOEC: No observed effect concentration

TBEP: Tri(butoxy ethyl)phosphate

TCEP: Tris(2-chloroethyl) phosphate

TCPP: Tris(1-chloro-2-propyl) phosphate

TDCPP: Tris(1,3-dichloroisopropyl) phosphate

TEP: Triethyl phosphate

TU: Toxic unit

UFZ: Umweltforschungszentrum

UV: Ultraviolet

WHC: Water hazard classes

WWTP: Wastewater treatment plant

Abstract

Wastewater treatment plant effluents and rainwater overflows serve as the main paths for organic micropollutants into aquatic ecosystems providing clear chemical fingerprints. However, due to the extraordinary complexity of wastewater-born chemicals, their analytical identification remains a big challenge.

The current study implemented a target analysis of 499 chemical compounds in two wastewater treatment plant effluents differentiating by size and wastewater treatment (conventional and advanced treatment by ozonation). The chemical target analysis provided a unique data set for implementing a Toxic Unit approach to identify chemical compounds potentially hazardous to aquatic organisms. However, the authors have faced a significant challenge by searching for toxicity data due to a big data gap. Available free tools could model acute data based on LC50 values without species specification. However, species-specific data could be found for at least 17% of detected chemical compounds. Fifteen chemicals were identified as potentially hazardous to particular aquatic organisms. However, many more chemical compounds can jeopardize chemical and ecological status in aquatic ecosystems.

Based on the current study design, a major part of the investigating micropollutants stems from households stressing the variance of chemical substances conventional users face each day. Ozonation (with the following sand filter) as an additional treatment step was sufficient to reduce 58% of chemical compounds. However, a suitable strategy for a complex ecotoxicological investigation of wastewater treatment plant effluents matching the interests of wastewater managers (simple applicability, low costs), politics, and NGOs is still needed.

Introduction

Currently, about 50.000 chemicals are in commerce worldwide (Bond and Garny, 2019). About 60% of them are potentially harmful to humans and the environment (Milieu Ltd et al., 2017). In 2004-2018, the production of chemicals with a significant acute environmental hazard increased by 77 % (Eurostat, 2020). Despite this fact, the last progress report of the European Water Framework Directive (EU WFD) records a positive trend in achieving a good chemical status in the European waterbodies without considering ubiquitous, persistent, bioaccumulative, and toxic substances (European Environment Agency, 2019). However, the chemical status in the EU WFD reflects information about a shortlist of chemicals without considering the entire chemical pollution on site. Multiple studies showed ecotoxicological risk outgoing from chemicals being not prioritized for the EU WFD monitoring (Alygizakis et al., 2018; Boxall et al., 2012; Brack et al., 2019; Carere et al., 2016; Loos et al., 2009; Malaj et al., 2014).

Hazard chemicals may originate from agriculture, households, urban sector and enter the aquatic environment via field runoff (Inostroza et al., 2016), wastewater treatment plant (WWTP) effluents, and rainwater overflows (Eganhouse and Sherblom, 2001; Selbig, 2009). Thus, Busch et al. (2016) recognized more than 200 chemical compounds potentially hazardous to aquatic organisms and a complex chemical mixture in European rivers with approximately 100 different interaction modes with biological systems. Coexisting in a complex mixture (Altenburger et al., 2015), environmental chemicals occur in very low (micropollutants) or even non-quantifiable concentrations, which are still sufficient to cause adverse effects in organisms (Hollert et al., 2005; Wernersson et al., 2015).

WWTP effluents and rainwater overflows serve as the main paths for organic micropollutants into aquatic ecosystems (Commission of the European Parliament and the Council, 2019). Thus, WWTP effluents were identified as sources of microplastic (Schmidt et al., 2020), halogenated compounds, surfactants (Beckers et al., 2020), estrogenic compounds (Adeyeye and Laub, 2020), pesticides, pharmaceuticals, and industrial chemicals (Müller et al., 2020). Beckers et al. (2020) showed complex spatial pollution patterns and source-related fingerprints comprised of non-target compounds in the Holtemme River (Germany), mainly originated from WWTPs. However, the

knowledge about the specificity grade of wastewater-born chemical fingerprints is still lacking.

For this purpose, the Centre for Environmental Research (*ger*. Umweltforschungszentrum, UFZ; Leipzig, Germany) initiated in 2017 a scientific activity within the NORMAN Network of reference laboratories, research centres, and related organizations for monitoring of emerging environmental substances on the identification of chemical and toxicological fingerprints of WWTP effluents. Thus, 57 WWTP effluents Europe-wide were sampled using the large-volume solid phase extraction (LVSPE). As part of the NORMAN network scientific activity, the current study aimed at evaluating and assessing the chemical effluent burden of two municipal WWTPs in Germany united by the same catchment area of the receiving water. As one of the studied WWTPs had advanced effluent treatment by ozonation, its effluent was sampled before entering the ozonation plant and after the passing of the ozonation plant and the sand filter.

Material and methods

Study design

50 L of each WWTP effluents were extracted on site using the LVSPE in according to Schulze et al. (2017). In addition, 1 traveling blank at each sampling site was prepared by extracting 1 L of water (LC-MS grade) by LVSPE. All extracts were re-dissolved in methanol (LC-MS grade) at a relative enrichment factor of 1000 and stored at –20°C until further analysis. A complete and comprehensive extraction methodology is laid down in a manuscript under review by Finckh et al. 2022b, under review.

The first sampling took place in January 2018 on the effluent of the WWTP_1. It is a small municipal WWTP with an annual treatment capacity of around 4 Mio. m^3 (see Table 1). After the initial mechanical separation, WWTP_1 comprises two conventional wastewater treatment steps (biological and chemical) following by sand filtration (see Figure 1). The resulting tertiary effluent enters the tributary of the main waterbody, making up to 72% of the total water flow in dry weather (ELWAS-WEB, 2021). The second sampling was provided on 04 July 2018 at the WWTP_2, located downstream of the WWTP_1. WWTP_2 is one of Germany's biggest WWTPs, with an annual treatment capacity of around 32 Mio. m^3. The wastewater treatment procedure is equal to this of the WWTP_1. 2018 the WWTP_2 achieved advanced effluent treatment by full stream ozonation. Therefore, the sampling design included the collection of the secondary effluent (after the secondary clarifier, before the ozonation plant) and advanced treated effluent (after the passage through the ozonation and a sand filter). WWTP_2 discharges its wastewater into the main waterbody providing around 84% of the total water flow (ELWAS-WEB, 2021).

For a better differentiation between different effluents, the effluents of the WWTP_1 and WWTP_2 without ozonation (WWTP_2_-O3) are signed as 'conventionally' effluents. Furthermore, the effluent of the WWTP_2 after the ozonation step and the sand filtration is signed as 'advanced treated' (WWTP_2_+O3).

Table 1: Information on studied WWTPs (Brückner et al., 2018; EC DGENV, 2017; ELWAS-WEB, 2021; Kompetenzzentrum Mikroschadstoffe. NRW, 2015).

	WWTP_1	**WWTP_2_-O3**	**WWTP_2_+O3**
Location	Tributary upstream of the WWTP_2	Main waterbody	
Sampling after treatment steps / sampled effluent type	Biological, chemical, sand filter = (tertiary) conventionally treated effluent	Biological, chemical = (secondary) conventionally treated effluent	Biological, chemical, ozonation, sand filter = advanced treated effluent
Treatment capacity, [$m^3\ y^{-1}$]	4 million	32 million	
Effluent portion in the average annual total water flow (normal hydrological conditions)	72%	84%	
Rain overflow system	Yes	Yes	
Release rate of rain overflow basin per year	3-4	40-45	
Data of the LVSPE	11.01.2018	04.07.2018	
Sampling weather	dry	dry	

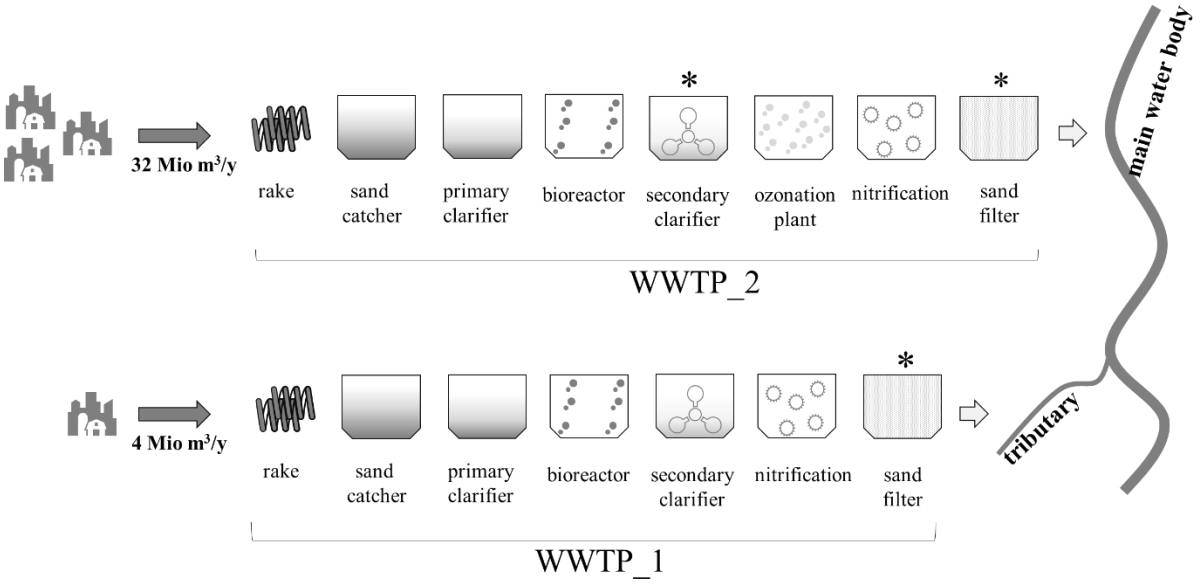

Figure 1: Scheme of the studied WWTPs and sampling sites (*). P.e.: population equivalent. Created using Power-user®.

Chemical analysis

In total, 499 chemicals (parent and their metabolites) from diverse chemical categories (plant protective agents, pharmaceuticals, industrial chemicals, ultraviolet light (UV)-filters, etc.) were analysed using Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) (Beckers et al., 2020; Muschket et al., 2018). For the chemical analysis, 100 µL of each LVSPE extract (REF 1000)/travel blank were transferred into a 2 mL autosampler vial with a 200 µL conical glass insert. Additionally, 10 µL of an internal standard mixture containing 38 isotope-labelled compounds (1 µg/mL), 30 µL of methanol (LC-MS grade) and 60 µL of water (LC-MS grade) were added. 5 µL extracts were used for the measurement applying a reversed-phase LC separation (Thermo Ultimate 3000 LC). HRMS was ran in full scan combined with data-independent mode using a quadrupole-Orbitrap MS (QExactive Plus, Thermo Scientific) with electrospray ionization (ESI) in positive and negative modes. Further information on settings, instrument parameters, data processing and evaluation are described in detail in Beckers et al. (2020).

The list of analysed chemical compounds with respective internal standards can be found in Supplementary Information (SI) S1, S2 and is also available on PANAGAEA (Finckh et al., 2022a). A complete and comprehensive methodology of the chemical analysis is laid down in a manuscript under review by Finckh et al. 2022b.

Evaluation of the toxicological hazard

The toxic unit (TU) approach was implemented for unravelling the samples' complexity and the prioritization of compounds of concern (Ohe et al., 2009; Ohe et al., 2011; Peterson, 1994). The calculation of TUs describes a relationship between the measured environmental concentration and an individual compound's effect level (see Eq. 1). $TU = \frac{c_i}{effect\ cocnentration_i}$ (Eq. 1), where c_i describes the measured concentration of the responsible chemical compound i and the $effect\ concentration_i$ as an experimental or simulated concentration of the compound i for a specific species. Therefore, the TU exceedance of one indicates a potential hazard for aquatic organisms.

Thus, chemical compounds of concern were prioritized using species-unspecific chronic LC50 (as TU_{LC50}) and species-specific acute EC50 (as TU_{EC50}) values. Species-unspecific effect concentrations were evaluated for daphnids and fish. In order to increase toxicological safety for all daphnids and fish species, TU_{LC50} was corrected by

the safety factors of 1000 for daphnids (TUch ≥ 0.001) and 100 for fish (TUch ≥ 0.01) (Ohe et al., 2009). The majority of all LC50 values stem from the Ecological Structure-Activity Relationships (ECOSAR) Predictive Model of the U.S. EPA v 2.0 (Mayo-Bean et al., 2017) basing on a combination of physicochemical properties of the target compounds with the experimental ecotoxicological data of structurally similar chemical compounds. For the minority of chemical compounds, ecotoxicological lethal data could not be predicted by ECOSAR. In that case, the ChemProp (TM) software OSIRIS Edition (UFZ Department of Ecological Chemistry, 2019) and VEGA *in silico* platform (v. 1.1.5) were applied.

For the species-specific acute toxicity, TU_{EC50} were calculated for a planktonic crustacean *Daphnia magna* as a freshwater deputy prey and a salmonid rainbow trout *Oncorhynchus mykiss*. For a sensitivity comparison, a zebrafish *Danio rerio* as a common investigative object in environmental science was also included in the acute chemical hazard assessment strategy. The central part of the EC50 values was obtained from the NORMAN Ecotoxicology Database (https://www.norman-network.com/nds/ecotox/) and the ECOTOX Knowledgebase of the U.S. EPA (https://cfpub.epa.gov/ecotox/). If more than one EC50 values were available for one species, the lowest EC50 value was chosen. For chemical compounds without any experimental ecotoxicological data, these were generated using the ECOSAR. Due to a vast toxicity data gap for most searched substances, such effect values as NOEC (TU_{NOEC}) or LOEC (TU_{LOEC}) were also considered.

Results

Detection of target chemicals in differentially treated effluents

200 and 220 compounds were identified in concentrations over the detection limit were identified in the effluent of the WWTP_1 and WWTP_2_-O3 (before the ozonation step) (see Supplementary Information, S3). Pesticides, pharmaceuticals, and industrial chemicals primarily made up the major groups of the chemical burden (see Figure 2). After the advanced effluent treatment by ozonation, 128 chemicals with the predominated pharmaceutical group were identified in the effluent of the WWTP_2. The full-stream effluent ozonation at the WWTP_2 showed a significant reduction of plant protective agents (74%), pharmaceuticals (89%), industrial chemicals (77%), and UV filters (80%). The group of other compounds reached due to low elimination of artificial sweeteners an elimination rate of 45% only (see SI, S4).

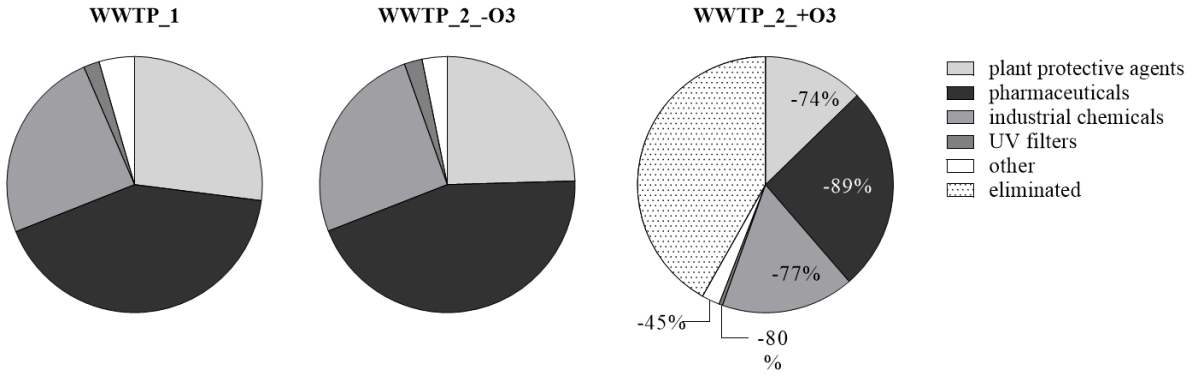

Figure 2: Identified groups of chemicals in the effluent of the WWTP 1, WWTP 2 before ozonation (-O3), and after the ozonation step (+O3). Percentual values show concentration reduction of the respective chemical compounds group after the ozonation treatment.

Prioritization of effluent chemical compounds with acute effect hazard potential

For calculating TUs for acute effect toxicity, EC50 values for 242 substances and three species (*Daphnia magna, Danio rerio, Oncorhynchus mykiss*) were derived from available databanks. Due to the absence of the effect data for many searched substances, NOEC or LOEC values were also evaluated, filling the effect data gap for 17% of the missing data.

The most acute effect toxicity data were available for *Daphnia magna* (61%), following by *Danio rerio* (31%) and the rainbow trout *Oncorhynchus mykiss* (8%). Thus, 12

chemical compounds exceeded the acute effect level and were prioritized as potentially acute hazardous to organisms in the receiving waterbody.

Two biocides, imazalil, and thiabendazole, were detected in the effluent of the WWTP_2_-O3 (4 ng/L and 8 ng/L, respectively), while the effluent of the WWTP_1 contained 3 ng/L imazalil only. Comparing with the acute effect data, these concentrations were evaluated as potentially acutely toxic to a zebrafish *Danio rerio* (see Table 2).

The conventionally treated effluent of the WWTP_2_-O3 showed a potentially acute toxic effect to *Danio rerio* with 16 ng/L of the industrial chemical 2,4-dichlorophenol. Its TU_{EC50} for *Daphnia magna* within the range of 0.1-0.99 categorized 2,4-dichlorophenol as potentially acute hazardous in case of a slight concentration exceedance.

Diethylatrazine, as a metabolite of atrazine, appeared in concentrations of high concern due to the TU_{EC50} for *Danio rerio* of 0.2 and 0.3 in the effluent of the WWTP_1 (5 ng/L) and WWTP_2_-O3 (9 ng/L), respectively. The fungicide trifloxystrobin and its metabolite NOA413161 in all effluent's samples showed acute toxicity to *Danio rerio*.

Six pharmaceutic drugs appeared in concentrations potentially acute toxic to aquatic organisms. Thus, atorvastatin (lipid blocker), clozapine (neuroleptic drug), ranitidine, and diphenhydramine (both anti-histaminic drugs) were identified in conventionally treated effluents of the WWTP_1 (27 ng/L, 26 ng/L, 149 ng/L, and 107 ng/L, respectively) and WWTP_2_-O3 (28 ng/L, 86 ng/L, 264 ng/L, and 156 ng/L, respectively) in concentrations potentially acute toxic to a zebrafish *Danio rerio*. Furthermore, the lipid blocker gemfibrozil was quantified in the effluent of the WWTP 2_-O3 indicating acute toxicity to a zebrafish *Danio rerio* (68 ng/L). The frequently prescribed diuretic medicine furosemide was classified as a chemical compound of a potential concern due to the TU_{EC50} value for *Danio rerio* of 0.15 and 0.32 in the effluent of the WWTP_1 and WWTP_2_-O3, respectively (see SI, S5).

Due to a big data gap for species-specific effect toxicity, the ranking procedure was not implemented.

Table 2: Substances prioritized by exceedance of the TU values the effect level (TU ≥ 1). The used effect level is given in brackets. TUs within the range of 0.1-0.99 were signed by '', emphasizing chemical effluent concentrations of high concern. –O3: effluent before ozonation, +O3: effluent after ozonation. Ind. chem.: industrial chemical. Pharm.: pharmaceuticals.*

	WWTP_1	WWTP_2_-O3	WWTP_2_+O3
Imazalil (biocide)	TU_{EC50} *D.rerio*	TU_{EC50} *D.rerio*	-
Thiabendazole (biocide)	-	TU_{EC50} *D.rerio*	-
2,4-dichlorophenol (ind. chem.)	TU_{EC50} *D.magna**	TU_{EC50} *D.magna**	-
	TU_{EC50} *D.rerio*	TU_{EC50} *D.rerio*	
Trifloxystrobin (pesticide)	TU_{EC50} *D.rerio*	TU_{EC50} *D.rerio*	TU_{EC50} *D.rerio*
NOA413161 (metabolite of trifloxystrobin)	TU_{EC50} *D.rerio*	TU_{EC50} *D.rerio*	-
Diethylatrazine (pesticide)	TU_{EC50} *D.rerio**	TU_{EC50} *D.rerio**	-
Gemfibrozil (pharm.)	-	TU_{NOEC} *D.rerio*	-
Clozapine (pharm.)	TU_{NOEC} *D.rerio*	TU_{NOEC} *D.rerio*	-
Ranitidine (pharm.)	TU_{NOEC} *D.rerio**	TU_{NOEC} *D.rerio*	-
Diphenhydramine (pharm.)	TU_{LOEC} *D.rerio**	TU_{LOEC} *D.rerio*	-
Atorvastatin (pharm.)	TU_{LOEC} *D.rerio*	TU_{LOEC} *D.rerio*	-
Furosemid (pharm.)	TU_{NOEC} *D.rerio**	TU_{NOEC} *D.rerio**	-

Prioritization of effluent chemical compounds with lethal hazard potential

Three chemical compounds were prioritized as compounds of concern: The industrial chemical 2,4-dichlorophenol showed in conventionally treated effluents of the WWTP_1 (12 ng/L), and WWTP_2_-O3 (16 ng/L) possible lethal toxicity to fish (see Table 3; see SI, S5). The antihypertensive drug telmisartan was identified in the effluent of the WWTP_1 and WWTP_2_-O3 with concentrations of 550 ng/L and 590 ng/L, respectively. These concentrations were potentially toxic to crustaceans and fish (1E-4 > TUch < 9.9E-4). After the effluent ozonation treatment, telmisartan concentration was reduced by 86 % to 85 ng/L, still remaining toxic to fish. The UV-filter phenyl benzimidazole sulfonic acid appeared in the conventionally treated effluents of the WWTP 1 (9.9 µg/L) and WWTP 2_-O3 (6,3 µg/L) in concentrations (9.9 µg/L and 6.3 µg/L, respectively), potentially causing lethality in the crustacean, which was sufficiently removed by ozonation (-86% to 1.5 µg/L).

Table 3: Substances prioritized by exceedance of the TU_{LC50} values to fish (TU_{LC50} ≥ 0.01) and crustacean (TU_{LC50} ≥ 0.001) (Ohe et al., 2009). TU_{LC50} within the range of 1E-3 - 9.9E-3 (fish) and 1E-4 - 9.9E-4 were marked by '' emphasizing chemical effluent concentrations of high concern. –O3: secondary effluent, no ozonation treatment; +O3: secondary effluent treated with ozone. Ind. chem.: industrial chemicals. Pharm.: pharmaceuticals.*

	WWTP_1	WWTP_2_-O3	WWTP_2_+O3
2,4-dichlorophenol (ind. chem.)	TU_{LC50} (fish)	TU_{LC50} (fish)	-
Telmisartan (pharm.)	TU_{LC50} (fish) TU_{LC50} (crustacea)*	TU_{LC50} (fish) TU_{LC50} (crustacea)*	TU_{LC50} (fish)*
Phenylbenzimidazole sulfonic acid (UV-filter)	TU_{LC50} (crustacea)	TU_{LC50} (crustacea)	-

Grouping of achieved TU_{LC50} according to their values unravelled the toxicity pattern similar to a normal distribution curve (see Figures 3 and 4). The range of the TU_{LC50} for crustacea was between 9E-10 and 9E-2, while the lowest TU_{LC50} for fish accounted for 7.4 E-12 due to the ozonated effluent WWTP_2_+O3 and the highest – for 4.54 due to the secondary effluent WWTP_2_-O3.

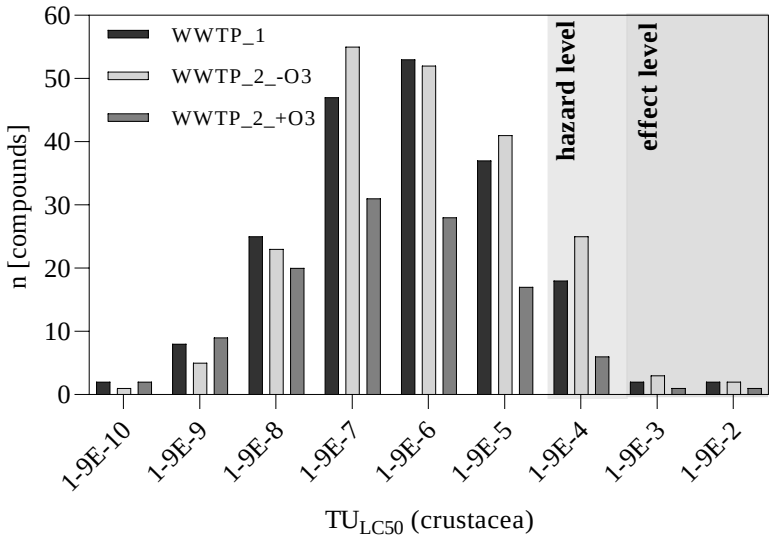

Figure 3: Species-unspecific TU calculated based on the LC50 toxicity values to crustacea in the studied effluent samples. Bright grey shadow marks the hazard level if the 1E-4 ≥ TU_{LC50} (crustacea) ≤ 9.9E-4. Dark grey shadow marks the effect level of the TU_{LC50} (crustacea) ≥ 0.001.

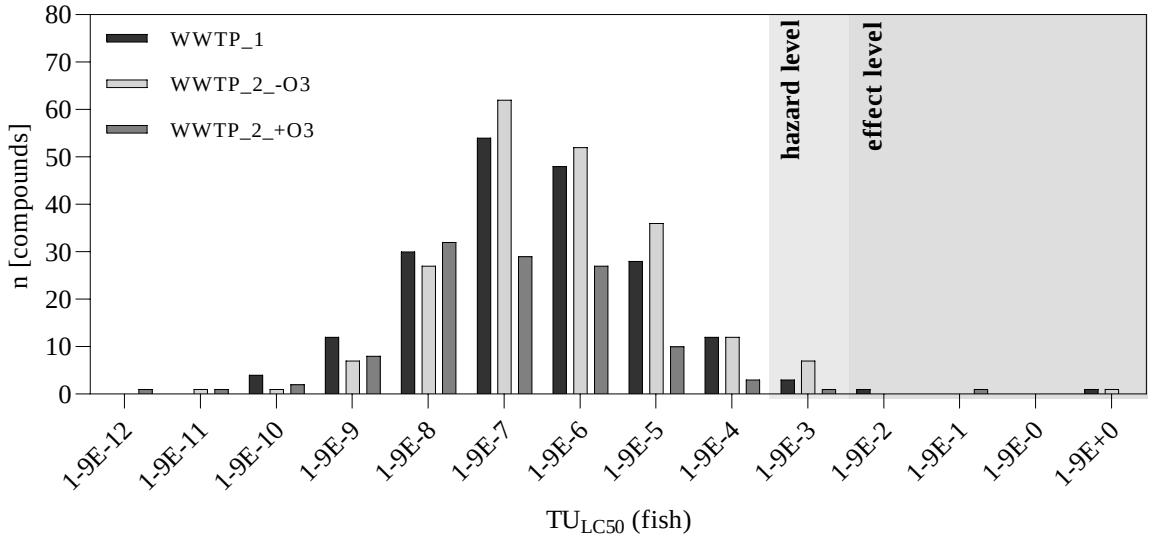

Figure 4: Species-unspecific TU calculated based on the LC50 toxicity values to fish in the studied effluent samples. Bright grey shadow marks the hazard level if the 1E-3 ≥ TU_{LC50} (fish) ≤ 9.9E-3. Dark grey shadow marks the effect level of the TU_{LC50} (fish) ≥ 0.01.

Annual load of identified target substances in receiving waterbody

The implemented target analysis allowed to assess a possible load of single substances on the receiving water. Thus, the WWTP_1 treats 8-timer smaller wastewater amount per year, discharging a much lower number of chemical compounds into the receiving water. The annual chemical burden of analysed target substances for 4.1 Mio. m^3/y treated wastewater per year in the WWTP_1 accounted for 283.32 g/y (see Figure 5). The WWTP_2 is located around 7 km downstream of the WWTP_1 and receives one part of the emitted chemical compounds by the WWTP_1 by adding its annual load of 3.7 kg/y (annual wastewater treatment: 31.8 Mio. m^3/y). Since the ozonation plant was launched, the total chemical burden discharging in the receiving water could be reduced by 2.1 kg/y resulting in 1.6 kg/y. Our results confirmed the suitability of ozone for the reduction and elimination of micropollutants. Normalizing by several detected substances (see Fig. 2), the WWTP_2 discharged a 14-times greater concentration of chemicals than the WWTP_1 (see SI, S6).

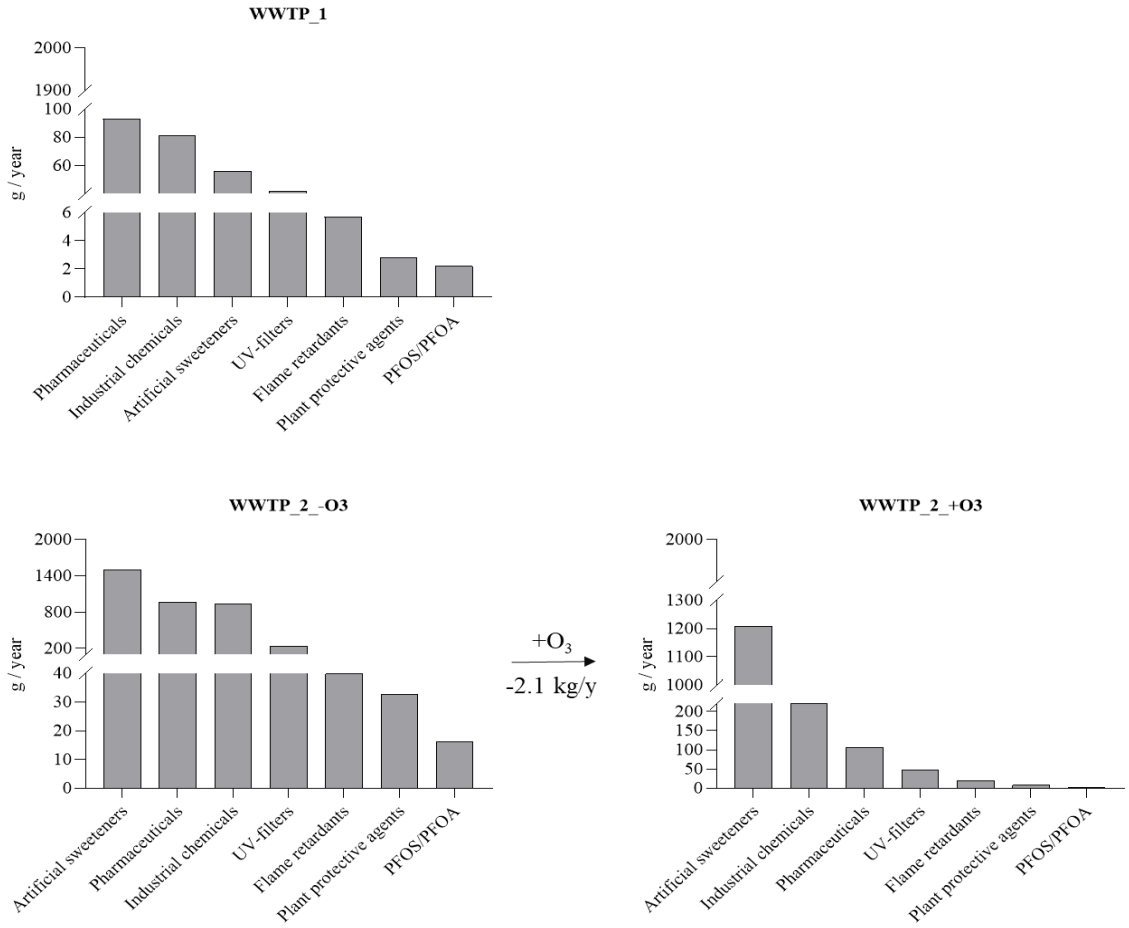

Figure 5: Annual load of chemical compounds detected by target analysis.

Substances hazardous to water

Each chemical substance directly entering the waterbody in Germany should be characterized by a responsible party according to its hazard to water resources. Accordingly, the German Water Recourses Act (*ger*. Gesetz zur Ordnung des Wasserhaushalts – Wasserhaushaltsgesetz), water hazard substances are defined as 'solid, liquid, or gaseous substances being able permanently or significantly adverse water quality characteristics (physically, chemically, or biologically)'. These are, for instance, heating oil, fuel, liquid manure, or plant-protective agents. Water hazard substances underlie evaluation criteria listed in the Ordinance on facilities for handling of water hazard substances (*ger*. Verordnung über Anlagen zum Umgang mit wassergefährdenden Stoffen) of 18 April 2017 (German Federal Office of Justice, 2017) and can be divided into three water hazard classes (WHC; *ger*. Wassergefährdungsklasse):

WHC 1: Substances slightly hazardous to water (*ger*. schwach wassergefährdend);

WHC 2: Substances obviously hazardous to water (*ger*. deutlich wassergefährdend);

WHC 3: Substances highly hazardous to water (*ger*. stark wassergefährdend).

Wastewater from households as user and industry as manufacturer/user of water hazard substances ends in WWTPs. According to the WHG, WWTPs are not obligated to the observation of water-hazard substance emissions. However, the end-of-pipe investigations of effluents can unravel information about the usage of the catchment area, unknown sources of pollution and contribute to the discussion about additional wastewater treatment. The chemical burden of the WWTP effluents regarding water hazardous substances was evaluated using the Rigoletto web database (v 3.0; https://webrigoletto.uba.de/rigoletto/public/welcome.do) conceived by the German Environmental Agency.

In total, 74 investigated target substances matched the list of water hazard substances in the Rigoletto database. In the conventionally treated effluents of WWTP_1 and WWTP_2, 22 (11 µg/L) and 23 (16 µg/L) slightly water-polluting substances (WHC 1) were detected (see SI, S7). Ozonation eliminated eight substances to 4 µg/L sum concentration of all WHC 1 substances. A similar number of hazardous WHC 2 substances were detected in the effluents of the WWTP_1 and WWTP_2_-O3 (21 and 28 substances, respectively). However, the sum concentrations were significantly different. Thus, 21 WHC 2-substances in the effluent of the WWTP_1 accounted for 9 µg/L, while 28 WHC 2-substances in the effluent of the WWTP_2_-O3 – for 50 µg/L. The ozonation step provided elimination of 12 WHC 2-substances up to 38 µg/L. Highly hazardous to water WHC 3-substances were detected in the effluents of the WWTP_1 and WWTP_2_-O3 in sum concentrations of 1.8 µg/L (18 substances) and 2.4 µg/L (17 substances), respectively. The applied full-stream ozonation was able to eliminate four WHC 3-substances contributing to a significant reduction of their concentration up to 164 ng/L in the effluent of the WWTP_2_+O3.

Classification of studied effluents according to national and international legislations

Domination of studied effluents on the total water flow in the receiving stream encouraged the authors to compare achieved data from the target analysis with consistent environment quality standards (EQS) recorded in the EU Water Framework Directive and national report by the German Environmental Agency (*ger*.

Umweltbundesamt) (see Table 5). The selected chemical compounds' concentrations were significantly lower than these of the prioritized substances by the EU WFD and the German Environmental Agency. Adding the dilution factor but also possible additional load from other sources of pollution in the waterbody, the EQS were expected not to be exceeded.

*Table 5: Concentration of single target substances in comparison to national and international threshold values. N.q.: Not quantifiable concentration, < limit of detection. EQS is marked with *. AA-EQS: Annual average EQS – is marked with **. MAC-EQS: Maximum allowable concentration of the EQS – is marked with ***. Reference 1: Directive 2013/39/EU. Reference 2: Umweltbundesamt, 2011.*

	WWTP_1	WWTP_2_-O3	WWTP_2_+O3	Quality standard	Ref
2-methyl-4-chlorophenoxy-acetic acid	1 ng/L	10 ng/L	n.q.	100 ng/L*	2
Atrazine	2 ng/L	n.q.	n.q.	600 ng/L**/ 2000 ng/L***	1
Bentazone	1 ng/L	1 ng/L	n.q.	100 ng/L*	2
Dichlorprop	2 ng/L	n.q.	n.q.	100 ng/L*	2
Diuron	18 ng/L	20 ng/L	8 ng/L	200 ng/L**/ 1800 ng/L***	1
Epoxiconazole	n.q.	2 ng/L	n.q.	200 ng/L*	2
Isoproturon	3 ng/L	26 ng/L	1 ng/L	300 ng/L**/ 1000 ng/L***	1
Mecoprop	16 ng/L	9 ng/L	n.q.	100 ng/L*	2
Metazachlor	n.q.	n.q.	n.q.	400 ng/L*	2
Metolachlor	n.q.	n.q.	n.q.	200 ng/L*	2
Metribuzin	1 ng/L	1 ng/L	n.q.	200 ng/L*	2
Propiconazole	7 ng/L	8 ng/L	2 ng/L	1000 ng/L*	2
Simazine	n.q.	6 ng/L	n.q.	1000 ng/L**/ 4000 ng/L***	1
Terbuthylazine	n.q.	3 ng/L	1 ng/L	200 ng/L*	2
Terbutryn	8 ng/L	30 ng/L	7 ng/L	65 ng/L**/ 34 ng/L***	1

Discussion

Prioritized and evaluated chemical compounds using the Toxic Unit approach

No acute lethal and sublethal toxicity was observed in chemical groups of artificial sweeteners, perfluorocarbons, plastic additives, flame retardants, surfactants, and UV filters. 15 chemicals were prioritized as being potentially toxic or hazardous to freshwater organisms, especially to fish (TU ≥ threshold value). In contrast, three chemical compounds (telmisartan, phenyl benzimidazole sulfonic acid, and 2,4-dichlorophenol) were detected in concentrations potentially toxic or hazardous to crustacea.

From 35 analysed biocides, ten were detected in effluent samples within the concentration range from 1 ng/L (e.g., warfarin in the effluent of the WWTP_1 and WWTP_2_-O3) till 166 ng/L (2-aminobenzimidazole in the effluent of the WWTP_2_-O3). The biocides terbutryn and carbendazim were detected in conventionally treated effluents of the WWTP_1 (8 ng/L and 5 ng/L, respectively) and WWTP_2 (30 ng/L and 24 ng/L, respectively). Due to potential toxic effects (mutagenic, endocrine-disruptive, etc.), terbutryn and carbendazim are not approved for use in the agriculture sector. However, they can use preservative and coating agents of textiles, papers, rubbers, and building facades (Burkhardt and Vonbank, 2011). Specific usage determines street runoffs, municipal and industrial wastewaters as the main paths of terbutryn and carbendazim into the aquatic environment. Both substances serve as benchmarks for wastewater-contaminated surface- and groundwaters (Fuchs et al., 2018; Merel et al., 2018). Current terbutryn concentrations were comparable with the German Environmental Agency average effluent concentration of 50 ng/L (modeled data) (Fuchs et al., 2018). The detected carbendazim concentration was consistent with the average measured effluent concentration in Germany of 24.9 ng/L (Merel et al., 2018). The current study confirmed high elimination rates of terbutryn and carbendazim by ozone (77% and 100%, respectively) (Hollender et al., 2009).

Azole fungicide imazalil was detected in the tertiary effluent of the WWTP_1 and the secondary effluent of the WWTP_2 in concentrations of 3 ng/L and 4 ng/L, respectively, prioritizing these biocides as acute hazardous to a zebrafish *Danio rerio*. Another azole fungicide, thiabendazole, was identified in the effluent of the WTWP_2_-O3 in a hazardous concentration to a zebrafish. Both fungicides are used

for postharvest treatment of fruits, increasing durability during transport and marketing (Castillo et al., 2000). Therefore, municipal wastewater was suggested as the main path for imazalil and thiabendazole in the environment. Detected effluent concentrations were in line with Wick et al. (2010) measured 5.1-6.9 ng/L of imazalil and 4.1-14 ng/L of thiabendazole in two WWTP effluents in Germany. Azole fungicides initially inhibit steroidogenesis, acting endocrine-disruptive (Zarn et al., 2003). However, low effect specificity can subsequently lead to neurotoxic (Jin et al., 2016) and embryotoxic effects in zebrafish larvae (Sişman and Türkez, 2010). Additionally, azole fungicides were shown to induce genotoxic changes in human peripheral lymphocytes (Sişman and Türkez, 2010). Imazalil and thiabendazole could be entirely removed by the effluent treatment with ozone (Genena et al., 2011).

Of 163 suggested pesticides, 33% (#53) were detected in the effluent samples. The majority of the single pesticides appeared in a concentration below 30 ng/L. However, 2,4-dichlorobenzoic acid was detected in a relatively high concentration in the effluent WWTP_2_-O3 of 0.1 µg/L. 2,4-dichlorobenzoic acid is one of the highly mobile metabolites of the insecticide Spirodiclofen (Envidor®) with a field of application in pome fruits, hops, strawberries, nuts, and vine cultures (Nauen et al., 2003). Although the area around Aachen is not known to be cultivated with the mentioned cultures, its origin from small-scale cultivation and use in households was suggested. Measured effluent concentration was comparable with Bernhard et al. detected 2,4-dichlorobenzoic acid in the effluent of one german WWTP in the concentration of 30-144 µg m^{-3} d^{-1} (Bernhard et al., 2006). 2,4-Dichlorobenzoic acid is expected to be bioaccumulated in algae (Freitag et al., 1982) and was responsible for disrupting fat metabolism in zebrafish (Zhang et al., 2019). In the European pesticide risk assessment, the presence of 2,4-dichlorobenzoic acid is relevant in soil and groundwater but not in surface water or sediment (EFSA, 2009). The latter argument is a potential reason for rare studies on the possible toxicity of 2,4-dichlorobenzoic acid or Spirodiclofen to non-target organisms. 2,4-dichlorobenzoic acid was eliminated by ozonation.

Trifloxystrobin was detected in all studied effluent samples in concentrations causing chronic toxicity in fish (1 ng/L in the effluent WWTP_1 and WWTP_2_+O3; 3 ng/L in the effluent WWTP_2_-O3). Its metabolite NOA413161 was detected in the effluent WWTP_1 and WWTP_2_-O3 in concentrations of 8 ng/L and 7 ng/L, respectively,

causing acute toxicity in zebrafish *Danio rerio*. Trifloxystrobin is a stable and highly lipophilic substance approved for use in the internal area only (BVL, 2011), indicating its household origin. Ozone treatment was not efficient in eliminating trifloxystrobin (67% elimination), bearing the risk for aquatic organisms in the receiving water. Thus, trifloxystrobin can inhibit algal growth (Shen et al., 2014), induce oxidative stress in aquatic organisms (Clasen et al., 2018; Shen et al., 2014), impact the predator-prey relationship (Junges et al., 2012), and bioaccumulate in fish (Ernst et al., 2018).

An interesting fund was detecting the non-selective herbicide imazapyr in the concentrations of 11 ng/L and 17 ng/L in the effluent of the WWTP_1 and WWTP_2_-O3, respectively. Imazapyr is one of the 101 pesticides that has been phased out in the EU (Council of the European Communities, 1991) with permitted approval in the European countries for especial use only. In Germany, imazapyr can be used to weed control at building surfaces, ways, and water-saturated areas. Imazapyr is suggested to be genotoxic in micronucleus assay based on experimental (Grisolia, 2002) and modeling data (VEGA *in silico* platform v. 1.1.5, model '*In silico* Micronucleus activity IRFMN/VERMEER' v. 1.0.0). The general toxicity to non-target organisms was evaluated as acceptable (Breckels and Kilgour, 2018). However, the monitoring of imazapyr is still essential due to surfactants and adjuvants in the formulation, which may reveal much higher toxicity to non-target organisms than imazapyr itself (Breckels and Kilgour, 2018).

A synthetic intermediate 2,4-dichlorophenol, which is widely used for manufacturing of chloride-based plant protective products, polyesters and also serve as a transformation product of triclosan, appeared in conventionally treated effluents in concentrations causing acute and chronic toxicity in fish (12 ng/L and 16 ng/L in the effluent of the WWTP_1 and WWTP_2_-O3, respectively). Several monitoring studies indicated that due to its vast industrial and agricultural use, the environmental concentrations of 2,4-dichlorophenol in receiving waters could reach µg/L (Chiron et al., 2007; Gao et al., 2008). Fish exposure to 2,4-dichlorophenol can cause mortality in early juvenile life stages (Holcombe et al., 1982), oxidative stress (Zhang et al., 2004), genotoxic effects (Chen et al., 2012), and feminization (Zhang et al., 2020). The effluent ozonation was effective in the complete elimination of 2,4-dichlorophenol (Contreras et al., 2003).

A chemical group of ubiquitously presented benzotriazoles was not identified as hazardous to aquatic organisms based on the TU strategy. However, benzotriazoles appeared in extremely high concentrations. Benzotriazoles as corrosion inhibitors are used in washing products for the durability of heating elements and corrosion-stable coating materials to protect facades and roofs (ECHA, 2021). Thus, the total concentration of the predecessor 1H-benzotriazole (1H-BT) and its successors (5-Methyl-1H-BT, 4-Hydroxy-BT, 5-Chloro-BT) was 7.4 µg/L and 10 µg/L in the effluent WWTP_1 and WWTP_2_-O3, respectively. High polarity, wide internal and external usage of benzotriazoles combining with the one-pipe canalization system of the studied WWTPs could explain detected concentrations. Additionally, one industrial detergent producer using benzotriazoles for manufacturing discharges its untreated effluent in the WWTP_1. Comparable values (12 µg/L of 1H-BT) were found in effluents in Germany (Weiss et al., 2006) and Switzerland (3,7 µg/L of 1H-BT) (Voutsa et al., 2006). Due to the incomplete elimination of benzotriazoles in WWTPs, an overall emission of benzotriazoles in Germany's receiving waters was estimated for 24 t annually (Vetter and Lorenz, 2013). The toxicity of benzotriazoles is still rarely explored. Thus, several studies showed low effect levels (mg/L) in organisms (Kadar et al., 2010; Liang et al., 2014; Seeland et al., 2012). However, benzotriazole showed anti-estrogenic activity *in vitro* in ng/L concentrations (Harris et al., 2007) and potential involvement in initial neurological pathways causing neurological disorders in fish at the concentration of 50 µg/L (Liang et al., 2016). The presence of benzotriazole in the environment means the formation of metabolites, which can be several times more toxic (Pillard et al., 2001). Ozonation could reduce the total concentration of benzotriazoles up to 2 µg/L. This result was in line with the finding from the big state project SchussenAktiv (and the following upgrade projects SchussenAktivplus, SchussenAktivplus+) (Triebskorn, 2017) and further studies (Nasuhoglu et al., 2018; Weiss et al., 2006).

Furthermore, high sum concentrations of per- and polyfluoroalkyl derivates (PFAS; the sum of 5 detected substances: Perfluorooctanoic acid (PFOA), perfluorooctanesulfonic acid (PFOS), perfluorohexanoic acid (PFC6H), perfluoroheptanoic acid (PFC7H), 6:2 fluorotelomer sulfonic acid (6:2 FTS)) was detected in the studied effluents. Thus, the effluent of the WWTP_1 contained 534 ng/L PFAS, and the effluent of the WWTP_2 without the ozonation treatment discharged 513 ng/L. The predominant part of detected PFAS was made up by 6:2 FTS

(500 ng/L and 474 ng/L in the effluent WWTP_1 and WWTP_2_-O3, respectively) as a substitute for long-chain PFAS in firefighting foams (Nicol et al., 2020). Due to multiple desirable unique characteristics of PFAS, such as extreme heat resistance and surfactant properties, PFAS were widely used in all fields of industry (Nicol et al., 2020; Taylor, 1999). The restriction of long-chain PFAS by the Stockholm Convention and under the REACH led to simultaneously increased short-chain PFAS and fluorotelomers as replacement compounds (Gonzalez et al., 2021), as confirmed by the results of the current study. The overall PFAS and especially 6:2 FTS removal by ozone accounted for 83% (85 ng/L) and 86% (66 ng/L), respectively. Our results were in good agreement with the results by Yang et al. detected almost 90% ozone elimination of 6:2 FTS (pH 11) (Yang et al., 2014).

A group of flame retardants and plasticizers, comprising 5 detected substances (tri(butoxyethyl)phosphate (TBEP), tris(1,3-dichloroisopropyl) phosphate (TDCPP), tris(1-chloro-2-propyl) phosphate (TCPP), triethylphosphate (TEP), tris(2-chloroethyl)phosphate (TCEP)), was quantified in a total sum concentration of 1356 ng/L in the tertiary effluent of the WWTP_1 and 1204 ng/L in the secondary effluent WWTP_2. A sum concentration of detected TDCPP, TCPP, TEP, and TCEP in conventionally treated effluents (985 ng/L and 1188 ng/L in the effluent of the WWTP_1 and WWTP_2_-O3, respectively) entering the study river (river basin size: 356 km²) was double so high as in surface water samples from the Elbe River (530±84 ng/L; river basin size: 148 268 km²) (Wolschke et al., 2015). Due to the oxidation resistance of flame retardants and plasticizers, the total elimination by ozone with the following sand filter accounted for around 50 % (601 ng/L in the effluent WWTP_2_+O3) (Sundaram et al., 2014). As studied effluents dominate the total water flow of the recipient water, it indicated high chemical risk, particularly outgoing from flame retardants and plasticizers due to low dilution capacity.

Although the WWTP_2 achieves effluents from 5 hospitals, the concentration of pharmaceuticals in the effluent is comparable to those in the effluent of the WWTP_1. The total concentration of all pharmaceuticals in the effluent of the WWTP_2_-O3 was 30.6 µg/L, while the total concentration of pharmaceuticals in the effluent of the WWTP_1 accounted for 31.5 µg/L. Advanced effluent treatment by ozonation resulted in 88% elimination.

As a widely used antihypertensive drug, telmisartan was quantified in all effluent samples, potentially causing mortality in crustacea (WWTP_1 and WWTP_2_-O3) and fish (WWTP_1, WWTP_2_-O3, WWTP_2_+O3). Thus, conventionally treated effluents of the WWTP_1 and WWTP_2 emitted 550 and 590 ng/L telmisartan, respectively. These concentrations were within the average telmisartan concentration of 368 ng/L measured in 57 European WWTPs (Loos et al., 2013) and even within the concentration range identified in hospital (100-1000 ng/L) (Beier et al., 2010) and municipal (635.5-1322.5 ng/L) (Gurke et al., 2015) wastewaters in Germany. Advanced effluent treatment by ozonation significantly reduced the telmisartan concentration up to 85 ng/L (86% reduction) (Szabová et al., 2020). However, this concentration was still recognized as being hazardous to fish. The knowledge about other possible adverse effects to aquatic organisms resulted in telmisartan exposure is rare.

Two anti-histaminic drugs, ranitidine, and diphenhydramine appeared in conventionally treated effluents in acutely toxic concentrations to a zebrafish *Danio rerio*. Thus, the effluent of the WWTP_1 discharged 149 ng/L ranitidine and 107 ng/L diphenhydramine. In the effluent of the WWTP_2_-O3, 264 ng/L ranitidine and 156 ng/L diphenhydramine were detected. Similar ranitidine concentration was found in one hospital effluent in Italy (240-2200 ng/L) (Verlicchi et al., 2012). The diphenhydramine concentration was in line with the effluent concentration in three tested Indian WWTPs (Anumol et al., 2016). The neuroleptic drug clozapine and the lipid blocker atorvastatin were detected in both conventionally treated effluents in similar concentrations of 26 ng/L and 27 ng/L in the effluent of the WWTP_1 and 86 ng/L and 28 ng/L in the effluent of the WWTP_2, respectively. These concentrations exceeded the no-effect concentration for zebrafish *Danio rerio*. Concentrations of clozapine and atorvastatin were comparable with those in secondary effluents of municipal WWTPs (Verlicchi et al., 2012; Yuan et al., 2013). Another lipid blocker, gemfibrozil, was identified in the secondary effluent of the WWTP_2 only in a concentration of 68 ng/L exceeding the no-effect concentration to a zebrafish *Danio rerio* (Verlicchi et al., 2012). The diuretic drug furosemide was identified in the conventionally treated effluents in concentrations of 90 ng/L (WWTP_1) and 197 ng/L (WWTP_2) recognized as potentially adverse to a zebrafish *Danio rerio* (Verlicchi et al., 2012).

The non-steroidal anti-inflammatory drug and a prominent wastewater benchmark substance diclofenac had the highest concentration in the tested effluents of 1.5 µg/L in the effluent WWTP_1 and 1.8 µg/L in the effluent WWTP_2_-O3, respectively. Detected diclofenac concentrations were in good agreement with the other measurements in effluents around Germany (0.4 - 3.3 µg/L) (Letzel et al., 2009; Meyer et al., 2016; Stülten et al., 2008). The maximal annual load of diclofenac in the river Rhine was assessed for almost 10 t/y (Sacher et al., 2008). Ozonation ensured nearly complete elimination of diclofenac (99.8%).

High concentrations of artificial sweeteners were detected in all conventionally treated effluent samples. Thus, the effluent of the WWTP_1 contained 6 µg/L of acesulfame, 7 µg/L of sucralose, and 296 ng/L of saccharin. In contrast, the effluent of the WWTP_2_-O3 contained a significantly lower concentration on acesulfame (164 ng/L) and a simultaneously higher concentration on sucralose (47 µg/L). No saccharin was detected in the effluent of the WWTP_2_-O3. The ozonation treatment of the WWTP_2 effluent was efficient for reducing 40 % acesulfame (Zhou et al., 2021) and 19 % sucralose (Hollender et al., 2009). According to the literature, artificial sweeteners have low or no adverse effects on aquatic organisms in very high concentrations of mg/L (Stolte et al., 2013). For example, sucralose had no chronic adverse effects on survival, reproduction, and growth of water flea *Daphnia magna* and mysid shrimp *Americamysis bahia* at concentrations of 1800 mg/L and 93 mg/L, respectively (Huggett and Stoddard, 2011). Acesulfame was shown to have very low toxicity. However, its transformation products seem to be more toxic (still in mg/L) to zebrafish embryos *Danio rerio* (Li et al., 2016) and goldfish *Carassius auratus* (Ren et al., 2016). The results from the current study showed that concentrations of artificial sweeteners in effluents might vary enormously. Also, comparable studies showed different results but with one agreement of ubiquitous occurrence of artificial sweeteners in WWTP effluents and surface waters in ng/L-µg/L concentrations underlying their high environmental relevance (Buerge et al., 2010; Loos et al., 2009; Scheurer et al., 2009; Seitz and Winzenbacher, 2017).

The bitterest agent denatonium (Bitrex®) was found in concentrations of 225 ng/L, being in line with the study by Lege et al. (2017). Denatonium is a frequent additive in personal care and household products, and denatured alcohol protects against poisoning by swallowing (Lege et al., 2017). Denatonium was successfully reduced by

ozonation (72% removal, 85 ng/L), confirming the study by Lege et al. (2019). It recorded the denatonium reduction rate by ozonation of 74% and the formation of unknown highly polar transformation products (Lege et al., 2019). The knowledge about the possible toxic effects of denatonium and its transformation products to aquatic organisms is very rare.

The UV-filter phenyl benzimidazole sulfonic acid occurred in conventionally treated effluents in high concentrations of 10 µg/L (WWTP_1) and 6.3 µg/L (WWTP_2_-O3), signalizing potential lethal toxicity to crustacea. The elimination rate of phenyl benzimidazole sulfonic acid accounted for 76.5%.

Emission of micropollutants by WWTPs

The WWTP_2 annually treats an 8-fold greater amount of wastewater than the WWTP_1 (31.8 Mio. m^3/y vs. 4.1 Mio. m^3/y). However, this rule could not be applied to the emission of water-hazardous substances. Thus, the emission difference was three-fold (~68 µg/L in the effluent of the WWTP_2_-O3 vs. ~22 µg/L in the effluent of the WWTP_1). The WWTP_2 collects wastewater in a middle-sized town with a high rate of young people (due to students) and innovative commercial enterprises. It achieves mineral oil containing wastewater from several small companies.

Additionally, five hospitals discharge their wastewater into the municipal canalization of the WWTP_2. The WWTP_1 collects wastewater from several small industries. Thus, a local waste disposal service provider produces pre-treated mineral oil-containing wastewater (ELWAS-WEB, 2020). Furthermore, two small industrial productions of paper tubes and laundry detergents release their wastewater directly into the WWTP_1. The current study showed that WWTP effluents contribute to a mixture of chemical compounds in the receiving water. Indeed, the study receiving waterbody had insufficient ecological status. However, due to severe structural changes in the receiving waterbody, the mixture toxicity alone could not be responsible for the current ecological status.

The effluent treatment upgrade by ozonation was sufficient to reduce 41.2% of studied chemical compounds. The total chemical burden discharging into the receiving water was, therefore, reduced by 2.1 kg/y.

From all quantified chemical compounds, 15 were prioritized as being potentially hazardous to aquatic organisms. The most exceedance of species-specific TU was

observed for a zebrafish *Danio rerio* due to its popularity as a model organism (Briggs, 2002; Lessman, 2011; Segner, 2009). For further conclusions on using a species-specific TU approach, the data gaps should be closed.

Water-hazard substances appeared in the effluent in ng/L-concentration, reaching lower concentrations in the environment due to the dilution effect. However, emitted water hazard substances continuously contribute to a permanent chronic exposure of aquatic organisms with a mixture of known hazard substances.

Conclusion

Currently, no wastewater ecotoxicological assessment strategy is available. Our study underlined the necessity of a combined approach comprising chemical analysis and bioassays for WWTPs effluent. However, available studies showed that effluents have a complex ecotoxicological profile. Even very low concentrations of micropollutants can still cause adverse effects in bioassays.

The authors are aware that a single target analysis is not suitable for a reliable creation of emission patterns for the studied WWTPs. However, such a comprehensive target analysis has not been implemented to the studied WWTPs before. The studied receiving water serves as a model and study river for multiple scientific institutions. Therefore, the current study provides precious knowledge about the emission of chemical compounds in the studied receiving water, supporting further scientific initiatives in water management and aquatic ecotoxicology. Moreover, the current study results should arouse interest for small waterbodies representing the most numerous groups of freshwater ecosystems worldwide.

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Supplementary Information

Title: Hazard evaluation of WWTPs effluents by a comprehensive target-screening: Valuable insights but also a big toxicological data gap

Contributing authors: Aliaksandra Shuliakevich[a], Saskia Finckh[c], Ira Brückner[d], Volker Linnemann[e], Regina Dolny[e], Jörg Ahlheim[c], Martin Krauss[c], Tobias Schulze[c], Sabrina Schiwy[a], Werner Brack[a,c], Henner Hollert[a]

a: Goethe University Frankfurt/Main, Department Evolutionary Ecology and Environmental Toxicology, Max-von-Laue-Strasse 13, 60438 Frankfurt/Main, Germany

b: RWTH Aachen University, Institute of Biology V, Worringerweg 1, 52074 Aachen, Germany

c: Helmholtz Centre for Environmental Research – UFZ, Department of Effect-Directed Analysis, Permoserstrasse 15, 04318 Leipzig, Germany

d: Eifel-Rur Waterboard (WVER), Eisenbahnstr. 5, 52354 Düren, Germany

e: RWTH Aachen University, Institute of Environmental Engineering, Krefelder Str. 299, 52070 Aachen

S1

Table S1: Analysed compounds for quantitative LC-HRMS screening analysis (Finckh et al. 2022a; Finckh et al. 2022b, under review).

Name	CAS No.	LC-HRMS mode	m/z	Retention time [min]	Formula	Ion	Primary or secondary
Naproxen	22204-53-1	ESI_pos	231.1016	11	C14H14O3	M+H	primary
Phenazone	60-80-0	ESI_pos	189.1022	6.14	C11H12N2O	M+H	primary
Dichlorvos	62-73-7	ESI_pos	220.9532	8.62	C4H7Cl2O4P	M+H	primary
Azinphos methyl	86-50-0	ESI_pos	318.013	10.87	C10H12N3O3PS2	M+H	primary
2,4-Dichlorophenoxyacetic acid	94-75-7	ESI_neg	218.9621	10.26	C8H6Cl2O3	M-H	primary
Clofibric acid	882-09-7	ESI_neg	213.0324	10.71	C10H11ClO3	M-H	primary
Bezafibrate	41859-67-0	ESI_neg	360.1008	11.46	C19H20ClNO4	M-H	primary
Monensin	17090-79-8	ESI_pos	693.419	14.95	C36H62O11	M+Na	primary
Clotrimazole	23593-75-1	ESI_pos	345.1153	10.06	C22H17ClN2	M+H	primary
Propyphenazone	479-92-5	ESI_pos	231.1492	9.73	C14H18N2O	M+H	primary
Dimethoate	60-51-5	ESI_pos	230.0069	6.05	C5H12NO3PS2	M+H	primary
Metoprolol	51384-51-1	ESI_pos	268.1907	6	C15H25NO3	M+H	primary
Bentazone	25057-89-0	ESI_neg	239.0496	9.42	C10H12N2O3S	M-H	primary
Diuron	330-54-1	ESI_pos	233.0243	10.18	C9H10Cl2N2O	M+H	primary
Isoproturon	34123-59-6	ESI_pos	207.1492	10.14	C12H18N2O	M+H	primary
Chloramphenicol	56-75-7	ESI_neg	321.0051	6.89	C11H12Cl2N2O5	M-H	primary
4-Nitrophenol	100-02-7	ESI_neg	138.0197	5.14	C6H5NO3	M-H	primary
Erythromycin	114-07-8	ESI_pos	734.4685	9.95	C37H67NO13	M+H	primary
Atenolol	29122-68-7	ESI_pos	267.1703	0.7	C14H22N2O3	M+H	primary
Cyclophosphamide	50-18-0	ESI_pos	261.0321	7.7	C7H15Cl2N2O2P	M+H	primary
Gemfibrozil	25812-30-0	ESI_pos	251.1642	13.05	C15H22O3	M+H	primary
Diazinon	333-41-5	ESI_pos	305.1083	12.59	C12H21N2O3PS	M+H	primary
Furosemide	54-31-9	ESI_neg	329.0004	9.2	C12H11ClN2O5S	M-H	primary
Perfluorodecanoic acid	335-76-2	ESI_neg	512.96	17	C10HF19O2	M-H	primary
Chlorotoluron	15545-48-9	ESI_pos	213.0789	9.73	C10H13ClN2O	M+H	primary
Paroxetine	61869-08-7	ESI_pos	330.15	9.14	C19H20FNO3	M+H	primary
Propranolol	525-66-6	ESI_pos	260.1645	7.83	C16H21NO2	M+H	primary
Verapamil	52-53-9	ESI_pos	455.2904	9.27	C27H38N2O4	M+H	primary
Pravastatin	81093-37-0	ESI_neg	423.2388	10.87	C23H36O7	M-H	primary
Myclobutanil	88671-89-0	ESI_pos	289.1215	11.56	C15H17ClN4	M+H	primary
Iminostilbene	256-96-2	ESI_pos	194.0964	11.96	C14H11N	M+H	primary
2-Aminobenzimidazole	934-32-7	ESI_pos	134.0713	0.7	C7H7N3	M+H	primary
Isophorone diamine	2855-13-2	ESI_pos	171.1856	0.7	C10H22N2	M+H	primary
3,3'-Dichlorobenzidine	91-94-1	ESI_pos	253.0294	10.79	C12H10Cl2N2	M+H	primary
N,N-Dimethyl-p-phenylenediamine	99-98-9	ESI_pos	137.1073	0.7	C8H12N2	M+H	primary
4'-Aminoacetanilide	122-80-5	ESI_pos	151.0866	0.7	C8H10N2O	M+H	primary
N,N-Dimethyldodecylamine N-oxide	1643-20-5	ESI_pos	230.2478	11.18	C14H31NO	M+H	primary
Triethylcitrate	77-93-0	ESI_pos	277.1282	9.2	C12H20O7	M+H	primary
Piperonyl butoxide	51-03-6	ESI_pos	356.2431	13.65	C19H30O5	M+NH4	primary
Imazalil	35554-44-0	ESI_pos	297.0556	8.9	C14H14Cl2N2O	M+H	primary
Fipronil	120068-37-3	ESI_neg	434.9314	12.32	C12H4Cl2F6N4OS	M-H	primary
Diflufenican	83164-33-4	ESI_pos	395.0813	13.25	C19H11F5N2O2	M+H	primary
Flusilazole	85509-19-9	ESI_pos	316.1076	12.2	C16H15F2N3Si	M+H	primary
Trifloxystrobin	141517-21-7	ESI_pos	409.137	13.25	C20H19F3N2O4	M+H	primary
Sulcotrione	99105-77-8	ESI_pos	329.0245	9.14	C14H13ClO5S	M+H	primary
Propoxycarbazone	145026-81-9	ESI_pos	399.0969	10.23	C15H18N4O7S	M+H	primary
4-Amino-N,N-dimethylbenzenesulfonamide	1709-59-7	ESI_pos	201.0692	4.24	C8H12N2O2S	M+H	primary
4-Aminobenzamide	2835-68-9	ESI_pos	137.0709	0.7	C7H8N2O	M+H	primary
2-Methylbenzothiazole	120-75-2	ESI_pos	150.0372	9.42	C8H7NS	M+H	primary
N-Acetyl-4-aminoantipyrine	83-15-8	ESI_pos	246.1237	4	C13H15N3O2	M+H	primary
4-Aminoantipyrine	83-07-8	ESI_pos	204.1131	1.1	C11H13N3O	M+H	primary
Benzophenone-3	131-57-7	ESI_pos	229.0859	12.06	C14H12O3	M+H	primary
Phenylbenzimidazole sulfonic acid	27503-81-7	ESI_neg	273.0339	4.1	C13H10N2O3S	M-H	primary
Thiabendazole	148-79-8	ESI_pos	202.0433	2.85	C10H7N3S	M+H	primary
Hydrochlorothiazide	58-93-5	ESI_neg	295.9572	1.42	C7H8ClN3O4S2	M-H	primary
N-isopropyl-N'-phenyl-p-phenylenediamine	101-72-4	ESI_pos	227.1543	7.6	C15H18N2	M+H	primary
Metribuzin	21087-64-9	ESI_pos	215.0961	8.49	C8H14N4OS	M+H	primary
Prochloraz	67747-09-5	ESI_pos	376.0381	12.02	C15H16Cl3N3O2	M+H	primary
Dimethenamid	87674-68-8	ESI_pos	276.082	11.07	C12H18ClNO2S	M+H	primary
Hexazinone	51235-04-2	ESI_pos	253.1659	8.99	C12H20N4O2	M+H	primary
Carbetamide	16118-49-3	ESI_pos	237.1234	8.46	C12H16N2O3	M+H	primary
Metalaxyl	57837-19-1	ESI_pos	280.1543	10.39	C15H21NO4	M+H	primary
Cyproconazole	94361-06-5	ESI_pos	292.1211	11.38	C15H18ClN3O	M+H	primary
Cyprodinil	121552-61-2	ESI_pos	226.1339	10.61	C14H15N3	M+H	primary
Dichlorprop	120-36-5	ESI_neg	232.9778	11.19	C9H8Cl2O3	M-H	primary
Imidacloprid	138261-41-3	ESI_pos	256.0596	6.33	C9H10ClN5O2	M+H	primary
Dimethachlor	50563-36-5	ESI_pos	256.1099	10.46	C13H18ClNO2	M+H	primary

Table S1: Continuation (1).

Dinoseb	88-85-7	ESI_neg	239.0673	12.46	C10H12N2O5	M-H	primary
Nicosulfuron	111991-09-4	ESI_pos	411.1081	9.33	C15H18N6O6S	M+H	primary
Flufenacet	142459-58-3	ESI_pos	364.0737	11.95	C14H13F4N3O2S	M+H	primary
Propachlor	1918-16-7	ESI_pos	212.0837	10.16	C11H14ClNO	M+H	primary
Prosulfocarb	52888-80-9	ESI_pos	252.1417	13.15	C14H21NOS	M+H	primary
Kresoxim-methyl	143390-89-0	ESI_pos	314.1387	12.38	C18H19NO4	M+H	primary
Pethoxamid	106700-29-2	ESI_pos	296.1412	11.9	C16H22ClNO2	M+H	primary
Ethofumesate	26225-79-6	ESI_pos	287.0948	11.11	C13H18O5S	M+H	primary
Metamitron	41394-05-2	ESI_pos	203.0927	5.7	C10H10N4O	M+H	primary
Chloridazon	1698-60-8	ESI_pos	222.0429	6.59	C10H8ClN3O	M+H	primary
Spiroxamine	118134-30-8	ESI_pos	298.2741	9.8	C18H35NO2	M+H	primary
Pyrazophos	13457-18-6	ESI_pos	374.0934	12.88	C14H20N3O5PS	M+H	primary
Simetryn	1014-70-6	ESI_pos	214.1121	7.82	C8H15N5S	M+H	primary
Azoxystrobin	131860-33-8	ESI_pos	404.1241	11.38	C22H17N3O5	M+H	primary
Fenpropimorph	67564-91-4	ESI_pos	304.2635	9.89	C20H33NO	M+H	primary
Epoxiconazole	133855-98-8	ESI_pos	330.0804	12	C17H13ClFN3O	M+H	primary
Bendiocarb	22781-23-3	ESI_pos	224.0917	9.06	C11H13NO4	M+H	primary
N-Butylbenzenesulfonamide	3622-84-2	ESI_pos	214.0896	9.81	C10H15NO2S	M+H	primary
Diazepam	439-14-5	ESI_pos	285.0789	11.05	C16H13ClN2O	M+H	primary
Tris(2-chloroethyl)phosphate	115-96-8	ESI_pos	284.9612	9.1	C6H12Cl3O4P	M+H	primary
Clarithromycin	81103-11-9	ESI_pos	748.4842	10.48	C38H69NO13	M+H	primary
Desethylatrazine	6190-65-4	ESI_pos	188.0697	6.75	C6H10ClN5	M+H	primary
Ketoprofen	22071-15-4	ESI_pos	255.1016	10.85	C16H14O3	M+H	primary
Sulfamethoxazole	723-46-6	ESI_pos	254.0594	5.9	C10H11N3O3S	M+H	primary
Sulfapyridine	144-83-2	ESI_pos	250.0645	2.1	C11H11N3O2S	M+H	primary
Desethylterbutylazine	30125-63-4	ESI_pos	202.0854	9.25	C7H12ClN5	M+H	primary
Ethion	563-12-2	ESI_pos	384.9949	13.85	C9H22O4P2S4	M+H	primary
Pirimiphos-methyl	29232-93-7	ESI_pos	306.1036	12.37	C11H20N3O3PS	M+H	primary
Ethyl azinphos	2642-71-9	ESI_pos	346.0443	11.96	C12H16N3O3PS2	M+H	primary
Desisopropylatrazine	1007-28-9	ESI_pos	174.0541	3.6	C5H8ClN5	M+H	primary
Perfluorooctanoic acid	335-67-1	ESI_neg	412.9664	14.8	C8HF15O2	M-H	primary
Caffeine	58-08-2	ESI_pos	195.0877	4.83	C8H10N4O2	M+H	primary
Octyl-methoxycinnamate	5466-77-3	ESI_pos	291.1955	14.75	C18H26O3	M+H	primary
2-(2-(Chlorophenyl)amino)benzaldehyde	71758-44-6	ESI_pos	232.0524	12.63	C13H10ClNO	M+H	primary
Carbendazim	10605-21-7	ESI_pos	192.0768	1.5	C9H9N3O2	M+H	primary
Acetaminophen	103-90-2	ESI_pos	152.0706	1.35	C8H9NO2	M+H	primary
Acesulfame	33665-90-6	ESI_neg	161.9867	1.28	C4H5NO4S	M-H	primary
Flutamide	13311-84-7	ESI_neg	275.0649	11.29	C11H11F3N2O3	M-H	primary
1,2-Benzisothiazolinone	2634-33-5	ESI_pos	152.0165	5.33	C7H5NOS	M+H	primary
Linuron	330-55-2	ESI_pos	249.0192	10.87	C9H10Cl2N2O2	M+H	primary
Terbuthylazine	5915-41-3	ESI_pos	230.1167	11.15	C9H16ClN5	M+H	primary
Terbutryn	886-50-0	ESI_pos	242.1434	10.04	C10H19N5S	M+H	primary
Carbamazepine	298-46-4	ESI_pos	237.1022	9.69	C15H12N2O	M+H	primary
Sucralose	56038-13-2	ESI_neg	441.0123	5	C12H19Cl3O8	M-H+FA	primary
Triclosan	3380-34-5	ESI_neg	286.9439	13.16	C12H7Cl3O2	M-H	primary
Triphenylphosphate	115-86-6	ESI_pos	327.0781	12.79	C18H15O4P	M+H	primary
Saccharin	81-07-2	ESI_neg	181.9917	2.63	C7H5NO3S	M-H	primary
Perfluorooctanesulfonic acid	1763-23-1	ESI_neg	498.9302	15.5	C8HF17O3S	M-H	primary
Cyclamate	100-88-9	ESI_neg	178.0543	3	C6H13NO3S	M-H	primary
2-Octyl-4-isothiazolin-3-one	26530-20-1	ESI_pos	214.126	11.83	C11H19NOS	M+H	primary
DEET	134-62-3	ESI_pos	192.1383	10.16	C12H17NO	M+H	primary
Atrazine	1912-24-9	ESI_pos	216.101	9.98	C8H14ClN5	M+H	primary
Irgarol	28159-98-0	ESI_pos	254.1434	10.47	C11H19N5S	M+H	primary
1H-Benzotriazole	95-14-7	ESI_pos	120.0556	3.15	C6H5N3	M+H	primary
Propiconazole	60207-90-1	ESI_pos	342.0771	12.49	C15H17Cl2N3O2	M+H	primary
Diclofenac	15307-86-5	ESI_pos	296.024	12.29	C14H11Cl2NO2	M+H	primary
Mefenamic acid	61-68-7	ESI_neg	240.103	12.99	C15H15NO2	M-H	primary
Metazachlor	67129-08-2	ESI_pos	278.1055	10.27	C14H16ClN3O	M+H	primary
N-Phenyl-1-naphthylamine	90-30-2	ESI_pos	220.1121	12.85	C16H13N	M+H	primary
Chlorfenvinphos	470-90-6	ESI_pos	358.9768	12.6	C12H14Cl3O4P	M+H	primary
Pirimicarb	23103-98-2	ESI_pos	239.1503	6.65	C11H18N4O2	M+H	primary
Perfluorohexanoic acid	307-24-4	ESI_neg	312.9728	13.14	C6HF11O2	M-H	primary
Metolachlor	51218-45-2	ESI_pos	284.1412	11.94	C15H22ClNO2	M+H	primary
Simazine	122-34-9	ESI_pos	202.0854	8.85	C7H12ClN5	M+H	primary
Primidone	125-33-7	ESI_pos	219.1128	9.45	C12H14N2O2	M+H	primary
Mecoprop	93-65-2	ESI_neg	213.0324	11.24	C10H11ClO3	M-H	primary
Enrofloxacin	93106-60-6	ESI_pos	360.1718	6.57	C19H22FN3O3	M+H	primary
Roxithromycin	80214-83-1	ESI_pos	837.5318	10.72	C41H76N2O15	M+H	primary
Ranitidine	66357-35-5	ESI_pos	315.1485	0.7	C13H22N4O3S	M+H	primary
Warfarin	81-81-2	ESI_pos	309.1121	11.35	C19H16O4	M+H	primary

Table S1: Continuation (2).

(3-Chloro-2-hydroxypropyl)trimethylammonium	82914-58-7	ESI_pos	152.0837	0.7	C6H15ClNO	M+	primary
2-Isopropylthioxanthone	5495-84-1	ESI_pos	255.0838	13.65	C16H14OS	M+H	primary
MCPA	94-74-6	ESI_neg	199.0167	10.41	C9H9ClO3	M-H	primary
4-Methylbenzylidene camphor	36861-47-9	ESI_pos	255.1743	13.65	C18H22O	M+H	primary
5-Methyl-1H-benzotriazole	136-85-6	ESI_pos	134.0713	7	C7H7N3	M+H	primary
Benzethonium	10172-60-8	ESI_pos	412.321	12.1	C27H42NO2	M+	primary
Benzophenone-4	4065-45-6	ESI_neg	307.0282	10.1	C14H12O6S	M-H	primary
Benzyldimethyldodecylammonium	10328-35-5	ESI_pos	304.2999	11.79	C21H38N	M+	primary
Benzyldimethylhexadecylammonium	10328-34-4	ESI_pos	360.3625	13.15	C25H46N	M+	primary
Hexadecyltrimethylammonium	01-10-1999	ESI_pos	284.3312	12.65	C19H42N	M+	primary
Chlorophene	120-32-1	ESI_neg	217.0426	12.31	C13H11ClO	M-H	primary
Chlorothalonil-4-hydroxy	28343-61-5	ESI_neg	244.9082	12.78	C8HCl3N2O	M-H	primary
Didecyldimethylammonium	20256-56-8	ESI_pos	326.3781	12.93	C22H48N	M+	primary
Ethyl 4-(dimethylamino)benzoate	10287-53-3	ESI_pos	194.1176	11.31	C11H15NO2	M+H	primary
Hexadecylpyridinium	7773-52-6	ESI_pos	304.2999	12.9	C21H38N	M+	primary
3-Iodopropynyl butylcarbamate	55406-53-6	ESI_pos	281.9985	10.18	C8H12INO2	M+H	primary
Lauryl diethanolamide	120-40-1	ESI_pos	288.2533	12.95	C16H33NO3	M+H	primary
p-Toluenesulfonamide	70-55-3	ESI_pos	189.0692	3.95	C7H9NO2S	M+NH4	primary
Tebuconazole	107534-96-3	ESI_pos	308.1524	12.35	C16H22ClN3O	M+H	primary
Thiacloprid	111988-49-9	ESI_pos	253.0309	7.89	C10H9ClN4S	M+H	primary
Tri(butoxyethyl)phosphate	78-51-3	ESI_pos	399.2506	13.45	C18H39O7P	M+H	primary
Triclocarban	101-20-2	ESI_neg	314.9681	13.17	C13H9Cl3N2O	M-H	primary
Tri-isobutylphosphate	126-71-6	ESI_pos	267.172	13.05	C12H27O4P	M+H	primary
Trimethyloctylammonium	15461-38-8	ESI_pos	172.206	7	C11H26N	M+	primary
TDCPP	13674-87-8	ESI_pos	428.8912	12.59	C9H15Cl6O4P	M+H	primary
Tris(1-chloro-2-propyl)phosphate	13674-84-5	ESI_pos	327.0081	11.42	C9H18Cl3O4P	M+H	primary
Metformin	657-24-9	ESI_pos	130.1087	0.7	C4H11N5	M+H	primary
Lauric isopropanolamide	142-54-1	ESI_pos	258.2428	13.25	C15H31NO2	M+H	primary
Hexa(methoxymethyl)melamine	3089-11-0	ESI_pos	391.23	10	C15H30N6O6	M+H	primary
Pindolol	13523-86-9	ESI_pos	249.1598	1.8	C14H20N2O2	M+H	primary
Fenuron	101-42-8	ESI_pos	165.1022	5.41	C9H12N2O	M+H	primary
Chloroxuron	1982-47-4	ESI_pos	291.0895	11.66	C15H15ClN2O2	M+H	primary
Lenacil	01-08-1964	ESI_pos	235.1441	10.17	C13H18N2O2	M+H	primary
Ametryn	834-12-8	ESI_pos	228.1277	8.94	C9H17N5S	M+H	primary
Pentobarbital	76-74-4	ESI_neg	225.1245	9.53	C11H18N2O3	M-H	primary
Sulfamethazine	57-68-1	ESI_pos	279.091	4.91	C12H14N4O2S	M+H	primary
Ketoconazole	65277-42-1	ESI_pos	531.156	9.79	C26H28Cl2N4O4	M+H	primary
2-Isopropyl-6-methyl-pyrimidin-4-ol	2814-20-2	ESI_pos	153.1022	1.8	C8H12N2O	M+H	primary
o-Dianisidine	119-90-4	ESI_pos	245.1285	1.5	C14H16N2O2	M+H	primary
Diphenylphosphate	838-85-7	ESI_pos	251.0468	10.95	C12H11O4P	M+H	primary
N-Ethyl-o-toluenesulfonamide	1077-56-1	ESI_pos	200.074	8.7	C9H13NO2S	M+H	primary
2-(Methylthio)benzothiazole	615-22-5	ESI_pos	182.0093	10.95	C8H7NS2	M+H	primary
Triglyme	112-49-2	ESI_pos	196.1542	3.7	C8H18O4	M+NH4	primary
Methylchloroisothiazolinone	26172-55-4	ESI_pos	149.9775	2.27	C4H4ClNOS	M+H	primary
4-Hydroxybenzotriazole	26725-51-9	ESI_pos	136.0505	1.8	C6H5N3O	M+H	primary
Aniline Yellow	no	ESI_pos	198.1026	10.72	C12H11N3	M+H	primary
4,4'-Methylene-bis(2-methyl aniline)	838-88-0	ESI_pos	227.1543	1.5	C15H18N2	M+H	primary
4,4'-Thiodianiline	139-65-1	ESI_pos	217.0794	3.25	C12H12N2S	M+H	primary
Tetrabromobisphenol A	79-94-7	ESI_neg	538.7498	13.36	C15H12Br4O2	M-H	primary
Perfluorobutanoic acid	375-22-4	ESI_neg	212.9792	7.05	C4HF7O2	M-H	primary
Michler's ketone	90-94-8	ESI_pos	269.1648	12.1	C17H20N2O	M+H	primary
Tetrachlorosalicylanilide	1154-59-2	ESI_neg	347.9158	14.42	C13H7Cl4NO2	M-H	primary
Benzothiazole	95-16-9	ESI_pos	136.0215	7.93	C7H5NS	M+H	primary
Desphenyl chloridazon	6339-19-1	ESI_pos	146.0116	0.7	C4H4ClN3O	M+H	primary
2,6-Dichlorobenzamide	2008-58-4	ESI_pos	189.9821	2.34	C7H5Cl2NO	M+H	primary
3,4-Dichlorophenylurea	08-02-2027	ESI_pos	204.993	9.34	C7H6Cl2N2O	M+H	primary
4-Isopropylaniline	99-88-7	ESI_pos	136.1121	3.15	C9H13N	M+H	primary
Metolachlor OA	152019-73-3	ESI_neg	278.1398	11.43	C15H21NO4	M-H	primary
Metolachlor ESA	171118-09-5	ESI_neg	328.1224	11.79	C15H23NO5S	M-H	primary
2,4-Dinitrophenol	51-28-5	ESI_neg	183.0047	6.36	C6H4N2O5	M-H	primary

Table S1: Continuation (3).

Lidocaine	137-58-6	ESI_pos	235.1805	1.75	C14H22N2O	M+H	primary
Tramadol	27203-92-5	ESI_pos	264.1958	5.4	C16H25NO2	M+H	primary
Mycophenolic acid	24280-93-1	ESI_pos	321.1333	10.86	C17H20O6	M+H	primary
Capecitabine	154361-50-9	ESI_pos	360.1565	9.58	C15H22FN3O6	M+H	primary
Dimethylaminophenazone	58-15-1	ESI_pos	232.1444	1.1	C13H17N3O	M+H	primary
Methotrexate	59-05-2	ESI_pos	455.1786	5.92	C20H22N8O5	M+H	primary
7-Hydroxymethotrexate	5939-37-7	ESI_pos	471.1735	7.42	C20H22N8O6	M+H	primary
Icaridin	119515-38-7	ESI_pos	230.1751	10.65	C12H23NO3	M+H	primary
Cotinine	486-56-6	ESI_pos	177.1022	0.7	C10H12N2O	M+H	primary
2-Benzothiazolesulfonic acid	941-57-1	ESI_neg	213.9638	6.71	C7H5NO3S2	M-H	primary
Lincomycin	154-21-2	ESI_pos	407.221	1.4	C18H34N2O6S	M+H	primary
Trimethoprim	738-70-5	ESI_pos	291.1452	2.75	C14H18N4O3	M+H	primary
4-Formyl-antipyrine	950-81-2	ESI_pos	217.0972	4.23	C12H12N2O2	M+H	primary
Crotamiton	483-63-6	ESI_pos	204.1383	11.07	C13H17NO	M+H	primary
10,11-Dihydro-10,11-dihydroxycarbamazepine	35079-97-1	ESI_pos	271.1077	8.05	C15H14N2O3	M+H	primary
Quinoxyfen	124495-18-7	ESI_pos	308.004	13.55	C15H8Cl2FNO	M+H	primary
Cetirizine	83881-51-0	ESI_pos	389.1626	10.18	C21H25ClN2O3	M+H	primary
2-Hydroxycarbamazepine	68011-66-5	ESI_pos	253.0972	8.54	C15H12N2O2	M+H	primary
10,11-Dihydro-10-hydroxycarbamazepine	29331-92-8	ESI_pos	255.1128	8.32	C15H14N2O2	M+H	primary
Amantadine	768-94-5	ESI_pos	152.1434	1.8	C10H17N	M+H	primary
Benzenesulfonic acid	98-11-3	ESI_neg	156.9965	1.34	C6H6O3S	M-H	primary
Clomazone	81777-89-1	ESI_pos	240.0786	10.7	C12H14ClNO2	M+H	primary
Methiocarb	2032-65-7	ESI_pos	226.0896	11.04	C11H15NO2S	M+H	primary
3,5,6-Trichloro-2-pyridinol	6515-38-4	ESI_neg	195.9129	9.83	C5H2Cl3NO	M-H	primary
Pendimethalin	40487-42-1	ESI_pos	282.1448	13.85	C13H19N3O4	M+H	primary
Boscalid	188425-85-6	ESI_pos	343.0399	11.36	C18H12Cl2N2O	M+H	primary
Prothioconazole-desthio	120983-64-4	ESI_pos	312.0665	11.9	C14H15Cl2N3O	M+H	primary
Sotalol	3930-20-9	ESI_pos	273.1267	0.7	C12H20N2O3S	M+H	primary
N-Formyl-4-aminoantipyrine	1672-58-8	ESI_pos	232.1081	3.35	C12H13N3O2	M+H	primary
2,4-Dichlorophenol	120-83-2	ESI_neg	160.9566	10.25	C6H4Cl2O	M-H	primary
Genistein	446-72-0	ESI_pos	271.0601	10.01	C15H10O5	M+H	primary
Daidzein	486-66-8	ESI_pos	255.0652	9.34	C15H10O4	M+H	primary
Amidosulfobetaine-14	216667-08-2	ESI_pos	435.3251	13.35	C22H47N2O4S	M+	primary
Bisphenol S	80-09-1	ESI_neg	249.0227	7.6	C12H10O4S	M-H	primary
Flurtamone	96525-23-4	ESI_pos	334.1049	11.27	C18H14F3NO2	M+H	primary
Picolinafen	137641-05-5	ESI_pos	377.0908	13.71	C19H12F4N2O2	M+H	primary
Finasteride	98319-26-7	ESI_pos	373.285	11.93	C23H36N2O2	M+H	primary
Diphenhydramine	58-73-1	ESI_pos	256.1696	8	C17H21NO	M+H	primary
Acetyl-sulfamethoxazole	21312-10-7	ESI_pos	296.07	7.73	C12H13N3O4S	M+H	primary
Citalopram	59729-33-8	ESI_pos	325.1711	8.22	C20H21FN2O	M+H	primary
Secobarbital	76-73-3	ESI_neg	237.1245	10.02	C12H18N2O3	M-H	primary
Lauramidopropylbetaine	08-10-1992	ESI_pos	343.2955	12.44	C19H39N2O3	M+	primary
4-Chloroaniline	106-47-8	ESI_pos	128.0262	1.45	C6H5ClO	M+H	primary
Bromoxynil	1689-84-5	ESI_neg	273.8509	9.52	C7H3Br2NO	M-H	primary
Amiodarone	1951-25-3	ESI_pos	646.031	12.2	C25H29I2NO3	M+H	primary
Amitriptyline	50-48-6	ESI_pos	278.1903	9.31	C20H23N	M+H	primary
Atorvastatin	134523-00-5	ESI_neg	557.2457	12.38	C33H35FN2O5	M-H	primary
Azelastine	58581-89-8	ESI_pos	382.1681	9.4	C22H24ClN3O	M+H	primary
Azithromycin	83905-01-5	ESI_pos	749.5158	8.15	C38H72N2O12	M+H	primary
Bisoprolol	66722-44-9	ESI_pos	326.2326	7.67	C18H31NO4	M+H	primary
Bupropion	34911-55-2	ESI_pos	240.115	6.6	C13H18ClNO	M+H	primary
Desloratadine	100643-71-8	ESI_pos	311.131	7.23	C19H19ClN2	M+H	primary
Duloxetine	116539-59-4	ESI_pos	298.126	9.24	C18H19NOS	M+H	primary
EDDP	30223-73-5	ESI_pos	278.1903	8.43	C20H23N	M+H	primary
Fluconazole	86386-73-4	ESI_pos	307.1113	7.16	C13H12F2N6O	M+H	primary
Glibenclamide	10238-21-8	ESI_pos	494.1511	12.24	C23H28ClN3O5S	M+H	primary
Glimepiride	93479-97-1	ESI_neg	489.2177	12.54	C24H34N4O5S	M-H	primary
Ketamine	6740-88-1	ESI_pos	238.0993	3.6	C13H16ClNO	M+H	primary
Loperamide	53179-11-6	ESI_pos	477.2303	10.3	C29H33ClN2O2	M+H	primary
Lorazepam	846-49-1	ESI_pos	321.0192	10.4	C15H10Cl2N2O2	M+H	primary
Memantine	19982-08-2	ESI_pos	180.1747	7.34	C12H21N	M+H	primary
Miconazole	22916-47-8	ESI_pos	414.9933	11.22	C18H14Cl4N2O	M+H	primary
Mirtazapine	61337-67-5	ESI_pos	266.1652	5.2	C17H19N3	M+H	primary
Oxazepam	604-75-1	ESI_pos	287.0582	10.28	C15H11ClN2O2	M+H	primary
Promethazin	60-87-7	ESI_pos	285.142	8.66	C17H20N2S	M+H	primary
Risperidone	106266-06-2	ESI_pos	411.2191	7.8	C23H27FN4O2	M+H	primary
Sertraline	79617-96-2	ESI_pos	306.0811	9.79	C17H17Cl2N	M+H	primary
Telmisartan	144701-48-4	ESI_pos	515.2442	10.73	C33H30N4O2	M+H	primary
Temazepam	846-50-4	ESI_pos	301.0738	10.6	C16H13ClN2O2	M+H	primary
Dicyclohexylphthalate	84-61-7	ESI_pos	331.1904	14.15	C20H26O4	M+H	primary

Table S1: Continuation (4).

Triethylphosphate	78-40-0	ESI_pos	183.0781	7.89	C6H15O4P	M+H	primary
Tris(2-ethylhexyl)phosphate	78-42-2	ESI_pos	435.3598	16.25	C24H51O4P	M+H	primary
Tricresylphosphate	1330-78-5	ESI_pos	369.125	14.05	C21H21O4P	M+H	primary
Bis(2-ethylhexyl)phosphate	298-07-7	ESI_pos	323.2346	13.5	C16H35O4P	M+H	primary
Fenthion	55-38-9	ESI_pos	279.0273	11.3	C10H15O3PS2	M+H	primary
Di-n-butyl phosphate	107-66-4	ESI_pos	211.1094	11.5	C8H19O4P	M+H	primary
Propamocarb	24579-73-5	ESI_pos	189.1598	0.7	C9H20N2O2	M+H	primary
Prosulfuron	94125-34-5	ESI_pos	420.0948	11.44	C15H16F3N5O4S	M+H	primary
Acetochlor	34256-82-1	ESI_pos	270.1255	11.84	C14H20ClNO2	M+H	primary
2-Hydroxyatrazine	2163-68-0	ESI_pos	198.1349	2.46	C8H15N5O	M+H	primary
3,4,5-Trichlorophenol	609-19-8	ESI_neg	194.9177	11.82	C6H3Cl3O	M-H	primary
Omethoate	1113-02-6	ESI_pos	214.0297	1.1	C5H12NO4PS	M+H	primary
Triadimenol	55219-65-3	ESI_pos	296.116	11.61	C14H18ClN3O2	M+H	primary
Ethylenethiourea	96-45-7	ESI_pos	103.0324	0.7	C3H6N2S	M+H	primary
Methomyl	16752-77-5	ESI_pos	163.0536	2.65	C5H10N2O2S	M+H	primary
Thiophanate-methyl	23564-05-8	ESI_pos	343.0529	9.1	C12H14N4O4S2	M+H	primary
Abamectin	71751-41-2	ESI_pos	895.482	14.75	C48H72O14	M+Na	primary
4-Chlorophenol	106-48-9	ESI_neg	126.9956	8.2	C6H5ClO	M-H	primary
(Benzothiazol-2-ylthio)methyl thiocyanate	21564-17-0	ESI_pos	238.9766	10.95	C9H6N2S3	M+H	primary
DCOIT	64359-81-5	ESI_pos	282.0481	13.45	C11H17Cl2NOS	M+H	primary
2,4-Dichlorobenzoic acid	50-84-0	ESI_neg	188.9516	9.79	C7H4Cl2O2	M-H	primary
Isophorone	78-59-1	ESI_pos	139.1117	8.79	C9H14O	M+H	primary
Benzidine	92-87-5	ESI_pos	185.1073	0.7	C12H12N2	M+H	primary
3,3'-Dimethylbenzidine	119-93-7	ESI_pos	213.1386	8	C14H16N2	M+H	primary
1-Naphthylamine	134-32-7	ESI_pos	144.0808	3.13	C10H9N	M+H	primary
2-Amino-3,8-dimethylimidazo-[4,5-f]quinoxaline (MeIQx)	77500-04-0	ESI_pos	214.1087	3.05	C11H11N5	M+H	primary
2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)	105650-23-5	ESI_pos	225.1135	6.18	C13H12N4	M+H	primary
Perfluorooctanesulfonamide	754-91-6	ESI_neg	497.9462	13.44	C8H2F17NO2S	M-H	primary
Perfluoroheptanoic acid	375-85-9	ESI_neg	362.9696	13.88	C7HF13O2	M-H	primary
6:2 fluorotelomer sulfonic acid	27619-97-2	ESI_neg	426.9679	14.7	C8H5F13O3S	M-H	primary
Bromochlorophen	15435-29-7	ESI_neg	422.8195	13.81	C13H8Br2Cl2O2	M-H	primary
Metobromuron	3060-89-7	ESI_pos	259.0077	9.81	C9H11BrN2O2	M+H	primary
Fenofibrate	49562-28-9	ESI_pos	361.1201	13.65	C20H21ClO4	M+H	primary
Metconazole	125116-23-6	ESI_pos	320.1524	12.61	C17H22ClN3O	M+H	primary
Bosentan	147536-97-8	ESI_pos	552.1911	12.17	C27H29N5O6S	M+H	primary
Celecoxib	169590-42-5	ESI_pos	382.0832	12.11	C17H14F3N3O2S	M+H	primary
Domperidone	57808-66-9	ESI_pos	426.1691	8.04	C22H24ClN5O2	M+H	primary
Efavirenz	154598-52-4	ESI_pos	316.0347	12.42	C14H9ClF3NO2	M+H	primary
Fluvoxamine	54739-18-3	ESI_pos	319.1628	9.44	C15H21F3N2O2	M+H	primary
Hydroxychloroquine	118-42-3	ESI_pos	336.1837	6.05	C18H26ClN3O	M+H	primary
L-Thyroxine	51-48-9	ESI_pos	777.694	11.17	C15H11I4NO4	M+H	primary
Losartan	114798-26-4	ESI_pos	423.1695	10.9	C22H23ClN6O	M+H	primary
Mebeverine	07-06-2025	ESI_pos	430.2588	8.5	C25H35NO5	M+H	primary
Montelukast	158966-92-8	ESI_pos	586.2177	14.22	C35H36ClNO3S	M+H	primary
Ondansetron	99614-02-5	ESI_pos	294.1601	6.98	C18H19N3O	M+H	primary
Pioglitazone	111025-46-8	ESI_pos	357.1267	8.24	C19H20N2O3S	M+H	primary
Ropinirole	91374-21-9	ESI_pos	261.1961	3.81	C16H24N2O	M+H	primary
Tacrolimus	104987-11-3	ESI_pos	826.4718	13.85	C44H69NO12	M+Na	primary
Valsartan	137862-53-4	ESI_neg	434.2198	11.9	C24H29N5O3	M-H	primary
Vardenafil	224785-90-4	ESI_pos	489.2279	8.82	C23H32N6O4S	M+H	primary
Ziprasidone	146939-27-7	ESI_pos	413.1197	8.54	C21H21ClN4OS	M+H	primary
Amoxicillin	26787-78-0	ESI_pos	366.1118	6.18	C16H19N3O5S	M+H	primary
Terbinafine	91161-71-6	ESI_pos	292.206	10.17	C21H25N	M+H	primary
Clopidogrel	113665-84-2	ESI_pos	322.0663	11.09	C16H16ClNO2S	M+H	primary
Clozapine	5786-21-0	ESI_pos	327.1371	7.56	C18H19ClN4	M+H	primary
Indometacin	53-86-1	ESI_pos	358.0841	12.39	C19H16ClNO4	M+H	primary
Benalaxyl	71626-11-4	ESI_pos	326.1751	12.55	C20H23NO3	M+H	primary
Tamoxifen	10540-29-1	ESI_pos	372.2322	11.44	C26H29NO	M+H	primary
4-Hydroxytamoxifen	68392-35-8	ESI_pos	388.2271	10.38	C26H29NO2	M+H	primary
Pentoxifylline	06-05-1993	ESI_pos	279.1452	8.06	C13H18N4O3	M+H	primary
Anastrozole	120511-73-1	ESI_pos	294.1713	9.2	C17H19N5	M+H	primary
Bicalutamide	90357-06-5	ESI_neg	429.0538	10.98	C18H14F4N2O4S	M-H	primary
Flumequine	42835-25-6	ESI_pos	262.0874	9.43	C14H12FNO3	M+H	primary
Propanil	709-98-8	ESI_pos	218.0134	10.84	C9H9Cl2NO	M+H	primary
Enalapril	75847-73-3	ESI_pos	377.2071	8.43	C20H28N2O5	M+H	primary
Acetamiprid	135410-20-7	ESI_pos	223.0745	7.07	C10H11ClN4	M+H	primary
Mebendazole	31431-39-7	ESI_pos	296.103	9.87	C16H13N3O3	M+H	primary
Mepiquat	15302-91-7	ESI_pos	114.1277	0.7	C7H16N	M+	primary
Albendazole	54965-21-8	ESI_pos	266.0958	9.45	C12H15N3O2S	M+H	primary
Terbuthylazine-2-hydroxy	66753-07-9	ESI_pos	212.1506	6	C9H17N5O	M+H	primary

Table S1: Continuation (5).

Tetraglyme	143-24-8	ESI_pos	240.1804	6.28	C10H22O5	M+NH4	primary
Raloxifene	84449-90-1	ESI_pos	474.1734	8.98	C28H27NO4S	M+H	primary
Clofibrate	637-07-0	ESI_pos	243.0782	10.7	C12H15ClO3	M+H	primary
Chlorpropham	101-21-3	ESI_pos	214.0629	11.41	C10H12ClNO2	M+H	primary
Denatonium	47324-98-1	ESI_pos	325.2274	8.29	C21H29N2O	M+	primary
Carbaryl	63-25-2	ESI_pos	202.0863	9.35	C12H11NO2	M+H	primary
Clonidine	4205-90-7	ESI_pos	230.0246	0.85	C9H9Cl2N3	M+H	primary
Tetracain	94-24-6	ESI_pos	265.1911	7.82	C15H24N2O2	M+H	primary
Picoxystrobin	117428-22-5	ESI_pos	368.1104	12.44	C18H16F3NO4	M+H	primary
2-Oxindole	59-48-3	ESI_pos	134.06	4.8	C8H7NO	M+H	primary
4-(Dimethylamino)pyridine	1122-58-3	ESI_pos	123.0917	0.7	C7H10N2	M+H	primary
Theophyllin	58-55-9	ESI_pos	181.072	2.1	C7H8N4O2	M+H	primary
Allopurinol	315-30-0	ESI_pos	137.0458	0.7	C5H4N4O	M+H	primary
Lamotrigine	84057-84-1	ESI_pos	256.0151	4.22	C9H7Cl2N5	M+H	primary
5-Chlorobenzotriazole	94-97-3	ESI_pos	154.0167	7.51	C6H4ClN3	M+H	primary
Quinoline N-oxide	1613-37-2	ESI_pos	146.06	3.17	C9H7NO	M+H	primary
Ifosfamide	3778-73-2	ESI_pos	261.0321	7.37	C7H15Cl2N2O2P	M+H	primary
Sulfathiazole	72-14-0	ESI_pos	256.0209	2	C9H9N3O2S2	M+H	primary
Bifenox free acid	53774-07-5	ESI_neg	325.9629	12.68	C13H7Cl2NO5	M-H	primary
Bupirimate	41483-43-6	ESI_pos	317.1642	11.03	C13H24N4O3S	M+H	primary
Clothianidin	210880-92-5	ESI_pos	250.016	6	C6H8ClN5O2S	M+H	primary
Cyromazine	66215-27-8	ESI_pos	167.104	0.7	C6H10N6	M+H	primary
Difenoconazole	119446-68-3	ESI_pos	406.072	12.95	C19H17Cl2N3O3	M+H	primary
Diflubenzuron	35367-38-5	ESI_pos	311.0393	12.1	C14H9ClF2N2O2	M+H	primary
Dodemorph	1593-77-7	ESI_pos	282.2791	9.46	C18H35NO	M+H	primary
Fenoxycarb	72490-01-8	ESI_pos	302.1387	12.28	C17H19NO4	M+H	primary
Fenpropidin	67306-00-7	ESI_pos	274.2529	9.75	C19H31N	M+H	primary
Fluoxastrobin	361377-29-9	ESI_pos	459.0866	12.05	C21H16ClFN4O5	M+H	primary
Imidacloprid-urea	120868-66-8	ESI_pos	212.0585	5.76	C9H10ClN3O	M+H	primary
Imidacloprid-guanidine	115970-17-7	ESI_pos	211.0745	0.7	C9H11ClN4	M+H	primary
Oryzalin	19044-88-3	ESI_pos	347.102	11.97	C12H18N4O6S	M+H	primary
Oxadiazone	19666-30-9	ESI_pos	345.0767	13.65	C15H18Cl2N2O3	M+H	primary
Propyzamide	23950-58-5	ESI_pos	256.029	11.22	C12H11Cl2NO	M+H	primary
Pyraclostrobin	175013-18-0	ESI_pos	388.1059	12.83	C19H18ClN3O4	M+H	primary
Quinmerac	90717-03-6	ESI_pos	222.0316	6.1	C11H8ClNO2	M+H	primary
Thiacloprid amide	676228-91-4	ESI_pos	271.0415	6.28	C10H11ClN4OS	M+H	primary
Thiamethoxam	153719-23-4	ESI_pos	292.0266	3.69	C8H10ClN5O3S	M+H	primary
Flufenoxuron	101463-69-8	ESI_pos	489.0435	14.18	C21H11ClF6N2O3	M+H	primary
Triallate	2303-17-5	ESI_pos	304.0091	13.85	C10H16Cl3NOS	M+H	primary
Nitrofurantoin	67-20-9	ESI_neg	237.0265	3.4	C8H6N4O5	M-H	primary
Orlistat	96829-58-2	ESI_pos	496.3996	15.55	C29H53NO5	M+H	primary
Sulfadimethoxine	122-11-2	ESI_pos	311.0809	8.11	C12H14N4O4S	M+H	primary
4-Fluorobenzoylpropionic acid	366-77-8	ESI_neg	195.0463	7.73	C10H9FO3	M-H	primary
Benzocain	94-09-7	ESI_pos	166.0863	7.73	C9H11NO2	M+H	primary
Metoprolol acid	56392-14-4	ESI_pos	268.1543	1.9	C14H21NO4	M+H	primary
Bifonazol	60628-96-8	ESI_pos	311.1543	10	C22H18N2	M+H	primary
Ambroxol	18683-91-5	ESI_pos	376.9859	6	C13H18Br2N2O	M+H	primary
Ebastin	90729-43-4	ESI_pos	470.3054	12.02	C32H39NO2	M+H	primary
Melperon	3575-80-2	ESI_pos	264.1758	7.02	C16H22FNO	M+H	primary
Pipamperone	1893-33-0	ESI_pos	376.2395	6.18	C21H30FN3O2	M+H	primary
Scopolamine-N-butyl	7182-53-8	ESI_pos	360.2169	6.93	C21H30BrNO4	M+	primary
Oxybutynin	5633-20-5	ESI_pos	358.2377	9.53	C22H31NO3	M+H	primary
Nitrendipin	39562-70-4	ESI_pos	361.1394	11.73	C18H20N2O6	M+H	primary
Dichlorophen	97-23-4	ESI_neg	266.9985	12.14	C13H10Cl2O2	M-H	primary
1-(3-carboxypropyl)-3,7-dimethylxanthine	08-07-1993	ESI_pos	267.1088	6.59	C11H14N4O4	M+H	primary
2-Hydroxybenzothiazole	934-34-9	ESI_neg	150.0019	7.63	C7H5NOS	M-H	primary
Dimethachlor OA	NOCAS_891458	ESI_neg	250.1085	8.96	C13H17NO4	M-H	primary
Metazachlor ESA	NOCAS_891454	ESI_neg	322.0867	9.09	C14H17N3O4S	M-H	primary
7-Amino-4-methylcoumarin	26093-31-2	ESI_pos	176.0706	7.07	C10H9NO2	M+H	primary
2(4-morpholinyl)benzothiazole	4225-26-7	ESI_pos	221.0743	9.39	C11H12N2OS	M+H	primary
2-Morpholinothiobenzothiazole	102-77-2	ESI_pos	253.0464	11.33	C11H12N2OS2	M+H	primary
7-Diethylamino-4-methylcoumarin	91-44-1	ESI_pos	232.1332	11.47	C14H17NO2	M+H	primary
Fipronil sulfide	120067-83-6	ESI_neg	418.9365	12.54	C12H4Cl2F6N4S	M-H	primary
Fipronil sulfone	120068-36-2	ESI_neg	450.9263	12.76	C12H4Cl2F6N4O2S	M-H	primary
N-Cyclohexyl-2-benzothiazole-amine	28291-75-0	ESI_pos	233.1107	9.75	C13H16N2S	M+H	primary
N-Cyclohexyl-2-benzothiazole-sulfenamide	95-33-0	ESI_pos	265.0828	13.38	C13H16N2S2	M+H	primary
Fipronil desulfinyl	205650-65-3	ESI_neg	386.9644	12.2	C12H4Cl2F6N4	M-H	primary
m-Xylene-4-sulfonic acid	88-61-9	ESI_neg	185.0278	7.06	C8H10O3S	M-H	primary
Dimethachlor ESA	NOCAS_891457	ESI_neg	300.0911	9.59	C13H19NO5S	M-H	primary

Table S1: Continuation (6).

Etofenprox	80844-07-1	ESI_pos	394.2376	15.05	C25H28O3	M+NH4	primary
Acridine	260-94-6	ESI_pos	180.0808	4.9	C13H9N	M+H	primary
4-Nitroquinoline-1-oxide	56-57-5	ESI_pos	191.0451	6.64	C9H6N2O3	M+H	primary
Acridone	578-95-0	ESI_pos	196.0757	9.21	C13H9NO	M+H	primary
2-Amino-3-methyl-imidazo[4,5-f]quinoline (IQ)	76180-96-6	ESI_pos	199.0978	1.95	C11H10N4	M+H	primary
Harman	486-84-0	ESI_pos	183.0917	5.5	C12H10N2	M+H	primary
2-Aminobiphenyl	90-41-5	ESI_pos	170.0964	8.52	C12H11N	M+H	primary
3-Aminoacetophenon	99-03-6	ESI_pos	136.0757	1.25	C8H9NO	M+H	primary
Azobenzene	103-33-3	ESI_pos	183.0917	10.35	C12H10N2	M+H	primary
Climbazole	38083-17-9	ESI_pos	293.1051	9.24	C15H17ClN2O2	M+H	primary
Labetalol	36894-69-6	ESI_pos	329.186	7.62	C19H24N2O3	M+H	primary
Torasemide	56211-40-6	ESI_pos	349.1329	8.67	C16H20N4O3S	M+H	primary
Trifloxystrobin CGA 321113	252913-85-2	ESI_pos	395.1213	12.85	C19H17F3N2O4	M+H	primary
Candesartan	139481-59-7	ESI_pos	441.167	11.29	C24H20N6O3	M+H	primary
Cyclohexylamine	108-91-8	ESI_pos	100.1121	0.7	C6H13N	M+H	primary
Decylsulfate	142-98-3	ESI_neg	237.1166	15.2	C10H22O4S	M-H	primary
Butocarboxim	34681-10-2	ESI_pos	191.0849	7.61	C7H14N2O2S	M+H	primary
Imazapyr	81334-34-1	ESI_pos	262.1186	6.24	C13H15N3O3	M+H	primary
(Methoxymethyl)triphenylphosphonium	no	ESI_pos	307.1246	7.55	C20H20OP	M+	primary
1,3-Dimethyl-2-imidazolidinone	80-73-9	ESI_pos	115.0866	1.1	C5H10N2O	M+H	primary
1,3-Diphenylguanidine	102-06-7	ESI_pos	212.1182	2.85	C13H13N3	M+H	primary
1-Butyl-3-methyl-imidazolium	80432-08-2	ESI_pos	139.123	0.7	C8H15N2	M+	primary
2-Hydroxyquinoline	59-31-4	ESI_pos	146.06	6.53	C9H7NO	M+H	primary
4-Hydroxyquinoline	611-36-9	ESI_pos	146.06	2	C9H7NO	M+H	primary
Ciclopirox	29342-05-0	ESI_pos	208.1332	12.57	C12H17NO2	M+H	primary
7-(Ethylamino)-4-methylcoumarin	28821-18-3	ESI_pos	204.1019	9.81	C12H13NO2	M+H	primary
Amidosulfuron	120923-37-7	ESI_pos	370.0486	9.83	C9H15N5O7S2	M+H	primary
5-Carboline	244-69-9	ESI_pos	169.076	2.3	C11H8N2	M+H	primary
Carboline	244-76-8	ESI_pos	169.076	3.2	C11H8N2	M+H	primary
Norharmane	244-63-3	ESI_pos	169.076	7.49	C11H8N2	M+H	primary
Harmine	442-51-3	ESI_pos	213.1022	7.12	C13H12N2O	M+H	primary
Dimoxystrobin	149961-52-4	ESI_pos	327.1703	12.28	C19H22N2O3	M+H	primary
Diphenylphosphine oxide	4559-70-0	ESI_pos	203.062	9.18	C12H11OP	M+H	primary
Flumioxazin	103361-09-7	ESI_pos	355.1089	10.95	C19H15FN2O4	M+H	primary
Gabapentin-Lactam	64744-50-9	ESI_pos	154.1226	8.18	C9H15NO	M+H	primary
Indoxacarb	173584-44-6	ESI_pos	528.078	13.25	C22H17ClF3N3O7	M+H	primary
Isoxaben	82558-50-7	ESI_pos	333.1809	11.58	C18H24N2O4	M+H	primary
Methyltriphenylphosphonium	15912-74-0	ESI_pos	277.1141	7.7	C19H18P	M+	primary
Metolachlor-NOA 413173	1418095-19-8	ESI_neg	328.086	10.85	C14H19NO6S	M-H	primary
Tetrapropylammonium	13010-31-6	ESI_pos	186.2216	1.35	C12H28N	M+	primary
Pyridaben	96489-71-3	ESI_pos	365.1449	14.45	C19H25ClN2OS	M+H	primary

Table S1: Continuation (7).

Pyriproxyfen	95737-68-1	ESI_pos	322.1438	13.75	C20H19NO3	M+H	primary
Tepraloxydim	149979-41-9	ESI_pos	342.1467	9.75	C17H24ClNO4	M+H	primary
Trichlorfon	52-68-6	ESI_pos	256.9299	5.14	C4H8Cl3O4P	M+H	primary
Azoxystrobin acid	1185255-09-7	ESI_pos	390.1084	10.68	C21H15N3O5	M+H	primary
Dimethenamid ESA	205939-58-8	ESI_neg	320.0632	10.56	C12H19NO5S2	M-H	primary
Metazachlor OA	1231244-60-2	ESI_neg	272.1041	8.7	C14H15N3O3	M-H	primary
Quinmerac BH518-2	90717-07-0	ESI_pos	252.0058	6.75	C11H6ClNO4	M+H	primary
2-Amido-3,5,6-trichloro-4-cyanobenzenesulfonic acid	1418095-02-9	ESI_neg	326.8806	6.6	C8H3Cl3N2O4S	M-H	primary
Methyldesphenylchloridazon	17254-80-7	ESI_pos	160.0272	1.25	C5H6ClN3O	M+H	primary
Prochloraz BTS44596	139520-94-8	ESI_pos	353.0221	12.42	C13H15Cl3N2O3	M+H	primary
2-Hydroxydesethylterbuthylazine	66753-06-8	ESI_pos	184.1193	0.7	C7H13N5O	M+H	primary
2-Trifluoromethyl-benzenesulfonamide	1869-24-5	ESI_pos	226.0144	5.1	C7H6F3NO2S	M+H	primary
Vancomycin	1404-90-6	ESI_pos	724.7224	5.73	C66H75Cl2N9O24	M+2H2+	primary
Tylosin	1401-69-0	ESI_pos	948.5526	10.03	C46H77NO17	M+CH3OH+H	primary
Spinosyn A	131929-60-7	ESI_pos	732.4681	11.93	C41H65NO10	M+H	primary
Emamectin B1a	121124-29-6	ESI_pos	886.5311	12.65	C49H75NO13	M+H	primary
Ivermectin B1a	71827-03-7	ESI_pos	892.5417	15.35	C48H74O14	M+NH4	primary
Methiocarb-sulfoxide phenol	22454-92-8	ESI_pos	185.0631	5.4	C9H12O2S	M+H	primary
2-(Piperazin-1-yl)ethanamine	140-31-8	ESI_pos	130.1339	0.7	C6H15N3	M+H	primary
4-Hydroxy-1-(2-hydroxyethyl)-2,2,6,6-tetramethylpiperidine	52722-86-8	ESI_pos	202.1802	0.7	C11H23NO2	M+H	primary
Dicyclohexyl sulfosuccinate	137361-04-7	ESI_neg	361.1326	14.16	C16H26O7S	M-H	primary
Dimethachlor CGA369873	NOCAS_1017801	ESI_neg	242.0493	5.6	C10H13NO4S	M-H	primary
Dimethyl-5-sulfoisophthalate	138-25-0	ESI_neg	273.0074	8.31	C10H10O7S	M-H	primary
Metazachlor BH479-12	NOCAS_1017810	ESI_pos	304.0928	7.96	C14H13N3O5	M+H	secondary
Phenylethylmalonamide	7206-76-0	ESI_pos	207.1128	2.85	C11H14N2O2	M+H	primary
Trifloxystrobin NOA413161	no	ESI_pos	425.0955	12.35	C19H15F3N2O6	M+H	primary
Trifloxystrobin NOA413163	no	ESI_pos	425.0955	11.87	C19H15F3N2O6	M+H	primary
Tripropyl phosphate	513-08-6	ESI_pos	225.125	11.2	C9H21O4P	M+H	primary
Thiadone	84352-75-0	ESI_neg	168.9689	5.8	C3HF3N2OS	M-H	primary
Dimethenamid OA	380412-59-9	ESI_neg	270.0806	10.23	C12H17NO4S	M-H	primary
Metolachlor CGA 357704	no	ESI_pos	280.1179	10.24	C14H17NO5	M+H	secondary
Metolachlor CGA 368208	446027-17-4	ESI_neg	256.0649	8.71	C11H15NO4S	M-H	primary
Prochloraz BTS40348	67747-01-7	ESI_pos	282.0214	8.14	C11H14Cl3NO	M+H	primary
Metazachlor BH 479-9	no	ESI_pos	350.1169	8.29	C16H19N3O4S	M+H	primary
Metazachlor BH 479-11	no	ESI_pos	306.1271	8.08	C15H19N3O2S	M+H	primary
Simazine 2-Hydroxy	03-11-1999	ESI_pos	184.1193	0.95	C7H13N5O	M+H	primary
Etoposide	no	ESI_pos	606.2181	9.56	C29H32O13	M+NH4	primary
Tributylamine	102-82-9	ESI_pos	186.2216	6.8	C12H27N	M+H	primary
N-Methyldodecylamine	7311-30-0	ESI_pos	200.2373	11.16	C13H29N	M+H	primary
2-Amino-9H-pyrido[2,3-b]indole (AalphaC)	26148-68-5	ESI_pos	184.0869	6.63	C11H9N3	M+H	primary
2-Naphthol-8-sulfonic acid	132-57-0	ESI_neg	223.0071	6.73	C10H8O4S	M-H	primary
Benzyldimethyltetradecylammonium	16287-71-1	ESI_pos	332.3312	12.59	C23H42N	M+	primary
N,N-Dimethyltetradecylamine-N-oxide	3332-27-2	ESI_pos	258.2791	12.22	C16H35NO	M+H	primary
N,N-Dimethyldodecylamine	112-18-5	ESI_pos	214.2529	10.95	C14H31N	M+H	primary
N,N-Dimethyldecylamine	1120-24-7	ESI_pos	186.2216	9.25	C12H27N	M+H	primary
N,N-Dimethyltetradecylamine	112-75-4	ESI_pos	242.2842	11.97	C16H35N	M+H	primary
Salinomycin	53003-10-4	ESI_neg	749.4845	15.47	C42H70O11	M-H	primary
Piperine	94-62-2	ESI_pos	286.1438	11.86	C17H19NO3	M+H	primary
4-Hydroxycoumarin	1076-38-6	ESI_pos	163.039	8.6	C9H6O3	M+H	primary
DINCH	318292-43-2	ESI_pos	425.3625	16.35	C26H48O4	M+H	primary
Quinoline	91-22-5	ESI_pos	130.0651	1	C9H7N	M+H	primary
Octyl-3,5-di-tert-butyl-4-hydroxyhydrocinnamate	13417-12-4	ESI_pos	391.3207	15.55	C25H42O3	M+H	primary

S2

Table S2: Internal standards - isotope-labelled compounds for quantitative LC-HRMS screening analysis (Finckh et al. 2022a; Finckh et al. 2022b, under review).

Name	Formula	LC-HRMS mode	adduct	m/z	RT [min]
Mono-isobutylphthalate-D4__M-H	C12H10[2]H4O4	ESI_neg	M-H	225.107	10.6
4-Nitrophenol-D4__M-H	C6H1[2]H4N1O3	ESI_neg	M-H	142.0448	5.7
Triclosan-D3__M-H	C12H4[2]H3Cl3O2	ESI_neg	M-H	289.9627	13.4
Mecoprop-D3__M-H	C10H8[2]H3Cl1O3	ESI_neg	M-H	216.0512	11.5
Diclofenac_d4__M-H	C14H7[2]H4Cl2N1O2	ESI_neg	M-H	298.0345	12.5
Laurylsulfate-D25__M-H	C12H1[2]H25O4S	ESI_neg	M-H	290.3048	24.4
Bezafibrate-D4__M-H	C19H16[2]H4Cl1N1O4	ESI_neg	M-H	364.1259	11.6
Acesulfame-D4__M-H	C4H1[2]H4N1O14S	ESI_neg	M-H	166.0118	1.5
Hydrochlorothiazide-13C6__M-H	C1[13]C6H8Cl1N3O4S2	ESI_neg	M-H	301.9773	1.7
Bentazone-D6__M-H	C10H6[2]H6N2O3S1	ESI_neg	M-H	245.0872	9.7
Cyclamate-D11__M-H	C6H2[2]H11N1O3S1	ESI_neg	M-H	189.1234	4.1
Mono-isobutylphthalate-D4___M+H	C12H10[2]H4O4	ESI_pos	M+H+	227.1216	10.6
Creatinine-D3 ___M+H	C4H4[2]H3N3O1	ESI_pos	M+H+	117.085	0.6
Diazinon-D10___M+H	C12H11[2]H10N2O3P1S1	ESI_pos	M+H+	315.1711	12.8
Benzophenone-3-D5__M+H	C14H7[2]H5O3	ESI_pos	M+H+	234.1173	12.3
p-Toluene-sulfonamide-D4__M+NH4	C7H5[2]H4NO2S1	ESI_pos	M+NH4+	193.0933	5
Cotinine-D3__M+H	C10H9[2]H3N2O1	ESI_pos	M+H+	180.1211	0.9
Diglyme-D6__M+H	C6H8[2]H6O3	ESI_pos	M+H+	141.1392	2.4
Chlormequat-D9__M+	C5H4[2]H9Cl1N1	ESI_pos	M+	131.1296	0.7
Carbamazepine-D10__M+H	C15H2[2]H10N2O1	ESI_pos	M+H+	247.165	9.8
Atrazine-13C3__M+H	C5[13]C3H14Cl1N5	ESI_pos	M+H+	219.1111	10.2
Benzotriazole-D4__M+H	C6H1[2]H4N3	ESI_pos	M+H+	124.0807	3.9
Carbendazim-D4__M+H	C9H5[2]H4N3O2	ESI_pos	M+H+	196.1019	2.2
Tri-n-butylphosphate-D27__M+H	C12[2]H27O4P1	ESI_pos	M+H+	294.3414	13.3
DEET-D7__M+H	C12H10[2]H7N1O1	ESI_pos	M+H+	199.1822	10.4
Metolachlor-D6__M+H	C15H16[2]H6Cl1N1O2	ESI_pos	M+H+	290.1788	12.2
Isoproturon-D3__M+H	C12H15[2]H3N2O1	ESI_pos	M+H+	210.168	10.4
Diclofenac_d4__M+H	C14H7[2]H4Cl2N1O2	ESI_pos	M+H+	300.0491	12.5
Caffeine-D3__M+H	C8H7[2]H3N4O2	ESI_pos	M+H+	198.1065	5.6
Clarithromycin-D3__M+H	C38H66[2]H3N1O13	ESI_pos	M+H+	751.503	10.3
Desisopropylatrazine-D5__M+H	C5H3[2]H5Cl1N5	ESI_pos	M+H+	179.0855	4.6
Decyltrimethylammonium-D30__M+	C13[2]H30N1	ESI_pos	M+	230.4256	9.4
Atenolol-D7__M+H	C14H15[2]H7N2O3	ESI_pos	M+H+	274.2143	1.1
Progesterone-D9__M+H	C21H21[2]H9O2	ESI_pos	M+H+	324.2883	12.6
Verapamil-D6__M+H	C27H32[2]H6N2O4	ESI_pos	M+H+	461.3281	9
Bezafibrate-D4__M+H	C19H16[2]H4Cl1N1O4	ESI_pos	M+H+	366.1405	11.6
Sulfamethoxazole-D4__M+H	C10H7[2]H4N3O3S1	ESI_pos	M+H+	258.0845	6.4
Tebuconazole-D9__M+H	C16H13[2]H9Cl1N3O1	ESI_pos	M+H+	317.2089	12.6
Imidacloprid-D4__M+H	C9H6[2]H4Cl1N5O2	ESI_pos	M+H+	260.0847	6.6

S3

Table S3: Concentrations [ng/L], results of the chemical target screening of 499 compounds (Finckh et al. 2022b, under review).

		ng/L			
Name	**CASRN**	**MDL**	**WWTP_1**	**WWTP_2_-O3**	**WWTP_2_+O3**
Naproxen	22204-53-1	15	69.8	70.6	
Phenazone	60-80-0	0.5	29.4	257	2.8
Dichlorvos	62-73-7	7			
Azinphos methyl	86-50-0	2			
2,4-Dichlorophenoxyacetic acid	94-75-7	1	7.1	11.8	
Clofibric acid	882-09-7	1			
Bezafibrate	41859-67-0	1	78.3	28.5	
Monensin	17090-79-8	2			
Clotrimazole	23593-75-1	8	11.9	9.8	
Propyphenazone	479-92-5	0.7		1.7	
Dimethoate	60-51-5	1			
Metoprolol	51384-51-1	3	578.2	757.1	79.8
Bentazone	25057-89-0	1			
Diuron	330-54-1	1	17.5	19.8	7.9
Isoproturon	34123-59-6	0.5	2.6	25.8	0.9
Chloramphenicol	56-75-7	1			
4-Nitrophenol	100-02-7	40			
Erythromycin	114-07-8	5	71.5	102	
Atenolol	29122-68-7	1	145.9	276.3	14.5
Cyclophosphamide	50-18-0	1		3.4	2.8
Gemfibrozil	25812-30-0	50		67.7	
Diazinon	333-41-5	0.2			
Furosemide	54-31-9	2	89.6	196.8	
Perfluorodecanoic acid	335-76-2	1			
Chlorotoluron	15545-48-9	1			
Paroxetine	61869-08-7	1			
Propranolol	525-66-6	1	29.7	38.9	1.2
Verapamil	52-53-9	1	69.9	32.4	1.4
Pravastatin	81093-37-0	2	4.8		
Myclobutanil	88671-89-0	0.7		1.6	
Iminostilbene	256-96-2	0.7			
2-Aminobenzimidazole	934-32-7	1	17.8	165.7	52.6
Isophorone diamine	2855-13-2	20	110.7	183	
3,3'-Dichlorobenzidine	91-94-1	16			
N,N-Dimethyl-p-phenylenediamine	99-98-9	500			
4'-Aminoacetanilide	122-80-5	15	1260.5	2258.2	955.5
N,N-Dimethyldodecylamine N-oxide	1643-20-5	1			
Triethylcitrate	77-93-0	5	22.6		
Piperonyl butoxide	51-03-6	5			
Imazalil	35554-44-0	1	2.5	4	
Fipronil	120068-37-3	0.5	3.2	6.6	2.4
Diflufenican	83164-33-4	10			
Flusilazole	85509-19-9	1			
Trifloxystrobin	141517-21-7	2			
Sulcotrione	99105-77-8	10			
Propoxycarbazone	145026-81-9	5			

Table S3: Continuation (1).

4-Amino-N,N-dimethylbenzenesulfonamide	1709-59-7	2			
4-Aminobenzamide	2835-68-9	20			
2-Methylbenzothiazole	120-75-2	450			
N-Acetyl-4-aminoantipyrine	83-15-8	5	443.9	867.6	5.9
4-Aminoantipyrine	83-07-8	2.5	354.9	932.6	29.6
Benzophenone-3	131-57-7	2		32.2	
Phenylbenzimidazole sulfonic acid	27503-81-7	5	9967.6	6332.1	1489.3
Thiabendazole	148-79-8	2.5		8.3	
Hydrochlorothiazide	58-93-5	1	3075.2	3104.5	245.2
N-isopropyl-N'-phenyl-p-phenylenediamine	101-72-4	500			
Metribuzin	21087-64-9	1	1.3		
Prochloraz	67747-09-5	0.5			
Dimethenamid	87674-68-8	1			
Hexazinone	51235-04-2	0.5			
Carbetamide	16118-49-3	1	2.7	5.8	1.8
Metalaxyl	57837-19-1	1	1.4	6.3	
Cyproconazole	94361-06-5	0.7			
Cyprodinil	121552-61-2	2			
Dichlorprop	120-36-5	1	1.5		
Imidacloprid	138261-41-3	1	7.1	18.6	9.8
Dimethachlor	50563-36-5	0.7			
Dinoseb	88-85-7	0.4			
Nicosulfuron	111991-09-4	2			
Flufenacet	142459-58-3	1			
Propachlor	1918-16-7	1			
Prosulfocarb	52888-80-9	1			
Kresoxim-methyl	143390-89-0	0.5			
Pethoxamid	106700-29-2	1	2		
Ethofumesate	26225-79-6	1	28.4	51.5	
Metamitron	41394-05-2	1		6.7	2.3
Chloridazon	1698-60-8	1			
Spiroxamine	118134-30-8	1			
Pyrazophos	13457-18-6	1			
Simetryn	1014-70-6	1			
Azoxystrobin	131860-33-8	1	1.8	3.2	
Fenpropimorph	67564-91-4	1			
Epoxiconazole	133855-98-8	0.7		1.8	
Bendiocarb	22781-23-3	2	3	5.9	
N-Butylbenzenesulfonamide	3622-84-2	50		59.5	
Diazepam	439-14-5	0.7		1.3	
Tris(2-chloroethyl)phosphate	115-96-8	0.7	29.6	48	42.8
Clarithromycin	81103-11-9	2.2	349.9	417.6	6
Desethylatrazine	6190-65-4	0.7	5	9	
Ketoprofen	22071-15-4	1	15.2	5.7	
Sulfamethoxazole	723-46-6	2	121.3	240.8	21.9
Sulfapyridine	144-83-2	5	54.6	47.3	7.7
Desethylterbutylazine	30125-63-4	1			
Ethion	563-12-2	1			
Pirimiphos-methyl	29232-93-7	1			
Ethyl azinphos	2642-71-9	1			

Table S3: Continuation (2).

Desisopropylatrazine	1007-28-9	2.5			
Perfluorooctanoic acid	335-67-1	0.4	10.2	9	3.7
Caffeine	58-08-2	25			
Octyl-methoxycinnamate	5466-77-3	1.5	3.2	3.4	
2-(2-(Chlorophenyl)amino)benzaldehyde	71758-44-6	10			
Carbendazim	10605-21-7	1	4.7	24	2
Acetaminophen	103-90-2	18	606.3	780.5	143.8
Acesulfame	33665-90-6	45	6240.5	164.3	98.1
Flutamide	13311-84-7	1			
1,2-Benzisothiazolinone	2634-33-5	17			
Linuron	330-55-2	2			
Terbuthylazine	5915-41-3	0.6		3.1	1
Terbutryn	886-50-0	0.7	7.6	29.7	7.2
Carbamazepine	298-46-4	0.5	515.3	585	2.9
Sucralose	56038-13-2	45	7092.8	46955.5	37981.7
Triclosan	3380-34-5	1			
Triphenylphosphate	115-86-6	5.8			
Saccharin	81-07-2	89	296.1		
Perfluorooctanesulfonic acid	1763-23-1	1	3.1	15	7
Cyclamate	100-88-9	55			
2-Octyl-4-isothiazolin-3-one	26530-20-1	1			
DEET	134-62-3	1.5	168.8	121.6	25
Atrazine	1912-24-9	1	2.4		
Irgarol	28159-98-0	0.3			
1H-Benzotriazole	95-14-7	5	6201	7453.6	1658.9
Propiconazole	60207-90-1	0.7	7	8.2	2.2
Diclofenac	15307-86-5	1	1523.2	1791.5	3.1
Mefenamic acid	61-68-7	1		3.1	
Metazachlor	67129-08-2	0.7	1.3		
N-Phenyl-1-naphthylamine	90-30-2	5			
Chlorfenvinphos	470-90-6	1			
Pirimicarb	23103-98-2	0.5			
Perfluorohexanoic acid	307-24-4	2	14.1	10.7	6.4
Metolachlor	51218-45-2	0.7			
Simazine	122-34-9	1	1.4	1.7	
Primidone	125-33-7	10	12.1	41.8	
Mecoprop	93-65-2	1	16.3	8.7	
Enrofloxacin	93106-60-6	20			
Roxithromycin	80214-83-1	2	120.5	105.4	
Ranitidine	66357-35-5	6.5	148.9	264.3	
Warfarin	81-81-2	0.4	0.6	0.5	
(3-Chloro-2-hydroxypropyl)trimethylammonium	82914-58-7	50			
2-Isopropylthioxanthone	5495-84-1	1			
MCPA	94-74-6	1	1.2	10	
4-Methylbenzylidene camphor	36861-47-9	2	2.4		
5-Methyl-1H-benzotriazole	136-85-6	15	1095.1	2393.5	336.2
Benzethonium	10172-60-8	2			
Benzophenone-4	4065-45-6	1	224.6	1115.9	
Benzyldimethyldodecylammonium	10328-35-5	20			
Benzyldimethylhexadecylammonium	10328-34-4	4			

Table S3: Continuation (3).

Hexadecyltrimethylammonium	01-10-1999	10			
Chlorophene	120-32-1	2			
Chlorothalonil-4-hydroxy	28343-61-5	1	1.3	3.4	
Didecyldimethylammonium	20256-56-8	15			
Ethyl 4-(dimethylamino)benzoate	10287-53-3	0.7			
Hexadecylpyridinium	7773-52-6	10			
3-Iodopropynyl butylcarbamate	55406-53-6	1.5			
Lauryl diethanolamide	120-40-1	4			
p-Toluenesulfonamide	70-55-3	5	60.4	635	156.6
Tebuconazole	107534-96-3	0.7	1	1.7	
Thiacloprid	111988-49-9	1			
Tri(butoxyethyl)phosphate	78-51-3	1	401.1	64.3	1.8
Triclocarban	101-20-2	1			
Tri-isobutylphosphate	126-71-6	45	62.9	173.3	
Trimethyloctylammonium	15461-38-8	5		9.2	
TDCPP	13674-87-8	6	36.8	53.3	42.3
Tris(1-chloro-2-propyl)phosphate	13674-84-5	10	447.6	849.9	442.4
Metformin	657-24-9	12	2476	1163.7	681.1
Lauric isopropanolamide	142-54-1	1			
Hexa(methoxymethyl)melamine	3089-11-0	1	261.4	168.1	68.6
Pindolol	13523-86-9	20			
Fenuron	101-42-8	0.7	11.3	28.4	14.3
Chloroxuron	1982-47-4	0.7			
Lenacil	01-08-1964	1	2.9	6.8	2
Ametryn	834-12-8	1			
Pentobarbital	76-74-4	50			
Sulfamethazine	57-68-1	3			
Ketoconazole	65277-42-1	10			
2-Isopropyl-6-methyl-pyrimidin-4-ol	2814-20-2	20			
o-Dianisidine	119-90-4	100	133.5	338.4	
Diphenylphosphate	838-85-7	1	65.2	53.8	
N-Ethyl-o-toluenesulfonamide	1077-56-1	1	37.4	89.3	15.9
2-(Methylthio)benzothiazole	615-22-5	14.9	350	260.2	92
Triglyme	112-49-2	8	8.3	39.2	
Methylchloroisothiazolinone	26172-55-4	4			
4-Hydroxybenzotriazole	26725-51-9	5	116.7	154.2	5.9
Aniline Yellow	b	10			
4,4'-Methylene-bis(2-methyl aniline)	838-88-0	20			
4,4'-Thiodianiline	139-65-1	100			
Tetrabromobisphenol A	79-94-7	10			
Perfluorobutanoic acid	375-22-4	20			
Michler's ketone	90-94-8	0.7			
Tetrachlorosalicylanilide	1154-59-2	1			
Benzothiazole	95-16-9	25	173.6	394.5	106.4
Desphenyl chloridazon	6339-19-1	20			
2,6-Dichlorobenzamide	2008-58-4	10			
3,4-Dichlorophenylurea	08-02-2027	10			
4-Isopropylaniline	99-88-7	10			
Metolachlor OA	152019-73-3	1	1.7	3.2	
Metolachlor ESA	171118-09-5	1	7.7	6	

Table S3: Continuation (4).

2,4-Dinitrophenol	51-28-5	2			
Lidocaine	137-58-6	5	131.3	252.6	12.7
Tramadol	27203-92-5	3	2827.6	735.3	57.7
Mycophenolic acid	24280-93-1	1	13.9	5.2	
Capecitabine	154361-50-9	10			
Dimethylaminophenazone	58-15-1	1	1.8	4.1	
Methotrexate	59-05-2	15			
7-Hydroxymethotrexate	5939-37-7	25			
Icaridin	119515-38-7	1			
Cotinine	486-56-6	2	96.7	172.6	36.4
2-Benzothiazolesulfonic acid	941-57-1	2	572.2	1310.5	683.9
Lincomycin	154-21-2	10		24.1	
Trimethoprim	738-70-5	2	36.6	135.8	
4-Formyl-antipyrine	950-81-2	10			
Crotamiton	483-63-6	1	7.7	76.9	
10,11-Dihydro-10,11-dihydroxycarbamazepine	35079-97-1	1	818.2	1284.5	359.7
Quinoxyfen	124495-18-7	1			
Cetirizine	83881-51-0	1	102.1	514.3	4.3
2-Hydroxycarbamazepine	68011-66-5	1	54.8	75.2	17.6
10,11-Dihydro-10-hydroxycarbamazepine	29331-92-8	1	343.3	264.3	81.8
Amantadine	768-94-5	2	55.9	109.4	37.1
Benzenesulfonic acid	98-11-3	100	275.8	133.9	196.6
Clomazone	81777-89-1	1			
Methiocarb	2032-65-7	1			
3,5,6-Trichloro-2-pyridinol	6515-38-4	1	12.3	13.4	
Pendimethalin	40487-42-1	2			
Boscalid	188425-85-6	0.7	10	6.7	2.2
Prothioconazole-desthio	120983-64-4	0.7		2.8	
Sotalol	3930-20-9	2.5	199.1	354.8	
N-Formyl-4-aminoantipyrine	1672-58-8	2	1318.4	6666.2	10.9
2,4-Dichlorophenol	120-83-2	8	12	16.1	
Genistein	446-72-0	1	12.1	6.9	
Daidzein	486-66-8	1			
Amidosulfobetaine-14	216667-08-2	1			
Bisphenol S	80-09-1	0.7			
Flurtamone	96525-23-4	1			
Picolinafen	137641-05-5	10			
Finasteride	98319-26-7	1			
Diphenhydramine	58-73-1	2	106.9	156.4	9.9
Acetyl-sulfamethoxazole	21312-10-7	1	3.7	13.9	1.4
Citalopram	59729-33-8	2.9	141.4	233	23.9
Secobarbital	76-73-3	80			
Lauramidopropylbetaine	08-10-1992	100			
4-Chloroaniline	106-47-8	5			
Bromoxynil	1689-84-5	1			
Amiodarone	1951-25-3	5			
Amitriptyline	50-48-6	1	77.7	77.7	5.4
Atorvastatin	134523-00-5	5	26.9	27.6	
Azelastine	58581-89-8	2			
Azithromycin	83905-01-5	10	487.5	623.2	

Table S3: Continuation (5).

Bisoprolol	66722-44-9	0.7	184.7	161.5	17.2
Bupropion	34911-55-2	3	3.3	147.3	42.3
Desloratadine	100643-71-8	15		16.6	
Duloxetine	116539-59-4	10		25	
EDDP	30223-73-5	5	48.1	194	83.4
Fluconazole	86386-73-4	12.2	47.1	122.2	60.2
Glibenclamide	10238-21-8	2			
Glimepiride	93479-97-1	1		14.8	
Ketamine	6740-88-1	5	11.9	35.4	
Loperamide	53179-11-6	1			
Lorazepam	846-49-1	2	3	13.2	3.5
Memantine	19982-08-2	1	21.4	43.1	16.5
Miconazole	22916-47-8	10			
Mirtazapine	61337-67-5	5	26.8	89.5	
Oxazepam	604-75-1	1	46.4	116.9	24.7
Promethazin	60-87-7	5		10.2	
Risperidone	106266-06-2	2			
Sertraline	79617-96-2	2	15.1	32.6	5.7
Telmisartan	144701-48-4	0.5	549.6	589.6	85.4
Temazepam	846-50-4	1	7.5	21.5	5.7
Dicyclohexylphthalate	84-61-7	1	3.6	1.3	
Triethylphosphate	78-40-0	0.7	470	236.9	73.9
Tris(2-ethylhexyl)phosphate	78-42-2	2			
Tricresylphosphate	1330-78-5	1			
Bis(2-ethylhexyl)phosphate	298-07-7	2			
Fenthion	55-38-9	5			
Di-n-butyl phosphate	107-66-4	20		49	
Propamocarb	24579-73-5	1	6.2		
Prosulfuron	94125-34-5	2			
Acetochlor	34256-82-1	2			
2-Hydroxyatrazine	2163-68-0	5	20.1		
3,4,5-Trichlorophenol	609-19-8	1			
Omethoate	1113-02-6	20			
Triadimenol	55219-65-3	1		1.6	
Ethylenethiourea	96-45-7	500			
Methomyl	16752-77-5	20			
Thiophanate-methyl	23564-05-8	1000			
Abamectin	71751-41-2	10			
4-Chlorophenol	106-48-9	15			
(Benzothiazol-2-ylthio)methyl thiocyanate	21564-17-0	12			
DCOIT	64359-81-5	1			
2,4-Dichlorobenzoic acid	50-84-0	55		97.6	
Isophorone	78-59-1	500			
Benzidine	92-87-5	200			
3,3'-Dimethylbenzidine	119-93-7	50			
1-Naphthylamine	134-32-7	35			
2-Amino-3,8-dimethylimidazo-[4,5-f]quinoxaline (MeIQx)	77500-04-0	5			
2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)	105650-23-5	5			
Perfluorooctanesulfonamide	754-91-6	1			
Perfluoroheptanoic acid	375-85-9	1	6.5	4.4	2.4

Table S3: Continuation (6).

6:2 fluorotelomer sulfonic acid	27619-97-2	150	499.9	473.7	
Bromochlorophen	15435-29-7	1			
Metobromuron	3060-89-7	5			
Fenofibrate	49562-28-9	5			
Metconazole	125116-23-6	1			
Bosentan	147536-97-8	1			
Celecoxib	169590-42-5	1	9	17	9.4
Domperidone	57808-66-9	2			
Efavirenz	154598-52-4	1		2.2	
Fluvoxamine	54739-18-3	2	7.1	101.7	
Hydroxychloroquine	118-42-3	10			
L-Thyroxine	51-48-9	20			
Losartan	114798-26-4	2.2	38.3	67.9	
Mebeverine	07-06-2025	1			
Montelukast	158966-92-8	2			
Ondansetron	99614-02-5	2		3	
Pioglitazone	111025-46-8	2			
Ropinirole	91374-21-9	5			8.4
Tacrolimus	104987-11-3	5			
Valsartan	137862-53-4	0.8	462	117.3	11.4
Vardenafil	224785-90-4	5			
Ziprasidone	146939-27-7	20			
Amoxicillin	26787-78-0	500			
Terbinafine	91161-71-6	2			
Clopidogrel	113665-84-2	1	13.7	23	
Clozapine	5786-21-0	2.5	25.7	86.1	
Indometacin	53-86-1	2	11.9	13.8	
Benalaxyl	71626-11-4	2			
Tamoxifen	10540-29-1	5			
4-Hydroxytamoxifen	68392-35-8	5			
Pentoxifylline	06-05-1993	2			
Anastrozole	120511-73-1	1	2.5	3.7	2.1
Bicalutamide	90357-06-5	0.7	28.7	64.4	44.5
Flumequine	42835-25-6	10			
Propanil	709-98-8	2			
Enalapril	75847-73-3	2			
Acetamiprid	135410-20-7	1	2.8	4.3	
Mebendazole	31431-39-7	1.5	1.9	9.2	
Mepiquat	15302-91-7	35	202.3	179.1	91.1
Albendazole	54965-21-8	2			
Terbuthylazine-2-hydroxy	66753-07-9	4	12.9	13.3	12.6
Tetraglyme	143-24-8	2.5	17.9	24.3	6.6
Raloxifene	84449-90-1	2			
Clofibrate	637-07-0	10			
Chlorpropham	101-21-3	10			
Denatonium	47324-98-1	1	147.9	306.6	85.3
Carbaryl	63-25-2	1			
Clonidine	4205-90-7	2			
Tetracain	94-24-6	1	5	11.9	2.1
Picoxystrobin	117428-22-5	2			

Table S3: Continuation (7).

2-Oxindole	59-48-3	25		70.6	
4-(Dimethylamino)pyridine	1122-58-3	5	32.9	56	6.3
Theophyllin	58-55-9	10	48.4	41.3	28.6
Allopurinol	315-30-0	10			
Lamotrigine	84057-84-1	0.5	310.8	1029.8	702
5-Chlorobenzotriazole	94-97-3	2	6.7	3.3	4.7
Quinoline N-oxide	1613-37-2	10			
Ifosfamide	3778-73-2	1		5.1	3.3
Sulfathiazole	72-14-0	2.5			
Bifenox free acid	53774-07-5	1			
Bupirimate	41483-43-6	1			
Clothianidin	210880-92-5	1			
Cyromazine	66215-27-8	2			
Difenoconazole	119446-68-3	0.7			
Diflubenzuron	35367-38-5	10			
Dodemorph	1593-77-7	1			
Fenoxycarb	72490-01-8	2			
Fenpropidin	67306-00-7	1			
Fluoxastrobin	361377-29-9	1			
Imidacloprid-urea	120868-66-8	1			
Imidacloprid-guanidine	115970-17-7	1			
Oryzalin	19044-88-3	4			
Oxadiazone	19666-30-9	5			
Propyzamide	23950-58-5	1			
Pyraclostrobin	175013-18-0	1			
Quinmerac	90717-03-6	1			
Thiacloprid amide	676228-91-4	1		1.8	
Thiamethoxam	153719-23-4	1			
Flufenoxuron	101463-69-8	5			
Triallate	2303-17-5	2			
Nitrofurantoin	67-20-9	7	30.1	25	
Orlistat	96829-58-2	5			
Sulfadimethoxine	122-11-2	1			
4-Fluorobenzoylpropionic acid	366-77-8	15			
Benzocain	94-09-7	2			
Metoprolol acid	56392-14-4	15	184.2	113.2	29.7
Bifonazol	60628-96-8	20			
Ambroxol	18683-91-5	1.5	196.5	77	
Ebastin	90729-43-4	2			
Melperon	3575-80-2	5	5.5	11.9	
Pipamperone	1893-33-0	10			
Scopolamine-N-butyl	7182-53-8	1	9.7	16.1	3.3
Oxybutynin	5633-20-5	1		1.1	
Nitrendipin	39562-70-4	1	4.2	4	1
Dichlorophen	97-23-4	1		1	
1-(3-carboxypropyl)-3,7-dimethylxanthine	08-07-1993	8	109	126.4	14.2
2-Hydroxybenzothiazole	934-34-9	50	60.9	102	
Dimethachlor OA	NOCAS_891458	2	12.6	22.2	7.4
Metazachlor ESA	NOCAS_891454	1	1.2	3.3	
7-Amino-4-methylcoumarin	26093-31-2	1	4	6.9	

Table S3: Continuation (8).

2(4-morpholinyl)benzothiazole	4225-26-7	2.9	5.3	10	
2-Morpholinothiobenzothiazole	102-77-2	1			
7-Diethylamino-4-methylcoumarin	91-44-1	0.7	7.8	5.6	0.8
Fipronil sulfide	120067-83-6	0.7			
Fipronil sulfone	120068-36-2	0.7			
N-Cyclohexyl-2-benzothiazole-amine	28291-75-0	1	3.6	1.4	
N-Cyclohexyl-2-benzothiazole-sulfenamide	95-33-0	5			
Fipronil desulfinyl	205650-65-3	0.7			
m-Xylene-4-sulfonic acid	88-61-9	5	367.6	1034.9	194.6
Dimethachlor ESA	NOCAS_891457	1			
Etofenprox	80844-07-1	1			
Acridine	260-94-6	20		33	
4-Nitroquinoline-1-oxide	56-57-5	10			
Acridone	578-95-0	0.5	12.9	6.4	10.3
2-Amino-3-methyl-imidazo[4,5-f]quinoline (IQ)	76180-96-6	5			
Harman	486-84-0	1	8.1	31.5	1.1
2-Aminobiphenyl	90-41-5	5			
3-Aminoacetophenon	99-03-6	35			
Azobenzene	103-33-3	450			
Climbazole	38083-17-9	1	52.8	67.8	2.3
Labetalol	36894-69-6	2	3	4.4	
Torasemide	56211-40-6	2	53.4	138	12
Trifloxystrobin CGA 321113	252913-85-2	0.4	1	3.4	1.1
Candesartan	139481-59-7	1	440	854.1	142.3
Cyclohexylamine	108-91-8	35	399.3	671.1	136.7
Decylsulfate	142-98-3	0.8	1.3		
Butocarboxim	34681-10-2	150			
Imazapyr	81334-34-1	5	11	16.7	
(Methoxymethyl)triphenylphosphonium	c	1			
1,3-Dimethyl-2-imidazolidinone	80-73-9	20	35.6		
1,3-Diphenylguanidine	102-06-7	5	42.9	152.7	17
1-Butyl-3-methyl-imidazolium	80432-08-2	0.5	18.2	47.9	5.2
2-Hydroxyquinoline	59-31-4	2	19.7	42.7	
4-Hydroxyquinoline	611-36-9	5	22.6	17.6	15
Ciclopirox	29342-05-0	8			
7-(Ethylamino)-4-methylcoumarin	28821-18-3	0.5	3.6	3.4	
Amidosulfuron	120923-37-7	20			
5-Carboline	244-69-9	10			
Carboline	244-76-8	20			
Norharmane	244-63-3	5			
Harmine	442-51-3	0.5	1.1	1.4	0.9
Dimoxystrobin	149961-52-4	0.5			
Diphenylphosphine oxide	4559-70-0	35			
Flumioxazin	103361-09-7	50			
Gabapentin-Lactam	64744-50-9	5	1909.1	1726.5	124.5
Indoxacarb	173584-44-6	2			
Isoxaben	82558-50-7	0.3		0.9	
Methyltriphenylphosphonium	15912-74-0	0.5	0.6		
Metolachlor-NOA 413173	1418095-19-8	2	12.4		
Tetrapropylammonium	13010-31-6	0.5			

Table S3: Continuation (9).

Pyridaben	96489-71-3	2			
Pyriproxyfen	95737-68-1	0.5			
Tepraloxydim	149979-41-9	0.5			
Trichlorfon	52-68-6	50			
Azoxystrobin acid	1185255-09-7	1	2.5	7	
Dimethenamid ESA	205939-58-8	2			
Metazachlor OA	1231244-60-2	7			
Quinmerac BH518-2	90717-07-0	500			
2-Amido-3,5,6-trichloro-4-cyanobenzenesulfonic acid	1418095-02-9	50			
Methyldesphenylchloridazon	17254-80-7	3			
Prochloraz BTS44596	139520-94-8	0.5			
2-Hydroxydesethylterbuthylazine	66753-06-8	1			
2-Trifluoromethyl-benzenesulfonamide	1869-24-5	500			
Vancomycin	1404-90-6	80		409.2	
Tylosin	1401-69-0	10			
Spinosyn A	131929-60-7	10			
Emamectin B1a	121124-29-6	2			
Ivermectin B1a	71827-03-7	10			
Methiocarb-sulfoxide phenol	22454-92-8	2			
2-(Piperazin-1-yl)ethanamine	140-31-8	180			
4-Hydroxy-1-(2-hydroxyethyl)-2,2,6,6-tetramethylpiperidine	52722-86-8	35			
Dicyclohexyl sulfosuccinate	137361-04-7	2		4	
Dimethachlor CGA369873	NOCAS_1017801	5	11.6	24.6	11.3
Dimethyl-5-sulfoisophthalate	138-25-0	2	109.1	159.4	14.8
Metazachlor BH479-12	NOCAS_1017810	200			
Phenylethylmalonamide	7206-76-0	1	154.4	119.1	72.8
Trifloxystrobin NOA413161	d	2	8.1	7.4	
Trifloxystrobin NOA413163	e	2			
Tripropyl phosphate	513-08-6	1			
Thiadone	84352-75-0	15			
Dimethenamid OA	380412-59-9	1.5			
Metolachlor CGA 357704	f	400			
Metolachlor CGA 368208	446027-17-4	160			
Prochloraz BTS40348	67747-01-7	0.5			
Metazachlor BH 479-9	g	8			
Metazachlor BH 479-11	h	0.5	0.8	2.1	
Simazine 2-Hydroxy	03-11-1999	5			
Etoposide	i	20			
Tributylamine	102-82-9	1.8	2.8	28.6	7.2
N-Methyldodecylamine	7311-30-0	1			
2-Amino-9H-pyrido[2,3-b]indole (AalphaC)	26148-68-5	0.5			
2-Naphthol-8-sulfonic acid	132-57-0	5			
Benzyldimethyltetradecylammonium	16287-71-1	0.5			
N,N-Dimethyltetradecylamine-N-oxide	3332-27-2	1			
N,N-Dimethyldodecylamine	112-18-5	5			
N,N-Dimethyldecylamine	1120-24-7	30			
N,N-Dimethyltetradecylamine	112-75-4	5			
Salinomycin	53003-10-4	2			
Piperine	94-62-2	0.5	1.9	3.7	
4-Hydroxycoumarin	1076-38-6	500			
DINCH	318292-43-2	40			
Quinoline	91-22-5	60		60.9	
Octyl-3,5-di-tert-butyl-4-hydroxyhydrocinnamate	13417-12-4	200	2	2	
Benzophenone-4	4065-45-6	1	225	1116	

S4

Table S4: Elimination of chemical compounds by ozone (with subsequent sand filtration).

Chemical substance	Chemical group	Ozone elimination rate
Diuron	biocide	60%
Fipronil	biocide	71%
Carbendazim	biocide	92%
2-Aminobenzimidazole	biocide	68%
Terbutryn	biocide	77%
Isoproturon	pesticide	96%
Trifloxystrobin	pesticide	67%
Carbetamide	pesticide	67%
Metalaxyl	pesticide	83%
Imidacloprid	pesticide	47%
Metamitron	pesticide	71%
Terbuthylazine	pesticide	67%
Terbuthylazine-2-hydroxy	pesticide	0%
Propiconazole	pesticide	75%
Metolachlor OA	pesticide	67%
Metolachlor ESA	pesticide	83%
Chlorothalonil-4-hydroxy	pesticide	67%
Tebuconazole	pesticide	50%
Thiacloprid amide	pesticide	50%
Fenuron	pesticide	50%
Lenacil	pesticide	71%
Boscalid	pesticide	71%
Acetamiprid	pesticide	75%
Mepiquat	pesticide	49%
Metazachlor ESA	pesticide	67%
Dimethachlor	pesticide	44%
Dimethachlor oxalamic acid (OA)	pesticide	68%
Diethyltoluamide (DEET)	pesticide	80%
Harman	food ingredient	97%
Harmine	food ingredient	0%
Acesulfame	food ingredient	40%
Sucralose	food ingredient	19%
Denatonium	industrial chemical	72%
1H-Benzotriazole	industrial chemical	78%
5-Methyl-1H-Benzotriazole	industrial chemical	86%
4-Hydroxybenzotriazole	industrial chemical	96%
7-Diethylamino-4-methylcoumarin	industrial chemical	83%
4'-Aminoacetanilide	industrial chemical	58%
p-Toluenesulfonamide	industrial chemical	75%
Hexa(methoxymethyl)melamine	industrial chemical	59%
Triphenylphosphine oxide	industrial chemical	78%
N-Ethyl-o-toluenesulfonamide	industrial chemical	82%
Tetraglyme	industrial chemical	71%
4-(Dimethylamino)pyridine	industrial chemical	89%
m-Xylene-4-sulfonic acid	industrial chemical	81%
Cyclohexylamine	industrial chemical	79%
1,3-Diphenylguanidine	industrial chemical	89%

Table S4: Continuation (1).

1-Butyl-3-methyl-imidazolium	industrial chemical	90%
4-Hydroxyquinoline	industrial chemical	17%
Dimethyl-5-sulfoisophthalate	industrial chemical	91%
Phenylethylmalonamide	industrial chemical	39%
Tributylamine	industrial chemical	76%
2-(Methylthio)benzothiazole	industrial chemical	65%
Benzothiazole	industrial chemical	73%
2-Benzothiazolesulfonic acid	industrial chemical	48%
Perfluorooctanoic acid	PFC	56%
Perfluorooctanesulfonic acid	PFC	53%
Perfluorohexanoic acid	PFC	45%
Perfluoroheptanoic acid	PFC	50%
6:2 fluorotelomer sulfonic acid	PFC	86%
Tris(2-chloroethyl)phosphate	PFC	10%
Celecoxib	pharmaceuticals	47%
Sulfapyridine	pharmaceuticals	83%
Trimethoprim	pharmaceuticals	99%
Sulfamethoxazole	pharmaceuticals	91%
Clarithromycin	pharmaceuticals	99%
Cyclophosphamide	pharmaceuticals	0%
Fluconazole	pharmaceuticals	51%
Amantadine	pharmaceuticals	66%
Metoprolol	pharmaceuticals	89%
Atenolol	pharmaceuticals	95%
Propranolol	pharmaceuticals	97%
Valsartan	pharmaceuticals	91%
Bisoprolol	pharmaceuticals	90%
Telmisartan	pharmaceuticals	86%
Candesartan	pharmaceuticals	83%
Nitrendipin	pharmaceuticals	75%
Fluvoxamine	pharmaceuticals	98%
Citalopram	pharmaceuticals	90%
Amitriptyline	pharmaceuticals	94%
Sertraline	pharmaceuticals	82%
Temazepam	pharmaceuticals	73%
Bupropion	pharmaceuticals	71%
Lorazepam	pharmaceuticals	69%
Memantine	pharmaceuticals	60%
Oxazepam	pharmaceuticals	79%
Lamotrigine	pharmaceuticals	32%
Carbamazepine	pharmaceuticals	99%
10,11-Dihydro-10,11-dihydroxycarbamazepine	pharmaceuticals	72%
2-Hydroxycarbamazepine	pharmaceuticals	76%
10,11-Dihydro-10-hydroxycarbamazepine	pharmaceuticals	69%
Verapamil	pharmaceuticals	97%
Hydrochlorothiazide	pharmaceuticals	92%
N-Acetyl-4-aminoantipyrine	pharmaceuticals	99%
4-Aminoantipyrine	pharmaceuticals	97%
Cetirizine	pharmaceuticals	99%
Diphenhydramine	pharmaceuticals	94%

Table S4: Continuation (2).

Metformin	pharmaceuticals	41%
Lidocaine	pharmaceuticals	95%
Tramadol	pharmaceuticals	92%
N-Formyl-4-aminoantipyrine	pharmaceuticals	99%
Acetyl-sulfamethoxazole	pharmaceuticals	93%
Edifenphos (EDDP)	pharmaceuticals	57%
Mebeverine	pharmaceuticals	0%
Anastrozole	pharmaceuticals	50%
Bicalutamide	pharmaceuticals	30%
Ifosfamide	pharmaceuticals	40%
Tetracaine	pharmaceuticals	83%
Metoprolol acid	pharmaceuticals	27%
Scopolamine-N-butyl	pharmaceuticals	81%
1-(3-carboxypropyl)-3,7-dimethylxanthine	pharmaceuticals	89%
Climbazole	pharmaceuticals	97%
Torasemide	pharmaceuticals	91%
Tri(butoxyethyl)phosphate	Plastic additives and flameretardants	97%
Tris(1,3-dichloroisopropyl)phosphate (TDCPP)	Plastic additives and flameretardants	21%
Tris(1-chloro-2-propyl)phosphate (TCPP)	Plastic additives and flameretardants	48%
Triethylphosphate (TEP)	Plastic additives and flameretardants	69%
Phenylbenzimidazole sulfonic acid	UV-filter	77%
Theophyllin	Other	29%
Cotinine	Other	79%

S5

Table S5: Toxic Units for different species. EC50: Effect concentration for 50% of species. LC50: Lethal concentration for 50% of species.

			mg/L				
			Daphnia magna	Daphnid	*Danio rerio*	*Oncorhynchus mykiss*	Fish
Diuron	330-54-1	EC50	1.20E-01		1.88E+00	4.30E+00	
		LC50		1.40E+00			1.90E+00
		WWTP_1	1.50E-04		9.57E-06	4.19E-06	
		WWTP_2_-O3	1.67E-04		1.06E-05	4.65E-06	
		WWTP_2_+O3	6.67E-05		4.26E-06	1.86E-06	
		WWTP_1		1.43E-05			9.47E-06
		WWTP_2_-O3		5.71E-06			1.05E-05
		WWTP_2_+O3		5.71E-06			4.21E-06
Imazalil	35554-44-0	EC50	3.54E+00		2.44E-09		
		LC50		1.06E+00		1.48E+00	1.35E-01
		WWTP_1	8.47E-07	2.83E-06	1.23E+03	2.03E-06	2.22E-05
		WWTP_2_-O3	1.13E-06	3.77E-06	1.64E+03	2.70E-06	2.96E-05
		WWTP_2_+O3					
Fipronil	120068-37-3	EC50	3.48E-02		1.62E-01		
		LC50		1.00E-01			3.00E-02
		WWTP_1	8.62E-05	3.00E-05	1.86E-05		1.00E-04
		WWTP_2_-O3	2.01E-04	7.00E-05	4.33E-05		2.33E-04
		WWTP_2_+O3	5.75E-05	2.00E-05	1.24E-05		6.67E-05
Thiabendazole	148-79-8	EC50	3.09E-01		3.03E-08		
		LC50		8.50E-01		5.60E-01	5.60E-01
		WWTP_1					
		WWTP_2_-O3	2.59E-05	9.41E-06	2.64E+02	1.43E-05	1.43E-05
		WWTP_2_+O3					
Bendiocarb	22781-23-3	EC50	2.35E-02				
		LC50		2.49E-01			3.52E+00
		WWTP_1	1.28E-04	1.20E-05			8.52E-07
		WWTP_2_-O3	2.55E-04	2.41E-05			1.70E-06
		WWTP_2_+O3					
Carbendazim	10605-21-7	EC50	2.29E-02		1.08E+00		
		LC50		2.32E+01		2.40E-03	2.85E+01
		WWTP_1	2.18E-04	2.16E-07	4.63E-06	2.08E-03	1.75E-07
		WWTP_2_-O3	1.05E-03	1.03E-06	2.22E-05	1.00E-02	8.42E-07
		WWTP_2_+O3	8.73E-05	8.62E-08	1.85E-06	8.33E-04	7.02E-08
2-Aminobenzimidazole	934-32-7	LC50		2.68E+00			7.77E+01
		WWTP_1		6.72E-06			2.32E-07
		WWTP_2_-O3		6.19E-05			2.14E-06
		WWTP_2_+O3		1.98E-05			6.82E-07
Warfarin	81-81-2	EC50	5.60E+01		5.98E+01		
		LC50		1.28E+01			5.02E+00
		WWTP_1	1.79E-08	7.81E-08	1.67E-08		1.99E-07
		WWTP_2_-O3	1.79E-08	7.81E-08	1.67E-08		1.99E-07
		WWTP_2_+O3					
Dichlorophen	97-23-4	EC50				9.90E-02	
		LC50		1.79E+00			1.20E-01
		WWTP_1					
		WWTP_2_-O3		5.59E-07		1.01E-05	8.33E-06
		WWTP_2_+O3					

Table S5: Continuation (1).

			mg/L				
			Daphnia magna	Daphnid	*Danio rerio*	*Oncorhynchus mykiss*	Fish
Genistein	446-72-0	EC50			1.83E+00		
		LC50		2.26E+00			5.41E-01
		WWTP_1		5.31E-06	6.56E-06		2.22E-05
		WWTP_2_-O3		3.10E-06	3.83E-06		1.29E-05
		WWTP_2_+O3					
Daidzein	486-66-8	LC50		3.52E+01			1.93E+01
		WWTP_1		2.84E-08			5.18E-08
		WWTP_2_-O3					
		WWTP_2_+O3					
Harman	486-84-0	LC50		1.93E+01			3.16E+01
		WWTP_1		4.15E-07			2.53E-07
		WWTP_2_-O3		1.66E-06			1.01E-06
		WWTP_2_+O3		5.18E-08			3.16E-08
Harmine	442-51-3	LC50		1.91E+01			3.12E+01
		WWTP_1		5.24E-08			3.21E-08
		WWTP_2_-O3		5.24E-08			3.21E-08
		WWTP_2_+O3		5.24E-08			3.21E-08
Piperine	94-62-2	LC50		7.80E+00			3.20E-01
		WWTP_1		2.56E-07			6.25E-06
		WWTP_2_-O3		5.13E-07			1.25E-05
		WWTP_2_+O3					
Acesulfame	33665-90-6	LC50		5.51E+04			1.32E+07
		WWTP_1		1.13E-07			4.73E-10
		WWTP_2_-O3		2.98E-09			1.24E-11
		WWTP_2_+O3		1.78E-09			7.42E-12
Sucralose	56038-13-2	LC50		6.99E+04			1.63E+05
		WWTP_1		1.01E-07			4.35E-08
		WWTP_2_-O3		6.72E-07			2.88E-07
		WWTP_2_+O3		5.43E-07			2.33E-07
Saccharin	81-07-2	EC50			4.75E+03		
		LC50		1.76E+00			1.33E+00
		WWTP_1		1.68E-04	6.23E-08		2.23E-04
		WWTP_2_-O3					
		WWTP_2_+O3					

Table S5: Continuation (2).

			mg/L				
			Daphnia magna	Daphnid	*Danio rerio*	*Oncorhynchus mykiss*	Fish
Denatonium	47324-98-1	LC50		8.91E+01			9.16E+01
		WWTP_1		1.66E-06			1.62E-06
		WWTP_2_-O3		3.45E-06			3.35E-06
		WWTP_2_+O3		9.54E-07			9.28E-07
1H-Benzotriazole	95-14-7	EC50		9.71E+01			
		LC50		1.42E+02			3.90E+01
		WWTP_1		6.39E-05			1.59E-04
		WWTP_2_-O3		7.68E-05			1.91E-04
		WWTP_2_+O3		1.71E-05			4.25E-05
		WWTP_1		4.38E-05			1.35E-03
		WWTP_2_-O3		5.26E-05			1.62E-03
		WWTP_2_+O3		1.17E-05			3.60E-04
5-Chlorobenzotriazole	94-97-3	LC50		9.23E+01			2.18E+01
		WWTP_1		7.58E-08			3.21E-07
		WWTP_2_-O3		3.25E-08			1.38E-07
		WWTP_2_+O3		5.42E-08			2.29E-07
7-Amino-4-methylcoumarin	26093-31-2	LC50		3.76E+01			7.02E+01
		WWTP_1		1.06E-07			5.70E-08
		WWTP_2_-O3		1.86E-07			9.97E-08
		WWTP_2_+O3					
7-Diethylamino-4-methylcoumarin	91-44-1	EC50			2.36E+01		
		LC50		1.09E+01			6.00E+00
		WWTP_1		7.34E-07	3.39E-07		1.33E-06
		WWTP_2_-O3		5.50E-07	2.54E-07		1.00E-06
		WWTP_2_+O3		9.17E-08	4.24E-08		1.67E-07
Iminostilbene	256-96-2	LC50		1.54E+00			2.25E+00
		WWTP_1					
		WWTP_2_-O3					
		WWTP_2_+O3					
Isophorone diamine	2855-13-2	EC50	1.74E+01				
		LC50		3.63E-01			2.29E+00
		WWTP_1		3.06E-04			4.85E-05
		WWTP_2_-O3		5.04E-04			7.99E-05
		WWTP_2_+O3					
4'-Aminoacetanilide	122-80-5	LC50		4.70E-02			2.25E+00
		WWTP_1		2.68E-02			5.60E-04
		WWTP_2_-O3		4.80E-02			1.00E-03
		WWTP_2_+O3		2.03E-02			4.25E-04
N-Butylbenzenesulfonamide	3622-84-2	LC50		1.54E+00			2.25E+00
		WWTP_1					
		WWTP_2_-O3					
		WWTP_2_+O3					
p-Toluenesulfonamide	70-55-3	LC50		1.54E+00			2.25E+00
		WWTP_1					
		WWTP_2_-O3					
		WWTP_2_+O3					
Triphenylphosphine oxide	791-28-6	LC50		8.00E-02			1.85E+01
		WWTP_1		3.50E-04			1.51E-06
		WWTP_2_-O3		3.46E-03			1.50E-05
		WWTP_2_+O3		7.63E-04			3.30E-06
o-Dianisidine	119-90-4	LC50		1.54E+00			2.25E+00
		WWTP_1		8.64E-05			5.91E-05
		WWTP_2_-O3		2.19E-04			1.50E-04
		WWTP_2_+O3					
N-Ethyl-o-toluenesulfonamide	1077-56-1	LC50		1.09E+01			8.60E-01
		WWTP_1		3.39E-06			4.30E-05
		WWTP_2_-O3		8.17E-06			1.03E-04
		WWTP_2_+O3		1.47E-06			1.86E-05

Table S5: Continuation (3).

Triglyme	112-49-2	LC50		9.25E+02			3.17E+03
		WWTP_1		8.65E-09			2.52E-09
		WWTP_2_-O3		4.22E-08			1.23E-08
		WWTP_2_+O3					
Benzenesulfonic acid	98-11-3	LC50		1.66E+04			6.26E+04
		WWTP_1		1.66E-08			4.41E-09
		WWTP_2_-O3		8.07E-09			2.14E-09
		WWTP_2_+O3		1.19E-08			3.15E-09
Tetraglyme	143-24-8	LC50		4.13E+04			9.62E+04
		WWTP_1		4.36E-10			1.87E-10
		WWTP_2_-O3		5.81E-10			2.49E-10
		WWTP_2_+O3		1.69E-10			7.28E-11
2,4-Dichlorophenol	120-83-2	EC50	2.00E-05		1.08E-05		
		LC50		2.60E+00		1.60E-05	4.20E+00
		NOEC					6.10E-09
		LOEC					6.10E-09
		WWTP_1	6.00E-01	4.62E-06	1.11E+00	7.50E-01	2.86E-06
		WWTP_2_-O3	8.00E-01	6.15E-06	1.48E+00	1.00E+00	3.81E-06
		WWTP_2_+O3					
		WWTP_1					1.97E+03
		WWTP_2_-O3					2.62E+03
		WWTP_2_+O3					
2-Oxindole	59-48-3	LC50		2.72E+01			1.78E+00
		WWTP_1					
		WWTP_2_-O3		2.61E-06			3.99E-05
		WWTP_2_+O3					
m-Xylene-4-sulfonic acid	88-61-9	LC50		2.72E+01			1.78E+00
		WWTP_1		1.35E-05			2.07E-04
		WWTP_2_-O3		3.81E-05			5.81E-04
		WWTP_2_+O3		7.17E-06			1.10E-04
Acridine	260-94-6	EC50	1.02E+03				
		LC50		6.18E+00			9.63E+00
		WWTP_1					
		WWTP_2_-O3	3.22E-08	5.34E-06			3.43E-06
		WWTP_2_+O3					
Acridone	578-95-0	LC50		4.46E+00			7.05E+00
		WWTP_1		2.91E-06			1.84E-06
		WWTP_2_-O3		1.35E-06			8.51E-07
		WWTP_2_+O3		2.24E-06			1.42E-06
Cyclohexylamine	108-91-8	EC50	4.90E+01				
		LC50		3.80E+00			3.33E+01
		WWTP_1	8.14E-06	1.05E-04			1.20E-05
		WWTP_2_-O3	1.37E-05	1.77E-04			2.02E-05
		WWTP_2_+O3	2.80E-06	3.61E-05			4.11E-06
1,3-Dimethyl-2-imidazolidinone	80-73-9	LC50		1.39E+00			1.05E+01
		WWTP_1		2.59E-05			3.43E-06
		WWTP_2_-O3					
		WWTP_2_+O3					
1,3-Diphenylguanidine	102-06-7	LC50		1.39E+00			1.05E+01
		WWTP_1		3.09E-05			4.10E-06
		WWTP_2_-O3		1.10E-04			1.46E-05
		WWTP_2_+O3		1.22E-05			1.62E-06
2-Hydroxyquinoline	59-31-4	EC50	5.13E+01				
		LC50		2.40E+01		1.07E+01	9.05E+00
		WWTP_1	3.90E-07	8.33E-07		1.87E-06	2.21E-06
		WWTP_2_-O3	8.38E-07	1.79E-06		4.02E-06	4.75E-06
		WWTP_2_+O3					
Phenylethylmalonamide	7206-76-0	LC50		1.27E+03			1.03E+03
		WWTP_1		1.21E-07			1.50E-07
		WWTP_2_-O3		9.37E-08			1.16E-07
		WWTP_2_+O3		5.75E-08			7.09E-08

Table S5: Continuation (4).

Tributylamine	102-82-9	EC50	8.00E+00				
		LC50		3.70E-01			8.64E-01
		WWTP_1	3.75E-07	8.11E-06			3.47E-06
		WWTP_2_-O3	3.63E-06	7.84E-05			3.36E-05
		WWTP_2_+O3	8.75E-07	1.89E-05			8.10E-06
Quinoline	91-22-5	EC50	5.13E+01				
		LC50		2.85E+01		1.07E+01	7.78E+01
		WWTP_1					
		WWTP_2_-O3	1.19E-06	2.14E-06		5.70E-06	7.84E-07
		WWTP_2_+O3					
2-(Methylthio)benzothiazole	615-22-5	LC50		7.52E+00			1.18E+01
		WWTP_1		4.65E-05			2.97E-05
		WWTP_2_-O3		3.46E-05			2.20E-05
		WWTP_2_+O3		1.22E-05			7.80E-06
Benzothiazole	95-16-9	LC50		4.52E+01			7.83E+01
		WWTP_1		3.85E-06			2.22E-06
		WWTP_2_-O3		8.74E-06			5.04E-06
		WWTP_2_+O3		2.35E-06			1.35E-06
2-Benzothiazolesulfonic acid	941-57-1	LC50		3.68E+05			8.55E+05
		WWTP_1		1.55E-09			6.69E-10
		WWTP_2_-O3		3.56E-09			1.53E-09
		WWTP_2_+O3		1.86E-09			8.00E-10
2-Hydroxybenzothiazole	934-34-9	LC50		7.22E+00			3.79E+00
		WWTP_1		8.45E-06			1.61E-05
		WWTP_2_-O3		1.41E-05			2.69E-05
		WWTP_2_+O3					
2-(4-morpholinyl)benzothiazole	4225-26-7	LC50		2.53E+01			4.17E+01
		WWTP_1		1.98E-07			1.20E-07
		WWTP_2_-O3		3.95E-07			2.40E-07
		WWTP_2_+O3					
2-Naphthol-8-sulfonic acid	132-57-0	LC50		2.16E+03			1.52E+04
		WWTP_1		2.31E-09			3.29E-10
		WWTP_2_-O3					
		WWTP_2_+O3					
Dimethyl-5-sulfoisophthalate	138-25-0	LC50		1.22E+05			4.03E+04
		WWTP_1		8.93E-10			2.70E-09
		WWTP_2_-O3		1.30E-09			3.95E-09
		WWTP_2_+O3		1.23E-10			3.72E-10
4-Hydroxybenzotriazole	26725-51-9	LC50		5.87E+01			8.32E+02
		WWTP_1		2.77E-07			1.41E-07
		WWTP_2_-O3		3.65E-07			1.85E-07
		WWTP_2_+O3		1.42E-08			7.21E-09
5-Methyl-1H-benzotriazole	136-85-6	EC50	4.97E+01				
		LC50		9.63E+01			2.16E+01
		WWTP_1	2.20E-05	1.14E-05			5.07E-05
		WWTP_2_-O3	4.82E-05	2.49E-05			1.11E-04
		WWTP_2_+O3	6.76E-06	3.49E-06			1.56E-05
Hexa(methoxymethyl)melamine	68002-20-0	LC50		1.23E+02			5.96E+02
		WWTP_1		2.12E-06			4.38E-07
		WWTP_2_-O3		1.36E-06			2.82E-07
		WWTP_2_+O3		5.60E-07			1.16E-07
1-Butyl-3-methyl-imidazolium	80432-08-2	LC50		6.24E+01			3.45E+02
		WWTP_1		2.89E-07			5.22E-08
		WWTP_2_-O3		7.70E-07			1.39E-07
		WWTP_2_+O3		8.02E-08			1.45E-08
Methyltriphenylphosphonium	15912-74-0	LC50		2.89E-01			1.64E-01
		WWTP_1		3.46E-06			6.08E-06
		WWTP_2_-O3					
		WWTP_2_+O3					
N-Cyclohexyl-2-benzothiazole-amine	40115-03-5	LC50		2.97E+00			4.33E+00
		WWTP_1		1.35E-06			9.25E-07
		WWTP_2_-O3		3.37E-07			2.31E-07
		WWTP_2_+O3					

Table S5: Continuation (5).

			mg/L				
			Daphnia magna	Daphnid	*Danio rerio*	*Oncorhynchus mykiss*	Fish
2,4-Dichlorophenoxyacetic acid	94-75-7	EC50	1.76E+01		1.61E+01		
		LC50		3.05E+02			5.07E+02
		WWTP_1	3.98E-07	2.30E-08	4.34E-07		1.38E-08
		WWTP_2_-O3	6.82E-07	3.93E-08	7.43E-07		2.37E-08
		WWTP_2_+O3					
Bentazone	25057-89-0	NOEL				5.00E+01	
		LC50		1.24E+02			1.22E+02
		WWTP_1		8.06E-09		2.00E-08	8.20E-09
		WWTP_2_-O3		8.06E-09		2.00E-08	8.20E-09
		WWTP_2_+O3					
Isoproturon	34123-59-6	NOEC				2.06E+00	
		EC50	2.06E+00				
		LC50		3.14E+00			1.16E+01
		WWTP_1	1.46E-06	9.55E-07		1.46E-06	2.59E-07
		WWTP_2_-O3	1.26E-05	8.28E-06		1.26E-05	2.24E-06
		WWTP_2_+O3	4.85E-07	3.18E-07		4.85E-07	8.62E-08
Myclobutanil	88671-89-0	EC50	7.22E+00		1.15E+01		
		LC50		1.10E+01			2.40E+00
		WWTP_1	1.39E-07	9.09E-08	8.70E-08		4.17E-07
		WWTP_2_-O3	2.77E-07	1.82E-07	1.74E-07		8.33E-07
		WWTP_2_+O3					
Metribuzin	21087-64-9	EC50	2.90E+00				
		LC50		2.33E+00			1.57E+00
		WWTP_1	3.45E-07	4.29E-07			6.37E-07
		WWTP_2_-O3	3.45E-07	4.29E-07			6.37E-07
		WWTP_2_+O3					
Carbetamide	16118-49-3	LC50		5.27E+01			6.11E+01
		WWTP_1		5.69E-08			4.91E-08
		WWTP_2_-O3		1.14E-07			9.82E-08
		WWTP_2_+O3		3.80E-08			3.27E-08
Metalaxyl	57837-19-1	EC50	2.10E+01				
		LC50		1.20E+02			5.58E+01
		WWTP_1	4.76E-08	8.33E-09			1.79E-08
		WWTP_2_-O3	2.86E-07	5.00E-08			1.08E-07
		WWTP_2_+O3	4.76E-08	8.33E-09			1.79E-08
Dichlorprop	15165-67-0	LC50		1.42E+02			2.27E+02
		WWTP_1		1.41E-08			8.81E-09
		WWTP_2_-O3					
		WWTP_2_+O3					
Imidacloprid	138261-41-3	EC50	3.32E-01		1.54E-01		
		LC50		8.52E+01			4.17E+02
		WWTP_1	2.11E-05	8.22E-08	4.55E-05		1.68E-08
		WWTP_2_-O3	5.72E-05	2.23E-07	1.23E-04		4.56E-08
		WWTP_2_+O3	3.01E-05	1.17E-07	6.49E-05		2.40E-08
Nicosulfuron	111991-09-4	EC50	1.00E+06				
		LC50		7.16E+03		1.00E+06	2.23E+03
		NOEL				1.00E+06	
		WWTP_1					
		WWTP_2_-O3					
		WWTP_2_+O3					
Flufenacet	142459-58-3	EC50			1.13E+01		
		LC50	7.00E+00	4.57E+01		2.70E+00	5.80E+00
		NOEL				4.70E-01	
		WWTP_1	1.43E-07	2.19E-08	8.87E-08	3.70E-07	1.72E-07
		WWTP_2_-O3					
		WWTP_2_+O3					
		WWTP_1		1.14E-07		2.13E-06	1.35E-06
		WWTP_2_-O3					
		WWTP_2_+O3					

Table S5: Continuation (6).

Ethofumesate	26225-79-6	EC50	6.40E+01				
		LC50		2.18E+01		7.50E-01	1.15E+01
		NOEL				3.70E+00	
		WWTP_1	4.38E-07	1.28E-06		3.73E-05	2.43E-06
		WWTP_2_-O3	8.13E-07	2.39E-06		6.93E-05	4.52E-06
		WWTP_2_+O3					
		WWTP_1				7.57E-06	
		WWTP_2_-O3				1.41E-05	
		WWTP_2_+O3					
Metamitron	41394-05-2	EC50	9.70E+01				
		LC50		2.26E+00			1.11E+00
		WWTP_1					
		WWTP_2_-O3	7.22E-08	3.10E-06			6.31E-06
		WWTP_2_+O3	2.06E-08	8.85E-07			1.80E-06
Spiroxamine	118134-30-8	EC50	2.50E+00				
		LC50		2.50E+00		1.70E+01	2.84E-01
		WWTP_1	4.00E-07	4.00E-07		5.88E-08	3.52E-06
		WWTP_2_-O3					
		WWTP_2_+O3					
Fenpropimorph	67564-91-4	EC50	1.72E+00				
		LC50		2.30E+00		2.26E+00	
		WWTP_1	5.81E-07	4.35E-07		4.42E-07	
		WWTP_2_-O3					
		WWTP_2_+O3					
Epoxiconazole	133855-98-8	EC50	2.64E+00				
		NOEC			1.00E-01		
		LC50		6.62E+00			5.17E+00
		WWTP_1					
		WWTP_2_-O3	7.58E-07	3.02E-07	2.00E-05		3.87E-07
		WWTP_2_+O3					
Terbuthylazine	5915-41-3	EC50	1.60E+01		1.30E+01		
		LC50		8.79E+00		2.40E+00	3.40E+00
		NOEL				1.90E+00	
		LOEL				4.58E-02	
		WWTP_1					
		WWTP_2_-O3	1.88E-07	3.41E-07	2.31E-07	1.25E-06	8.82E-07
		WWTP_2_+O3	6.25E-08	1.14E-07	7.69E-08	4.17E-07	2.94E-07
		WWTP_1					
		WWTP_2_-O3				1.58E-06	
		WWTP_2_+O3				5.26E-07	
		WWTP_1					
		WWTP_2_-O3				6.55E-05	
		WWTP_2_+O3				2.18E-05	
Atrazine	1912-24-9	EC50	4.20E-01		1.85E+01		
In der Datei von Tobias nachscauen		LC50				6.30E-01	4.50E+00
		WWTP_1	4.76E-06		1.08E-07	3.17E-06	4.44E-07
		WWTP_2_-O3					
		WWTP_2_+O3					
2-Hydroxyatrazine	2163-68-0	LC50		1.05E+01			2.32E+01
		WWTP_1		1.90E-06			8.62E-07
		WWTP_2_-O3					
		WWTP_2_+O3					
Deethylatrazine	6190-65-4	EC50	3.56E+01				
		LC50		2.22E+00			2.82E+01
		NOEC				1.47E-01	
		LOEC			3.00E-05		
		WWTP_1	1.40E-07	2.25E-06	1.67E-01	3.40E-05	1.77E-07
		WWTP_2_-O3	2.53E-07	4.05E-06	3.00E-01	6.12E-05	3.19E-07
		WWTP_2_+O3					

Table S5: Continuation (7).

Metolachlor	51218-45-2	EC50	4.25E+00		9.57E+00		
		LC50		5.85E+00		3.16E-03	1.75E+00
		WWTP_1	2.82E-06	2.05E-06	1.25E-06	3.80E-03	6.86E-06
		WWTP_2_-O3					
		WWTP_2_+O3					
Simazine	122-34-9	EC50	5.60E-01		8.00E-01		
		LC50		1.10E+00		3.40E+01	1.60E+01
		NOEC				1.57E-01	
		WWTP_1					
		WWTP_2_-O3	1.07E-05	5.45E-06	7.50E-06	1.76E-07	3.75E-07
		WWTP_2_+O3					
		WWTP_1					
		WWTP_2_-O3		6.54E-06		3.82E-05	4.00E-06
		WWTP_2_+O3					
Mecoprop	16484-77-8	EC50	9.09E+01				
		LC50		1.57E+02			2.54E+02
		WWTP_1	1.76E-07	1.02E-07			6.30E-08
		WWTP_2_-O3	9.90E-08	5.73E-08			3.54E-08
		WWTP_2_+O3					
2-methyl-4-chlorophenoxyacetic acid (MCPA)	94-74-6	EC50	1.80E+02				
		LC50		3.35E+02		7.20E+01	5.62E+02
		WWTP_1	5.56E-09	2.99E-09		1.39E-08	1.78E-09
		WWTP_2_-O3	5.56E-08	2.99E-08		1.39E-07	1.78E-08
		WWTP_2_+O3					
Tebuconazole	107534-96-3	EC50	2.10E+00				
		LC50		2.80E+00		3.80E+00	4.40E+00
		NOEC				1.50E+00	
		WWTP_1	4.76E-07	3.57E-07		2.63E-07	2.27E-07
		WWTP_2_-O3	9.52E-07	7.14E-07		5.26E-07	4.55E-07
		WWTP_2_+O3	4.76E-07	3.57E-07		2.63E-07	2.27E-07
		WWTP_1				6.67E-07	
		WWTP_2_-O3				1.33E-06	
		WWTP_2_+O3				6.67E-07	
Fenuron	101-42-8	LC50		7.20E+01		2.04E+02	1.46E+02
		WWTP_1		1.53E-07		5.39E-08	7.53E-08
		WWTP_2_-O3		3.89E-07		1.37E-07	1.92E-07
		WWTP_2_+O3		1.94E-07		6.86E-08	9.59E-08
3,5,6-Trichloro-2-pyridinol	6515-38-4	LC50		8.36E-01		1.50E+00	1.10E+00
		WWTP_1		1.44E-05		8.00E-06	1.09E-05
		WWTP_2_-O3		1.56E-05		8.67E-06	1.18E-05
		WWTP_2_+O3					
Boscalid	188425-85-6	EC50	4.00E+00		3.90E-01		
		LC50		9.81E-01		2.70E+00	1.14E+00
		NOEL				1.88E+00	
		WWTP_1	2.50E-06	1.02E-05	2.56E-05	3.70E-06	8.77E-06
		WWTP_2_-O3	1.75E-06	7.14E-06	1.79E-05	2.59E-06	6.14E-06
		WWTP_2_+O3	5.00E-07	2.04E-06	5.13E-06	7.41E-07	1.75E-06
		WWTP_1				5.32E-06	
		WWTP_2_-O3				3.72E-06	
		WWTP_2_+O3				1.06E-06	
Propamocarb	24579-73-5	EC50	1.06E+02				
		LC50		1.46E+01			1.14E+02
		WWTP_1	5.66E-08	4.11E-07			5.26E-08
		WWTP_2_-O3					
		WWTP_2_+O3					
Triadimenol	55219-65-3	EC50	2.10E+00		8.85E+00		
		LC50		2.50E+00		1.20E+01	1.40E+01
		NOEC				6.90E+00	
		WWTP_1					
		WWTP_2_-O3	9.52E-07	8.00E-07	2.26E-07	1.67E-07	1.43E-07
		WWTP_2_+O3					
		WWTP_1					
		WWTP_2_-O3		1.25E-05		2.90E-07	3.33E-05
		WWTP_2_+O3					

Table S5: Continuation (8).

2,4-Dichlorobenzoic acid	50-84-0	LC50		1.76E+02			2.87E+02
		WWTP_1					
		WWTP_2_-O3		5.57E-07			3.41E-07
		WWTP_2_+O3					
Acetamiprid	135410-20-7	EC50	4.60E+01				
		LC50		2.38E+00	7.45E+00	1.00E+02	1.87E+01
		NOEC			6.70E-01		
		NOEL				3.50E+01	
		WWTP_1	6.52E-08	1.26E-06	1.34E-07	3.00E-08	1.60E-07
		WWTP_2_-O3	8.70E-08	1.68E-06		4.00E-08	2.14E-07
		WWTP_2_+O3	2.17E-08	4.20E-07		1.00E-08	5.35E-08
		WWTP_1				8.57E-08	
		WWTP_2_-O3				1.14E-07	
		WWTP_2_+O3				2.86E-08	
Mepiquat	15302-91-7	LC50		1.40E+04			3.20E+04
		WWTP_1		1.44E-08			6.31E-09
		WWTP_2_-O3		1.28E-08			5.59E-09
		WWTP_2_+O3		6.50E-09			2.84E-09
Clothianidin	210880-92-5	EC50	5.00E-01				
		LC50		3.79E+01		1.06E+02	3.73E+02
		NOEL				1.06E+02	
		WWTP_1					
		WWTP_2_-O3					
		WWTP_2_+O3					
Trifloxystrobin	141517-21-7	EC50	1.70E+00		2.66E-07		
		LC50		1.50E-02		1.40E+01	7.30E-02
		NOEL	1.80E+01			7.20E+00	
		WWTP_1	5.88E-07	6.67E-05	3.76E+00	7.14E-08	1.37E-05
		WWTP_2_-O3	1.76E-06	2.00E-04	1.13E+01	2.14E-07	4.11E-05
		WWTP_2_+O3	5.88E-07	6.67E-05	3.76E+00	7.14E-08	1.37E-05
		WWTP_1	5.56E-08			1.39E-07	
		WWTP_2_-O3	1.67E-07			4.17E-07	
		WWTP_2_+O3	5.56E-08			1.39E-07	
Imazapyr	81334-34-1	EC50	1.00E+02				
		LC50		1.65E+03		1.00E+02	1.59E+03
		NOEC			1.00E+01		
		NOEL				1.00E+02	
		WWTP_1	1.10E-07	6.67E-09	1.10E-06	1.10E-07	6.92E-09
		WWTP_2_-O3	1.70E-07	1.03E-08	1.70E-06	1.70E-07	1.07E-08
		WWTP_2_+O3					
Isoxaben	82558-50-7	EC50	1.30E+00		1.13E+01		
		LC50		1.93E+00		1.10E+00	2.67E+00
		NOEL				9.25E+01	
		WWTP_1					
		WWTP_2_-O3	7.69E-07	5.18E-07	8.83E-08	9.09E-07	3.75E-07
		WWTP_2_+O3					
		WWTP_1					
		WWTP_2_-O3				1.08E-08	
		WWTP_2_+O3					

Table S5: Continuation (9).

Dimethenamid	87674-68-8	EC50	1.20E+01		8.41E+00		
		LC50		1.60E+01		1.70E+00	2.60E+00
		WWTP_1	1.67E-07	1.25E-07	2.38E-07	1.18E-06	7.69E-07
		WWTP_2_-O3					
		WWTP_2_+O3					
Metazachlor	67129-08-2	LC50		1.08E+01			5.16E+00
		WWTP_1					
		WWTP_2_-O3					
		WWTP_2_+O3					
Dimethachlor	50563-36-5	LC50		1.34E+01			5.05E+00
		WWTP_1		8.96E-07			2.38E-06
		WWTP_2_-O3		1.87E-06			4.95E-06
		WWTP_2_+O3		8.21E-07			2.18E-06
Trifloxystrobin NOA413161	141517-21-7	EC50	1.70E+00		2.66E-07		
		LC50		1.50E-02		1.40E+01	7.30E-02
		NOEL				7.20E+00	
		WWTP_1	4.71E-06	5.33E-04	3.01E+01	5.71E-07	1.10E-04
		WWTP_2_-O3	4.12E-06	4.67E-04	2.63E+01	5.00E-07	9.59E-05
		WWTP_2_+O3					
		WWTP_1				1.11E-06	
		WWTP_2_-O3				9.72E-07	
		WWTP_2_+O3					
Diethyltoluamide (DEET)	134-62-3	EC50	5.60E+01				
		LC50		3.14E+01		7.13E+01	3.36E+01
		NOEL				5.60E+01	
		WWTP_1	3.02E-06	5.38E-06		2.37E-06	5.03E-06
		WWTP_2_-O3	2.18E-06	3.89E-06		1.71E-06	3.63E-06
		WWTP_2_+O3	4.46E-07	7.96E-07		3.51E-07	7.44E-07
		WWTP_1				3.02E-06	
		WWTP_2_-O3				2.18E-06	
		WWTP_2_+O3				4.46E-07	
Chlorothalonil-4-hydroxy	28343-61-5	LC50		4.25E+00			6.00E+00
		WWTP_1		2.35E-07			1.67E-07
		WWTP_2_-O3		7.06E-07			5.00E-07
		WWTP_2_+O3		2.35E-07			1.67E-07
Lenacil	2164-08-1	LC50		1.16E+00			7.58E+00
		WWTP_1		2.59E-06			3.96E-07
		WWTP_2_-O3		6.03E-06			9.23E-07
		WWTP_2_+O3		1.72E-06			2.64E-07
Pethoxamid	106700-29-2	LC50		1.26E+01			2.90E+01
		WWTP_1		1.58E-07			6.90E-08
		WWTP_2_-O3					
		WWTP_2_+O3					
Desphenyl chloridazon	6339-19-1	LC50		9.27E+01			5.85E+02
		WWTP_1					
		WWTP_2_-O3					
		WWTP_2_+O3					
Methyldesphenylchloridazon	17254-80-7	LC50		4.55E+02			5.03E+03
		WWTP_1					
		WWTP_2_-O3					
		WWTP_2_+O3					
Azoxystrobin	1185255-09-7	LC50		1.19E+01			2.36E+01
		WWTP_1		1.69E-07			8.47E-08
		WWTP_2_-O3		2.53E-07			1.27E-07
		WWTP_2_+O3					
Terbuthylazine-2-hydroxy	66753-07-9	LC50		1.27E+02			7.81E+02
		WWTP_1		1.03E-07			1.66E-08
		WWTP_2_-O3		1.03E-07			1.66E-08
		WWTP_2_+O3		1.03E-07			1.66E-08
Desethylterbutylazine	30125-63-4	LC50		5.52E+01			2.54E+02
		WWTP_1					
		WWTP_2_-O3					
		WWTP_2_+O3					

Table S5: Continuation (10).

Thiacloprid amide	676228-91-4	LC50		9.30E+01			4.65E+02
		WWTP_1					
		WWTP_2_-O3		2.15E-08			4.30E-09
		WWTP_2_+O3		1.08E-08			2.15E-09
Prothioconazole-desthio	120983-64-4	LC50		1.60E+01			3.94E+01
		WWTP_1		6.24E-08			2.54E-08
		WWTP_2_-O3		1.87E-07			7.62E-08
		WWTP_2_+O3					
Dimethachlor oxalamic acid (OA)	1086384-49-7	LC50		8.83E+01			4.45E+02
		WWTP_1		1.47E-07			2.92E-08
		WWTP_2_-O3		2.49E-07			4.94E-08
		WWTP_2_+O3		7.93E-08			1.57E-08

			mg/L				
			Daphnia magna	Daphnid	*Danio rerio*	*Oncorhynchus mykiss*	Fish
Perfluorooctanoic acid	335-67-1	EC50	5.11E-04		1.13E+02		
		LC50		7.44E+00			1.01E+01
		LOEC				5.00E+02	
		WWTP_1	1.96E-02	1.34E-06	8.85E-08	2.00E-08	9.90E-07
		WWTP_2_-O3	1.76E-02	1.21E-06	7.96E-08	1.80E-08	8.91E-07
		WWTP_2_+O3	7.83E-03	5.38E-07	3.54E-08	8.00E-09	3.96E-07
Perfluorooctanesulfonic acid	1763-23-1	EC50	3.74E+01				
		LC50		1.69E+01			2.37E+01
		WWTP_1	8.03E-08	1.78E-07			1.27E-07
		WWTP_2_-O3	4.01E-07	8.88E-07			6.33E-07
		WWTP_2_+O3	1.87E-07	4.14E-07			2.95E-07
Perfluorohexanoic acid	307-24-4	EC50	8.02E+02				
		LC50		7.93E+01			1.22E+02
		WWTP_1	1.75E-08	1.77E-07			1.15E-07
		WWTP_2_-O3	1.37E-08	1.39E-07			9.02E-08
		WWTP_2_+O3	7.48E-09	7.57E-08			4.92E-08
Perfluoroheptanoic acid	375-85-9	EC50	1.02E+03				
		LC50		2.45E+01			3.54E+01
		WWTP_1	6.87E-09	2.86E-07			1.98E-07
		WWTP_2_-O3	3.92E-09	1.63E-07			1.13E-07
		WWTP_2_+O3	1.96E-09	8.16E-08			5.65E-08
Tris(2-chloroethyl)phosphate	115-96-8	EC50			1.79E+02		
		LC50		1.60E-01			9.00E+01
		WWTP_1		1.88E-04	1.68E-07		3.33E-07
		WWTP_2_-O3		3.00E-04	2.68E-07		5.33E-07
		WWTP_2_+O3		2.69E-04	2.40E-07		4.78E-07
6:2 fluorotelomer sulfonic acid	27619-97-2	LC50		5.44E+02			9.01E+02
		WWTP_1		9.19E-07			5.55E-07
		WWTP_2_-O3		8.71E-07			5.26E-07
		WWTP_2_+O3		1.21E-07			7.33E-08

Table S5: Continuation (11).

			mg/L				
			Daphnia magna	Daphnid	*Danio rerio*	*Oncorhynchus mykiss*	Fish
Naproxen	22204-53-1	EC50	1.74E+02			5.00E-01	
		LC50		1.22E+02			1.93E+02
		WWTP_1	4.02E-07	5.74E-07		1.40E-04	3.63E-07
		WWTP_2_-O3	4.08E-07	5.82E-07		1.42E-04	3.68E-07
		WWTP_2_+O3					
Phenazone	60-80-0	LC50		3.47E+00			2.32E+00
		WWTP_1		8.36E-06			1.25E-05
		WWTP_2_-O3		7.41E-05			1.11E-04
		WWTP_2_+O3		8.65E-07			1.29E-06
Propyphenazone	479-92-5	LC50		1.80E+00			7.06E-01
		WWTP_1					
		WWTP_2_-O3		1.11E-06			2.83E-06
		WWTP_2_+O3					
Ketoprofen	22071-15-4	LC50		1.64E+02			2.64E+02
		EC50				5.33E-02	
		WWTP_1		9.15E-08		2.81E-04	5.68E-08
		WWTP_2_-O3		3.66E-08		1.12E-04	2.27E-08
		WWTP_2_+O3					
Acetaminophen (Paracetamol)	103-90-2	EC50	3.30E+00		9.42E+02		
		LC50		8.74E-01			1.55E+01
		LOEC				2.42E+03	
		WWTP_1	1.84E-04	6.93E-04	8.19E-07	2.51E-07	3.91E-05
		WWTP_2_-O3	2.37E-04	8.94E-04	6.43E-07	3.23E-07	5.04E-05
		WWTP_2_+O3	4.36E-05	1.65E-04	8.29E-07	5.95E-08	9.29E-06
Diclofenac	60207-90-1	EC50	8.90E-01		9.12E+00		
		NOEL				3.20E-01	
		LC50		2.79E+00		6.70E-01	1.78E+00
		WWTP_1	1.71E-03	5.46E-04	1.67E-04	4.76E-03	8.56E-04
		WWTP_2_-O3	2.01E-03	6.42E-04	1.96E-04	5.60E-03	1.01E-03
		WWTP_2_+O3	3.37E-06	1.08E-06	3.29E-07	9.38E-06	1.69E-06
		WWTP_1				2.27E-03	
		WWTP_2_-O3				2.67E-03	
		WWTP_2_+O3				4.48E-06	
Mefenamic acid	15307-86-5	EC50	6.70E+01			4.45E-02	
		LC50		2.58E+01			3.77E+01
		NOEC			2.00E+00		
		WWTP_1	1.49E-08	3.88E-08	5.00E-07	2.25E-05	2.65E-08
		WWTP_2_-O3	4.48E-08	1.16E-07	1.50E-06	6.74E-05	7.96E-08
		WWTP_2_+O3					
Dimethylaminophenazone	58-15-1	LC50		4.23E+00			2.82E+00
		WWTP_1		4.73E-07			7.09E-07
		WWTP_2_-O3		9.46E-07			1.42E-06
		WWTP_2_+O3					
Celecoxib	169590-42-5	LOEC			3.81E+00		
		NOEC			3.81E+00		
		LC50		3.23E+00			7.21E-01
		WWTP_1		2.79E-06	2.36E-06		1.25E-05
		WWTP_2_-O3		5.26E-06	4.46E-06		2.36E-05
		WWTP_2_+O3		2.79E-06	2.36E-06		1.25E-05
Indometacin	53-86-1	EC50			5.37E+01		
		LC50		1.07E+01			1.22E+00
		WWTP_1		1.12E-06	2.24E-07		9.84E-06
		WWTP_2_-O3		1.31E-06	2.61E-07		1.15E-05
		WWTP_2_+O3					
Sulfadimethoxine	122-11-2	EC50	1.57E+02				
		LC50		5.24E+00			1.16E+02
		WWTP_1	6.37E-09	1.91E-07			8.62E-09
		WWTP_2_-O3					
		WWTP_2_+O3					

Table S5: Continuation (12).

Erythromycin	114-07-8	EC50	1.69E+01				
		LC50		8.62E+00			1.52E+01
		LOEC			3.67E+02		
		WWTP_1	4.26E-06	8.35E-06	1.96E-07		4.74E-06
		WWTP_2_-O3	6.04E-06	1.18E-05	2.78E-07		6.71E-06
		WWTP_2_+O3					
Sulfapyridine	144-83-2	LC50		6.17E+00			2.46E+02
		WWTP_1		8.91E-06			2.24E-07
		WWTP_2_-O3		7.62E-06			1.91E-07
		WWTP_2_+O3		1.30E-06			3.25E-08
Lincomycin	154-21-2	EC50	1.90E+01				
		LC50		1.02E+02			1.04E+03
		NOEC			1.00E+03		
		WWTP_1					
		WWTP_2_-O3	1.27E-06	2.35E-07	2.40E-08		2.31E-08
		WWTP_2_+O3					
Trimethoprim	738-70-5	EC50	7.26E+01				
		LC50		6.38E+00			2.12E+02
		NOEC			1.57E-01		
		LOEC			1.57E-01		
		WWTP_1	5.09E-07	5.80E-06	2.36E-04		1.75E-07
		WWTP_2_-O3	1.87E-06	2.13E-05	8.66E-04		6.42E-07
		WWTP_2_+O3	2.75E-08	3.13E-07	1.27E-05		9.43E-09
Sulfamethoxazole	723-46-6	EC50	1.29E+00		1.09E+02	2.74E+01	
		LC50		6.43E+00			2.67E+02
		WWTP_1	9.38E-05	1.88E-05	1.11E-06	4.42E-06	4.53E-07
		WWTP_2_-O3	1.87E-04	3.75E-05	2.21E-06	8.81E-06	9.03E-07
		WWTP_2_+O3	1.71E-05	3.42E-06	2.02E-07	8.04E-07	8.24E-08
Clarithromycin	81103-11-9	EC50	2.17E+01				
		NOEC			1.00E+03		
		LC50		3.31E+00			2.05E+01
		WWTP_1	1.61E-05	1.06E-04	3.50E-07		1.71E-05
		WWTP_2_-O3	1.92E-05	1.26E-04	4.18E-07		2.04E-05
		WWTP_2_+O3	2.76E-07	1.81E-06	6.00E-09		2.93E-07
Roxithromycin	80214-83-1	LC50		6.72E+00			4.08E+01
		WWTP_1		1.80E-05			2.97E-06
		WWTP_2_-O3		1.56E-05			2.57E-06
		WWTP_2_+O3					
Azithromycin	83905-01-5	LC50		3.02E+00			1.88E+01
		WWTP_1		1.62E-04			2.60E-05
		WWTP_2_-O3		2.06E-04			3.31E-05
		WWTP_2_+O3					
Chloramphenicol	56-75-7	EC50	2.27E+02				
		LC50		7.21E+01			3.88E+01
		WWTP_1					
		WWTP_2_-O3					
		WWTP_2_+O3					
Cyclophosphamide	50-18-0	EC50			1.23E+03		
		LC50		2.60E-02			1.30E+01
		WWTP_1					
		WWTP_2_-O3		1.15E-04	2.44E-09		2.31E-07
		WWTP_2_+O3		1.15E-04	2.44E-09		2.31E-07
Fluconazole	86386-73-4	LC50		5.30E+02			1.63E+03
		WWTP_1		8.87E-08			2.88E-08
		WWTP_2_-O3		2.30E-07			7.48E-08
		WWTP_2_+O3		1.13E-07			3.68E-08
Efavirenz	154598-52-4	LC50		7.25E-01			9.96E-01
		WWTP_1					
		WWTP_2_-O3		2.76E-06			2.01E-06
		WWTP_2_+O3					
Nitrofurantoin	67-20-9	LC50		6.86E+00			6.09E+00
		WWTP_1		4.37E-06			4.93E-06
		WWTP_2_-O3		3.64E-06			4.11E-06
		WWTP_2_+O3					
Amantadine	768-94-5	EC50	1.74E+01				
		LC50		1.90E+00			6.09E+00
		WWTP_1	3.22E-06	2.95E-05			9.20E-06
		WWTP_2_-O3	6.26E-06	5.74E-05			1.79E-05
		WWTP_2_+O3	2.13E-06	1.95E-05			6.08E-06

Table S5: Continuation (13).

Mebendazole	31431-39-7	LC50		7.25E+00			3.20E+00
		WWTP_1		2.76E-07			6.25E-07
		WWTP_2_-O3		1.24E-06			2.81E-06
		WWTP_2_+O3					
Albendazole	54965-21-8	EC50	4.28E+01		4.20E+01		
		LC50		3.59E+00			1.08E+00
		WWTP_1					
		WWTP_2_-O3	4.67E-08	5.57E-07	4.76E-08		1.85E-06
		WWTP_2_+O3					
Atenolol	29122-68-7	EC50	3.13E+02			5.00E-01	
		NOEC			5.00E+00		
		LOEC			1.00E+01		
		LC50		1.03E+02			1.10E+03
		WWTP_1	4.66E-07	1.42E-06	2.92E-05	2.92E-04	1.33E-07
		WWTP_2_-O3	8.82E-07	2.68E-06	5.52E-05	5.52E-04	2.51E-07
		WWTP_2_+O3	4.79E-08	1.46E-07	3.00E-06	3.00E-05	1.36E-08
		WWTP_1			1.46E-05		
		WWTP_2_-O3			2.76E-05		
		WWTP_2_+O3			1.50E-06		
Propranolol	525-66-6	EC50	7.50E+00			2.52E+02	
		NOEC				2.59E+01	
		LOEC				2.59E+01	
		LC50		2.58E+00			2.02E+01
		WWTP_1	4.00E-06	1.16E-05		1.19E-07	1.49E-06
		WWTP_2_-O3	5.20E-06	1.51E-05		1.55E-07	1.93E-06
		WWTP_2_+O3	1.33E-07	3.88E-07		3.98E-09	4.95E-08
		WWTP_1		1.32E-04		1.16E-06	3.15E-05
		WWTP_2_-O3		1.72E-04		1.51E-06	4.10E-05
		WWTP_2_+O3		4.41E-06		3.86E-08	1.05E-06
Sotalol	3930-20-9	LC50		6.08E+01			6.17E+02
		WWTP_1		3.27E-06			3.23E-07
		WWTP_2_-O3		5.84E-06			5.75E-07
		WWTP_2_+O3					
Losartan	114798-26-4	LC50		1.69E+00			2.34E-01
		WWTP_1		2.25E-05			1.62E-04
		WWTP_2_-O3		4.02E-05			2.91E-04
		WWTP_2_+O3					
Valsartan	137862-53-4	LC50		4.76E+01			6.28E+01
		WWTP_1		9.71E-06			7.36E-06
		WWTP_2_-O3		2.46E-06			1.86E-06
		WWTP_2_+O3		2.31E-07			1.75E-07
Bisoprolol	66722-44-9	LC50		9.35E+00			7.99E+01
		WWTP_1		1.98E-05			2.32E-06
		WWTP_2_-O3		1.73E-05			2.03E-06
		WWTP_2_+O3		1.82E-06			2.13E-07
Telmisartan	144701-48-4	LC50		4.60E-02			1.30E-04
		WWTP_1		1.20E-02			4.23E+00
		WWTP_2_-O3		1.28E-02			4.54E+00
		WWTP_2_+O3		1.85E-03			6.54E-01
Labetalol	36894-69-6	LC50		4.21E+00			2.32E+01
		WWTP_1		7.13E-07			1.29E-07
		WWTP_2_-O3		9.50E-07			1.72E-07
		WWTP_2_+O3					
Candesartan	139481-59-7	LC50		6.01E+00			4.16E-01
		WWTP_1		7.32E-05			1.06E-03
		WWTP_2_-O3		1.42E-04			2.05E-03
		WWTP_2_+O3		2.36E-05			3.41E-04
Nitrendipin	39562-70-4	LC50		2.07E+00			1.27E+01
		WWTP_1		1.93E-06			3.15E-07
		WWTP_2_-O3		1.93E-06			3.15E-07
		WWTP_2_+O3		4.83E-07			7.87E-08

Table S5: Continuation (14).

Bezafibrate	41859-67-0	EC50	8.00E+01				
		NOEC			3.40E-05	1.45E+02	
		LOEC			3.40E-05		
		LC50		1.22E+01			1.76E+01
		WWTP_1	9.75E-07	6.39E-06	2.29E+00	5.39E-07	4.43E-06
		WWTP_2_-O3	3.62E-07	2.38E-06	8.53E-01	2.00E-07	1.65E-06
		WWTP_2_+O3					
Gemfibrozil	25812-30-0	EC50	1.20E-04				
		NOEC			1.00E-07	1.50E+00	
		LC50		4.93E+00	3.40E-06		6.73E+00
		WWTP_1					
		WWTP_2_-O3	5.67E-01	1.38E-05	6.80E+02	4.53E-05	1.01E-05
		WWTP_2_+O3					
		WWTP_1					
		WWTP_2_-O3			2.00E+01		
		WWTP_2_+O3					
Atorvastatin	134523-00-5	LOEC			1.30E-05		
		NOEC	1.00E-03				
		LC50		3.12E-01			1.50E-02
		WWTP_1	2.70E-02	8.65E-05	2.08E+00		1.80E-03
		WWTP_2_-O3	2.80E-02	8.97E-05	2.15E+00		1.87E-03
		WWTP_2_+O3					
Clotrimazole	23593-75-1	LOEC				5.17E+01	
		NOEC				5.17E+00	
		EC50	2.10E-01				
		LC50		6.10E-02			1.20E-03
		WWTP_1	5.71E-05	1.97E-04		2.32E-07	1.00E-02
		WWTP_2_-O3	4.76E-05	1.64E-04		1.93E-07	8.33E-03
		WWTP_2_+O3					
Diazepam	439-14-5	LOEC			2.73E+02		
		NOEC			2.73E-01		
		EC50	1.50E-05				
		LC50	3.20E-05	1.98E+01			2.26E+01
		WWTP_1					
		WWTP_2_-O3	6.67E-02	5.05E-08	3.66E-09		4.42E-08
		WWTP_2_+O3					
		WWTP_1					
		WWTP_2_-O3	3.13E-02		3.66E-06		
		WWTP_2_+O3					
Pravastatin	81093-37-0	LC50		8.91E+01			3.87E+01
		WWTP_1		5.61E-08			1.29E-07
		WWTP_2_-O3					
		WWTP_2_+O3					
Primidone	125-33-7	LC50		7.10E+02			6.02E+02
		WWTP_1		1.69E-08			1.99E-08
		WWTP_2_-O3		5.92E-08			6.98E-08
		WWTP_2_+O3					
Fluvoxamine	54739-18-3	LC50		1.60E+00			1.18E+01
		WWTP_1		4.38E-06			5.93E-07
		WWTP_2_-O3		6.38E-05			8.64E-06
		WWTP_2_+O3		1.25E-06			1.69E-07
Citalopram	59729-33-8	LC50		6.52E-01			4.47E+00
		WWTP_1		2.16E-04			3.15E-05
		WWTP_2_-O3		3.57E-04			5.21E-05
		WWTP_2_+O3		3.68E-05			5.37E-06
Clozapine	5786-21-0	NOEL			1.00E-08		
		LOEL			1.00E-06		
		LC50		2.32E+00			1.77E+01
		WWTP_1		1.12E-05	2.60E+03		1.47E-06
		WWTP_2_-O3		3.71E-05	8.60E+03		4.86E-06
		WWTP_2_+O3					
		WWTP_1			2.60E+01		
		WWTP_2_-O3			8.60E+01		
		WWTP_2_+O3					
Amitriptyline	50-48-6	EC50	1.15E+00				
		LC50		1.03E-01			6.16E-01
		WWTP_1	6.78E-05	7.57E-04			1.27E-04
		WWTP_2_-O3	6.78E-05	7.57E-04			1.27E-04
		WWTP_2_+O3	4.35E-06	4.85E-05			8.12E-06

Table S5: Continuation (15).

Sertraline	79617-96-2	EC50	5.60E-01				
		LOEC			1.00E-03	3.20E-01	
		NOEC				1.00E-01	
		LC50		7.10E-02		3.80E-01	4.08E-01
		WWTP_1	2.68E-05	2.11E-04	1.50E-02	4.69E-05	3.68E-05
		WWTP_2_-O3	5.89E-05	4.65E-04	3.30E-02	1.03E-04	8.09E-05
		WWTP_2_+O3	1.07E-05	8.45E-05	6.00E-03	1.88E-05	1.47E-05
		WWTP_1				1.50E-04	
		WWTP_2_-O3				3.30E-04	
		WWTP_2_+O3				6.00E-05	
		WWTP_1				3.95E-05	
		WWTP_2_-O3				8.68E-05	
		WWTP_2_+O3				1.58E-05	
Ropinirole	91374-21-9	LC50		1.42E+00			1.05E+01
		WWTP_1					
		WWTP_2_-O3					
		WWTP_2_+O3		5.63E-06			7.62E-07
Temazepam	846-50-4	LC50		6.11E+01			6.42E+01
		WWTP_1		1.31E-07			1.25E-07
		WWTP_2_-O3		3.60E-07			3.43E-07
		WWTP_2_+O3		9.82E-08			9.35E-08
Bupropion	34911-55-2	LC50		4.10E-01			2.79E+00
		WWTP_1		7.32E-06			1.08E-06
		WWTP_2_-O3		3.59E-04			5.27E-05
		WWTP_2_+O3		1.02E-04			1.51E-05
Lorazepam	846-49-1	LC50		6.11E+01			6.42E+01
		WWTP_1		4.91E-08			4.67E-08
		WWTP_2_-O3		2.13E-07			2.02E-07
		WWTP_2_+O3		6.55E-08			6.23E-08
Memantine	19982-08-2	LC50		6.36E-01			4.57E+00
		WWTP_1		3.30E-05			4.60E-06
		WWTP_2_-O3		6.76E-05			9.41E-06
		WWTP_2_+O3		2.67E-05			3.72E-06
Oxazepam	604-75-1	LC50		4.16E+01			4.49E+01
		WWTP_1		1.11E-06			1.02E-06
		WWTP_2_-O3		2.81E-06			2.61E-06
		WWTP_2_+O3		6.01E-07			5.57E-07
Melperone	3575-80-2	LC50		4.60E-01			3.12E+00
		WWTP_1		1.30E-05			1.92E-06
		WWTP_2_-O3		2.61E-05			3.85E-06
		WWTP_2_+O3					
Lamotrigine	84057-84-1	LC50		4.83E+00			1.26E+02
		WWTP_1		6.44E-05			2.47E-06
		WWTP_2_-O3		2.13E-04			8.17E-06
		WWTP_2_+O3		1.45E-04			5.57E-06
Carbamazepine	298-46-4	EC50	6.44E+01		5.25E+01	7.51E+01	
		LC50		1.41E+01			4.09E+01
		WWTP_1	8.00E-06	3.65E-05	9.82E-06	6.85E-06	1.26E-05
		WWTP_2_-O3	9.08E-06	4.15E-05	1.12E-05	7.79E-06	1.43E-05
		WWTP_2_+O3	4.66E-08	2.13E-07	5.72E-08	3.99E-08	7.33E-08
Furosemide	54-31-9	NOEC			6.10E-04		
		LOEC			6.10E-04		
		LC50		4.86E+02			5.24E+02
		WWTP_1		1.85E-07	1.48E-01		1.72E-07
		WWTP_2_-O3		4.05E-07	3.23E-01		3.76E-07
		WWTP_2_+O3					

Table S5: Continuation (16).

Verapamil	52-53-9	NOEC			1.36E+01	2.70E-01	
		LOEC				1.35E+00	
		LC50	1.13E+01	2.10E-01			7.74E-01
		WWTP_1	6.18E-06	3.33E-04	5.13E-06	2.59E-04	9.04E-05
		WWTP_2_-O3	2.82E-06	1.52E-04	2.35E-06	1.19E-04	4.13E-05
		WWTP_2_+O3	8.83E-08	4.76E-06	7.33E-08	3.70E-06	1.29E-06
		WWTP_1				5.19E-05	
		WWTP_2_-O3				2.37E-05	
		WWTP_2_+O3				7.41E-07	
Hydrochlorothiazide	58-93-5	EC50				5.00E-01	
		LC50		4.81E+03			3.60E+03
		WWTP_1		6.39E-07		6.15E-03	8.54E-07
		WWTP_2_-O3		6.45E-07		6.21E-03	8.62E-07
		WWTP_2_+O3		5.09E-08		4.90E-04	6.81E-08
N-Acetyl-4-aminoantipyrine	83-15-8	LC50		6.91E+00			6.05E+00
		WWTP_1		6.43E-05			7.34E-05
		WWTP_2_-O3		1.26E-04			1.43E-04
		WWTP_2_+O3		8.68E-07			9.92E-07
4-Aminoantipyrine	83-07-8	LC50		5.53E+00			4.74E+00
		WWTP_1		6.42E-05			7.49E-05
		WWTP_2_-O3		1.69E-04			1.97E-04
		WWTP_2_+O3		5.42E-06			6.33E-06
Ranitidine	66357-35-5	NOEC			2.50E-04		
		LOEC			2.50E-04		
		LC50		7.80E+01			7.98E+02
		WWTP_1		1.91E-06	5.96E-01		1.87E-07
		WWTP_2_-O3		3.38E-06	1.06E+00		3.31E-07
		WWTP_2_+O3					
Cetirizine	83881-51-0	LC50		3.41E+03			3.87E+03
		WWTP_1		2.99E-08			2.64E-08
		WWTP_2_-O3		1.51E-07			1.33E-07
		WWTP_2_+O3		1.17E-09			1.03E-09
Diphenhydramine	58-73-1	EC50			1.50E-04		
		LC50		1.25E+00			9.20E+00
		WWTP_1		8.56E-05	7.13E-01		1.16E-05
		WWTP_2_-O3		1.25E-04	1.04E+00		1.70E-05
		WWTP_2_+O3		8.00E-06	6.67E-02		1.09E-06
Promethazin	60-87-7	LC50		2.02E-01			1.27E+00
		WWTP_1					
		WWTP_2_-O3		4.95E-05			7.87E-06
		WWTP_2_+O3					
Lidocaine	137-58-6	LC50		8.64E+00			7.54E+01
		WWTP_1		1.52E-05			1.74E-06
		WWTP_2_-O3		2.93E-05			3.36E-06
		WWTP_2_+O3		1.50E-06			1.72E-07
Metformin	657-24-9	EC50	6.40E+01				
		LC50		1.93E+03			2.77E+04
		WWTP_1	3.87E-05	1.28E-06			8.94E-08
		WWTP_2_-O3	1.82E-05	6.03E-07			4.20E-08
		WWTP_2_+O3	1.06E-05	3.53E-07			2.46E-08

Table S5: Continuation (17).

Tramadol	27203-92-5	NOEC			6.60E-01		
		LOEC			6.60E-01		
		LC50		1.47E+00			1.02E+01
		WWTP_1		1.92E-03	4.28E-03		2.77E-04
		WWTP_2_-O3		5.00E-04	1.11E-03		7.21E-05
		WWTP_2_+O3		3.95E-05	8.79E-05		5.69E-06
Methotrexate	59-05-2	LC50		3.27E+02			6.76E+04
		WWTP_1					
		WWTP_2_-O3					
		WWTP_2_+O3					
Mycophenolic acid	483-60-3	LC50		1.54E+01			1.31E+01
		WWTP_1		9.09E-07			1.07E-06
		WWTP_2_-O3		3.25E-07			3.82E-07
		WWTP_2_+O3					
Crotamiton	483-63-6	LC50		5.89E+00			2.13E+00
		WWTP_1		1.36E-06			3.76E-06
		WWTP_2_-O3		1.31E-05			3.62E-05
		WWTP_2_+O3					
N-Formyl-4-aminoantipyrine	1672-58-8	LC50		4.51E+00			3.13E+00
		WWTP_1		2.92E-04			4.21E-04
		WWTP_2_-O3		1.48E-03			2.13E-03
		WWTP_2_+O3		2.44E-06			3.51E-06
Acetyl-sulfamethoxazole	21312-10-7	LC50		3.76E+02			3.43E+02
		WWTP_1		1.06E-08			1.17E-08
		WWTP_2_-O3		3.72E-08			4.08E-08
		WWTP_2_+O3		2.66E-09			2.92E-09
Duloxetine	116539-59-4	LC50		1.61E-01			9.89E-01
		WWTP_1					
		WWTP_2_-O3		1.55E-04			2.53E-05
		WWTP_2_+O3					
Edifenphos (EDDP)	17109-49-8	LC50		8.34E+00			4.77E+00
		WWTP_1		5.76E-06			1.01E-05
		WWTP_2_-O3		2.33E-05			4.07E-05
		WWTP_2_+O3		9.95E-06			1.74E-05
Ketamine	6740-88-1	LC50		1.13E+00			8.34E+00
		WWTP_1		1.06E-05			1.44E-06
		WWTP_2_-O3		3.10E-05			4.20E-06
		WWTP_2_+O3					
Pioglitazone	111025-46-8	LC50		1.84E+00			2.13E+00
		WWTP_1					
		WWTP_2_-O3		1.09E-06			9.39E-07
		WWTP_2_+O3					
Clopidogrel	113665-84-2	LC50		5.79E-01			3.93E+00
		WWTP_1		2.42E-05			3.56E-06
		WWTP_2_-O3		3.97E-05			5.85E-06
		WWTP_2_+O3					
Anastrozole	120511-73-1	LC50		4.12E+01			1.56E+01
		WWTP_1		7.28E-08			1.92E-07
		WWTP_2_-O3		9.71E-08			2.56E-07
		WWTP_2_+O3		4.85E-08			1.28E-07
Bicalutamide	90357-06-5	LC50		6.55E+01			7.05E+01
		WWTP_1		4.43E-07			4.11E-07
		WWTP_2_-O3		9.77E-07			9.08E-07
		WWTP_2_+O3		6.87E-07			6.38E-07
Ifosfamide	3778-73-2	EC50			8.09E+02		
		LC50		3.22E+02			1.39E+02
		WWTP_1					
		WWTP_2_-O3		1.55E-08	6.18E-09		3.60E-08
		WWTP_2_+O3		9.32E-09	3.71E-09		2.16E-08
Tetracaine	94-24-6	LC50		1.45E+00			1.08E+01
		WWTP_1		3.45E-06			4.63E-07
		WWTP_2_-O3		8.28E-06			1.11E-06
		WWTP_2_+O3		1.38E-06			1.85E-07
Ambroxol	18683-91-5	LC50		1.79E+00			1.32E+01
		WWTP_1		1.10E-04			1.49E-05
		WWTP_2_-O3		4.30E-05			5.83E-06
		WWTP_2_+O3					

Table S5: Continuation (18).

Scopolamine-N-butyl	149-64-4	LC50		6.20E+02		1.77E+02
		WWTP_1		1.61E-08		5.65E-08
		WWTP_2_-O3		2.58E-08		9.04E-08
		WWTP_2_+O3		4.84E-09		1.69E-08
Oxybutynin	5633-20-5	LC50		5.29E-01		3.53E+00
		WWTP_1		1.89E-06		2.83E-07
		WWTP_2_-O3		1.89E-06		2.83E-07
		WWTP_2_+O3				
Climbazole	38083-17-9	LC50		1.66E+00		2.88E-01
		WWTP_1		3.19E-05		1.84E-04
		WWTP_2_-O3		4.10E-05		2.36E-04
		WWTP_2_+O3		1.20E-06		6.94E-06
Torasemide	56211-40-6	LC50		5.45E+01		5.12E+01
		WWTP_1		9.72E-07		1.04E-06
		WWTP_2_-O3		2.53E-06		2.70E-06
		WWTP_2_+O3		2.20E-07		2.34E-07
Vancomycin	1404-90-6	LC50		1.76E+04		2.05E+05
		WWTP_1				
		WWTP_2_-O3		2.32E-08		2.00E-09
		WWTP_2_+O3				
Mebeverine	3625-06-7	LC50		1.30E-01		7.40E-01
		WWTP_1		7.69E-06		1.35E-06
		WWTP_2_-O3		7.69E-06		1.35E-06
		WWTP_2_+O3		7.69E-06		1.35E-06
Metoprolol	56392-14-4	LC50		6.20E+01		2.68E+02
		WWTP_1		9.33E-06		2.16E-06
		WWTP_2_-O3		1.22E-05		2.83E-06
		WWTP_2_+O3		1.29E-06		2.99E-07
Mirtazapine	85650-52-8	LC50		1.27E+01		3.04E+01
		WWTP_1		2.12E-06		8.89E-07
		WWTP_2_-O3		7.08E-06		2.96E-06
		WWTP_2_+O3				
10,11-Dihydro-10,11-dihydroxycarbamazepine	35079-97-1	LC50		2.61E+02		1.92E+03
		WWTP_1		3.13E-06		4.25E-07
		WWTP_2_-O3		4.92E-06		6.67E-07
		WWTP_2_+O3		1.38E-06		1.87E-07
2-Hydroxycarbamazepine	68011-66-5	LC50		3.76E+01		1.38E+02
		WWTP_1		1.46E-06		4.00E-07
		WWTP_2_-O3		1.99E-06		5.45E-07
		WWTP_2_+O3		4.79E-07		1.31E-07
Desloratadine	100643-71-8	LC50		2.78E+00		3.54E+00
		WWTP_1				
		WWTP_2_-O3		6.12E-06		4.80E-06
		WWTP_2_+O3				
Glimepiride	93479-97-1	LC50		3.46E+01		9.55E+01
		WWTP_1				
		WWTP_2_-O3		4.34E-07		1.57E-07
		WWTP_2_+O3				
Ondansetron	99614-02-5	LC50		1.55E+01		3.83E+01
		WWTP_1				
		WWTP_2_-O3		1.94E-07		7.83E-08
		WWTP_2_+O3				
Metoprolol acid	56392-14-4	LC50		1.63E+02		1.01E+03
		WWTP_1		1.13E-06		1.83E-07
		WWTP_2_-O3		6.95E-07		1.12E-07
		WWTP_2_+O3		1.84E-07		2.98E-08
1-(3-carboxypropyl)-3,7-dimethylxanthine	6493-07-8	LC50		1.00E+03		1.23E+04
		WWTP_1		1.09E-07		8.87E-09
		WWTP_2_-O3		1.26E-07		1.03E-08
		WWTP_2_+O3		1.40E-08		1.14E-09
Gabapentin-Lactam	64744-50-9	LC50		9.22E+01		5.69E+02
		AC Eilendorf		2.07E-05		3.35E-06
		AC Soers_2_vO		1.87E-05		3.03E-06
		AC Soers_nO		1.36E-06		2.20E-07

Table S5: Continuation (19).

			mg/L				
			Daphnia magna	Daphnid	*Danio rerio*	*Oncorhynchus mykiss*	Fish
Triethylcitrate	77-93-0	LC50		8.62E+02			3.47E+02
		WWTP_1		2.67E-08			6.63E-08
		WWTP_2_-O3					
		WWTP_2_+O3					
Tri-isobutylphosphate	126-71-6	LC50		7.28E+00			4.15E+00
		WWTP_1		8.65E-06			1.52E-05
		WWTP_2_-O3		2.38E-05			4.17E-05
		WWTP_2_+O3					
Tris(1,3-dichloroisopropyl)phosphate (TDCPP)	13674-87-8	EC50			1.65E+00		
		LC50		1.09E+01			6.28E+00
		WWTP_1		3.39E-06	2.24E-05		5.89E-06
		WWTP_2_-O3		4.86E-06	3.21E-05		8.44E-06
		WWTP_2_+O3		3.85E-06	2.55E-05		6.69E-06
Tris(1-chloro-2-propyl)phosphate (TCPP)	13674-84-5	NOEC			8.90E-02		
		LOEC			4.75E-01		
		LC50		2.51E+01			1.33E+01
		WWTP_1		1.78E-05	5.03E-03		3.37E-05
		WWTP_2_-O3		3.39E-05	9.55E-03		6.39E-05
		WWTP_2_+O3		1.76E-05	4.97E-03		3.32E-05
Diphenylphosphate	838-85-7	LC50		2.06E+01			3.35E+01
		WWTP_1		3.16E-06			1.94E-06
		WWTP_2_-O3		2.62E-06			1.61E-06
		WWTP_2_+O3					
Triethylphosphate (TEP)	78-40-0	EC50	9.00E+02		1.24E+03		
		LC50		2.60E+02			9.83E+00
		WWTP_1	5.22E-07	1.81E-06	3.79E-07		4.78E-05
		WWTP_2_-O3	2.63E-07	9.12E-07	1.91E-07		2.41E-05
		WWTP_2_+O3	8.22E-08	2.85E-07	5.97E-08		7.53E-06
Di-n-butyl phosphate	107-66-4	EC50	3.50E+01				
		LC50		5.54E+01			9.50E+01
		WWTP_1					
		WWTP_2_-O3	1.40E-06	8.84E-07			5.16E-07
		WWTP_2_+O3					
Dicyclohexylphthalate	84-61-7	LC50		2.10E-01			1.60E-01
		WWTP_1		1.90E-05			2.50E-05
		WWTP_2_-O3		4.76E-06			6.25E-06
		WWTP_2_+O3					

			mg/L				
			Daphnia magna	Daphnid	*Danio rerio*	*Oncorhynchus mykiss*	Fish
Trimethyloctylammonium	15461-38-8	LC50		350			660
		WWTP_1					
		WWTP_2_-O3		2.57143E-08			1.3636E-08
		WWTP_2_+O3					
Decylsulfate	142-98-3	LC50		336			624
		WWTP_1		2.97619E-09			1.6026E-09
		WWTP_2_-O3					
		WWTP_2_+O3		2.97619E-09			1.6026E-09
Dicyclohexyl sulfosuccinate	137361-04-7	LC50		63.3			37.3
		WWTP_1					
		WWTP_2_-O3		6.31912E-08			1.0724E-07
		WWTP_2_+O3					

Table S5: Continuation (20).

			mg/L				
			Daphnia magna	Daphnid	*Danio rerio*	*Oncorhynchus mykiss*	Fish
Benzophenone-3	131-57-7	NOEC	1.01E-01			5.50E-02	
		LC50		2.40E+00			2.78E+00
		WWTP_1					
		WWTP_2_-O3	3.18E-04	1.33E-05		5.82E-04	1.15E-05
		WWTP_2_+O3					
Phenylbenzimidazole sulfonic acid	716-79-0	LC50		3.18E+00			1.08E+00
		WWTP_1		3.13E-03			9.23E-03
		WWTP_2_-O3		1.99E-03			5.86E-03
		WWTP_2_+O3		4.68E-04			1.38E-03
4-Methylbenzylidene camphor (Enzacamene)	36861-47-9	LC50		5.20E-02			2.64E-01
		WWTP_1		3.85E-05			7.58E-06
		WWTP_2_-O3		3.85E-05			7.58E-06
		WWTP_2_+O3					
Benzophenone-4 (Sulisobenzone)	4065-45-6	NOEC			3.00E-02		
		LOEC			3.00E+00		
		LC50		1.16E+03			5.58E+03
		WWTP_1		1.94E-07	7.50E-03		4.03E-08
		WWTP_2_-O3		9.62E-07	3.72E-02		2.00E-07
		WWTP_2_+O3					
Octyl-methoxycinnamate	83834-59-7	LC50		8.42E-01			7.03E-01
		WWTP_1		3.56E-06			4.27E-06
		WWTP_2_-O3		3.56E-06			4.27E-06
		WWTP_2_+O3					

			mg/L				
			Daphnia magna	Daphnid	*Danio rerio*	*Oncorhynchus mykiss*	Fish
Theophyllin	58-55-9	NOEC			1.00E-03		
		LOEC			2.00E-03		
		EC50	8.61E-04				
		LC50		3.24E+02	2.50E-02		2.23E+03
		WWTP_1	5.57E-02	1.48E-07	4.80E-02		2.15E-08
		WWTP_2_-O3	4.76E-02	1.27E-07	4.10E-02		1.84E-08
		WWTP_2_+O3	3.37E-02	8.95E-08	2.90E-02		1.30E-08
		WWTP_1		8.65E-06	2.40E-02		3.20E-05
		WWTP_2_-O3		7.39E-06	2.05E-02		2.73E-05
		WWTP_2_+O3		5.23E-06	1.45E-02		1.93E-05
		WWTP_1			1.92E-03		
		WWTP_2_-O3			1.64E-03		
		WWTP_2_+O3			1.16E-03		
Cotinine	486-56-6	LC50		1.22E+03			9.73E+02
		WWTP_1		7.95E-08			9.97E-08
		WWTP_2_-O3		1.42E-07			1.78E-07
		WWTP_2_+O3		2.95E-08			3.70E-08

Table S6: Annual load of target substances in the receiving waterbody, mg y^{-1}.

Chemical compounds	WWTP_1	WWTP_2_-O3	WWTP_2_+O3
1-(3-carboxypropyl)-3,7-dimethylxanthine	448.58	4002.1	444.68
1,3-Dimethyl-2-imidazolidinone	148.16	<MLD	<MLD
1,3-Diphenylguanidine	176.96	4859.7	539.97
10,11-Dihydro-10,11-dihydroxycarbamazepine	3366.44	40783.34	11434.58
10,11-Dihydro-10-hydroxycarbamazepine	1411.6	8385.36	2604.54
1-Butyl-3-methyl-imidazolium	74.08	1524.61	158.81
1H-Benzotriazole	25519.89	236759.4	52694.37
2-(4-morpholinyl)benzothiazole	20.58	317.63	<MLD
2-(Methylthio)benzothiazole	1440.41	8258.31	2922.17
2,4-Dichlorobenzoic acid	<MLD	3112.75	<MLD
2,4-Dichlorophenol	49.39	508.2	<MLD
2,4-Dichlorophenoxyacetic acid	28.81	381.15	<MLD
2-Aminobenzimidazole	74.08	5,272,613	1,683,425
2-Benzothiazolesulfonic acid	2354.04	41640.94	21725.71
2-Hydroxyatrazine	82.31	<MLD	<MLD
2-Hydroxybenzothiazole	251.04	3,239,798	<MLD
2-Hydroxycarbamazepine	226.35	2,382,205	5,717,291
2-Hydroxyquinoline	82.31	1,365,797	<MLD
2-methyl-4-chlorophenoxyacetic acid (MCPA)	4.12	3,176,273	<MLD
2-Naphthol-8-sulfonic acid	20.58	<MLD	<MLD
2-Oxindole	<MLD	2,255,154	<MLD
3,5,6-Trichloro-2-pyridinol	49.39	4,129,155	<MLD
4-(Dimethylamino)pyridine	1,358,098	1,778,713	1,905,764
4'-Aminoacetanilide	5189.58	71720.24	30365.17
4-Aminoantipyrine	1,460,984	29634.63	9,528,819
4-Hydroxybenzotriazole	4,815,074	4891.46	1,905,764
4-Hydroxyquinoline	946,553	5,717,291	4,764,409
4-Methylbenzylidene camphor	8,230,896	6,352,546	<MLD
5-Chlorobenzotriazole	2,880,814	9,528,819	1,588,136
5-Methyl-1H-benzotriazole	4,506,416	76039.97	10672.28
6:2 fluorotelomer sulfonic acid	2,057,724	15055.53	2096.34
7-(Ethylamino)-4-methylcoumarin	1,646,179	9,528,819	<MLD
7-Amino-4-methylcoumarin	1,646,179	2,223,391	<MLD
7-Diethylamino-4-methylcoumarin	3,292,358	1,905,764	3,176,273
Acesulfame	25680.4	5,209,088	3,112,747
Acetaminophen (Paracetamol)	2,493,961	24806.69	4,573,833
Acetamiprid	1,234,634	1,270,509	3,176,273
Acetyl-sulfamethoxazole	1,646,179	4,446,782	3,176,273
Acridine	<MLD	1048.17	<MLD
Acridone	5,350,082	1,905,764	3,176,273
Albendazole	<MLD	6,352,546	<MLD
Amantadine	2,304,651	3,462,137	1,175,221

Table S6: Continuation (1).

Ambroxol	8,107,433	2445.73	<MLD
Amitriptyline	3,210,049	2,477,493	1,588,136
Anastrozole	1,234,634	1,270,509	6,352,546
Atenolol	6,008,554	8,766,513	4,764,409
Atorvastatin	1,111,171	8,893,564	<MLD
Atrazine	8,230,896	<MLD	<MLD
Azithromycin	2,008,339	19788.18	<MLD
Azoxystrobin	8,230,896	9,528,819	<MLD
Azoxystrobin acid	1,234,634	2,223,391	<MLD
Bendiocarb	1,234,634	1,905,764	<MLD
Bentazone	4,115,448	3,176,273	<MLD
Benzenesulfonic acid	1,135,864	4,256,206	6,257,258
Benzophenone-3	<MLD	1,016,407	<MLD
Benzophenone-4 (Sulisobenzone)	9,259,758	35447.21	<MLD
Benzothiazole	716,088	12546.28	3,366,849
Bezafibrate	3,210,049	9,211,191	<MLD
Bicalutamide	119,348	2,032,815	1,429,323
Bisoprolol	7,613,579	5,145,562	5,399,664
Boscalid	4,115,448	2,223,391	6,352,546
Bupropion	1,234,634	4,669,121	1,334,035
Candesartan	1,810,797	27125.37	4,510,308
Carbamazepine	2,119,456	18581.2	9,528,819
Carbendazim	2,057,724	7,623,055	6,352,546
Carbetamide	1,234,634	1,905,764	6,352,546
Celecoxib	3,703,903	5,399,664	2,858,646
Cetirizine	4,197,757	16326.04	1,270,509
Chlorothalonil-4-hydroxy	4,115,448	9,528,819	3,176,273
Citalopram	5,802,782	7,400,716	7,623,055
Clarithromycin	1,440,407	13276.82	1,905,764
Climbazole	2,181,187	2,159,866	6,352,546
Clopidogrel	5,761,627	7,305,428	<MLD
Clotrimazole	4,938,538	3,176,273	<MLD
Clozapine	1,070,016	2,731,595	<MLD
Cotinine	3,991,985	5,494,952	1,143,458
Crotamiton	3,292,358	2445.73	<MLD
Cyclohexylamine	1,642,064	21312.79	4,351,494
Cyclophosphamide	<MLD	9,528,819	9,528,819
Daidzein	4,115,448	<MLD	<MLD
Decylsulfate	4,115,448	<MLD	3,176,273
Denatonium	6,090,863	9,751,158	2,699,832
Desethylatrazine	2,057,724	2,858,646	<MLD
Desloratadine	<MLD	5,399,664	<MLD
Diazepam	<MLD	3,176,273	<MLD
Dichlorophen	<MLD	3,176,273	<MLD
Dichlorprop	8,230,896	<MLD	<MLD

Table S6: Continuation (2).

Diclofenac	6,267,827	56918.81	9,528,819
Dicyclohexyl sulfosuccinate	<MLD	1,270,509	<MLD
Dicyclohexylphthalate	1,646,179	3,176,273	<MLD
Diethyltoluamide (DEET)	6,955,107	3,875,053	7,940,682
Dimethachlor	4,938,538	7,940,682	349.39
Dimethachlor oxalamic acid (OA)	5,350,082	698.78	2,223,391
Dimethenamid	8,230,896	<MLD	<MLD
Dimethyl-5-sulfoisophthalate	4,485,838	5,050,274	4,764,409
Dimethylaminophenazone	8,230,896	1,270,509	<MLD
Di-n-butyl phosphate	<MLD	1,556,374	<MLD
Diphenhydramine	4,403,529	4,954,986	3,176,273
Diphenylphosphate	2,675,041	1,715,187	<MLD
Diuron	7,407,806	6,352,546	2,541,018
Duloxetine	<MLD	7,940,682	<MLD
Edifenphos (EDDP)	1,975,415	6,161,969	2,636,307
Efavirenz	<MLD	6,352,546	<MLD
Epoxiconazole	<MLD	6,352,546	<MLD
Erythromycin	2,963,123	3,239,798	<MLD
Ethofumesate	1,152,325	1,651,662	<MLD
Fenpropimorph	4,115,448	<MLD	<MLD
Fenuron	4,526,993	8,893,564	4,446,782
Fipronil	1,234,634	2,223,391	6,352,546
Fluconazole	1,934,261	3,875,053	1,905,764
Flufenacet	4,115,448	<MLD	<MLD
Fluvoxamine	2,880,814	3,239,798	6,352,546
Furosemide	3,703,903	6,257,258	<MLD
Gabapentin-Lactam	7856.39	54822.47	3,970,341
Gemfibrozil	<MLD	2,159,866	<MLD
Genistein	4,938,538	2,223,391	<MLD
Glimepiride	<MLD	4,764,409	<MLD
Harman	3,292,358	1,016,407	3,176,273
Harmine	4,115,448	3,176,273	3,176,273
Hexa(methoxymethyl)melamine	1,074,132	5,336,138	2,191,628
Hydrochlorothiazide	12655	98591.51	7,781,869
Ifosfamide	<MLD	1,588,136	9,528,819
Imazalil	1,234,634	1,270,509	<MLD
Imazapyr	4,526,993	5,399,664	<MLD
Imidacloprid	2,880,814	6,034,919	3,176,273
Indometacin	4,938,538	4,446,782	<MLD
Isophorone diamine	4,568,147	5,812,579	<MLD
Isoproturon	1,234,634	825,831	3,176,273
Isoxaben	<MLD	3,176,273	<MLD
Ketamine	4,938,538	1,111,696	<MLD
Ketoprofen	6,173,172	1,905,764	<MLD
Labetalol	1,234,634	1,270,509	<MLD

Table S6: Continuation (3).

Lamotrigine	1,279,904	32715.61	22297.44
Lenacil	1,234,634	2,223,391	6,352,546
Lidocaine	5,391,237	8035.97	4,129,155
Lincomycin	<MLD	7,623,055	<MLD
Lorazepam	1,234,634	4,129,155	1,270,509
Losartan	156,387	2,159,866	<MLD
Mebendazole	8,230,896	2,858,646	<MLD
Mebeverine	4,115,448	3,176,273	3,176,273
Mecoprop	6,584,717	2,858,646	<MLD
Mefenamic acid	4,115,448	9,528,819	<MLD
Melperone	2,469,269	3,811,527	<MLD
Memantine	8,642,441	1,365,797	5,399,664
Mepiquat	8,313,205	5,685,528	2,890,408
Metalaxyl	4,115,448	1,905,764	3,176,273
Metamitron	<MLD	2,223,391	6,352,546
Metazachlor	4,115,448	<MLD	<MLD
Metazachlor BH 479-11	4,115,448	6,352,546	<MLD
Metazachlor ESA	4,115,448	9,528,819	3,176,273
Metformin	10189.85	36971.82	21630.42
Methyltriphenylphosphonium	4,115,448	<MLD	<MLD
Metolachlor	4,938,538	<MLD	<MLD
Metolachlor ESA	3,292,358	1,905,764	3,176,273
Metolachlor OA	8,230,896	9,528,819	3,176,273
Metoprolol	2,378,729	24044.39	2,541,018
Metoprolol acid	7,572,424	3,589,188	9,528,819
Metribuzin	4,115,448	3,176,273	<MLD
Mirtazapine	1,111,171	2,858,646	<MLD
m-Xylene-4-sulfonic acid	1,514,485	32874.42	6,193,732
Myclobutanil	4,115,448	6,352,546	<MLD
Mycophenolic acid	5,761,627	1,588,136	<MLD
N-Acetyl-4-aminoantipyrine	1,827,259	27570.05	1,905,764
Naproxen	2,880,814	2,255,154	<MLD
N-Butylbenzenesulfonamide	<MLD	1,905,764	<MLD
N-Cyclohexyl-2-benzothiazole-amine	1,646,179	3,176,273	<MLD
N-Ethyl-o-toluenesulfonamide	1,522,716	2,826,883	5,082,037
N-Formyl-4-aminoantipyrine	5424.16	211730.4	349.39
Nitrendipin	1,646,179	1,270,509	3,176,273
Nitrofurantoin	1,234,634	7,940,682	<MLD
Octyl-methoxycinnamate	1,234,634	9,528,819	<MLD
o-Dianisidine	5,473,546	10735.8	<MLD
Ondansetron	<MLD	9,528,819	<MLD
Oxazepam	1,893,106	3,716,239	7,940,682
Oxybutynin	4,115,448	3,176,273	<MLD
Perfluoroheptanoic acid	2,880,814	1,270,509	6,352,546
Perfluorohexanoic acid	5,761,627	349.39	1,905,764

Table S6: Continuation (4).

Perfluorooctanesulfonic acid	1,234,634	4,764,409	2,223,391
Perfluorooctanoic acid	4,115,448	2,858,646	1,270,509
Pethoxamid	8,230,896	<MLD	<MLD
Phenazone	119,348	8,163,021	9,528,819
Phenylbenzimidazole sulfonic acid	41022.79	201121.6	47294.7
Phenylethylmalonamide	633,779	3,779,765	2,318,679
Pioglitazone	<MLD	6,352,546	<MLD
Piperine	8,230,896	1,270,509	<MLD
Pravastatin	2,057,724	<MLD	<MLD
Primidone	4,938,538	1,334,035	<MLD
Promethazin	<MLD	3,176,273	<MLD
Propamocarb	2,469,269	<MLD	<MLD
Propiconazole	2,880,814	2,541,018	6,352,546
Propranolol	1,234,634	1,238,746	3,176,273
Propyphenazone	<MLD	6,352,546	<MLD
Prothioconazole-desthio	4,115,448	9,528,819	<MLD
p-Toluenesulfonamide	2,469,269	20169.33	4,986,748
Quinoline	<MLD	1,937,526	<MLD
Ranitidine	6,132,018	8385.36	<MLD
Ropinirole	<MLD	<MLD	2,541,018
Roxithromycin	4,979,692	3,335,087	<MLD
Saccharin	1,218,173	<MLD	<MLD
Scopolamine-N-butyl	4,115,448	5,082,037	9,528,819
Sertraline	6,173,172	1048.17	1,905,764
Simazine	<MLD	1,905,764	<MLD
Sotalol	8,189,742	11275.77	<MLD
Spiroxamine	4,115,448	<MLD	<MLD
Sucralose	29190.87	1491419	1206412
Sulfadimethoxine	4,115,448	<MLD	<MLD
Sulfamethoxazole	4,979,692	7,654,818	698.78
Sulfapyridine	2,263,496	1,492,848	2,541,018
Summe Benzotriazole	30536.62	317786.1	63716.03
Tebuconazole	4,115,448	6,352,546	3,176,273
Telmisartan	2,263,496	18740.01	2,699,832
Temazepam	3,292,358	698.78	1,905,764
Terbuthylazine	<MLD	9,528,819	3,176,273
Terbuthylazine-2-hydroxy	5,350,082	4,129,155	4,129,155
Terbutryn	3,292,358	9,528,819	2,223,391
Tetracaine	2,057,724	3,811,527	6,352,546
Tetraglyme	7,407,806	7,623,055	2,223,391
Theophyllin	1,975,415	1,302,272	9,211,191

Table S6: Continuation (5).

Thiabendazole	<MLD	2,541,018	<MLD
Thiacloprid amide	<MLD	6,352,546	3,176,273
Torasemide	2,181,187	4,383,257	3,811,527
Tramadol	11638.49	23345.61	1,842,238
Tri(butoxyethyl)phosphate (TBEP)	1,650,295	2,032,815	6,352,546
Triadimenol	<MLD	6,352,546	<MLD
Tributylamine	1,234,634	9,211,191	2,223,391
Triethylcitrate	946,553	<MLD	<MLD
Triethylphosphate (TEP)	1,934,261	7,527,767	2,350,442
Trifloxystrobin	4,115,448	9,528,819	3,176,273
Trifloxystrobin NOA413161	3,292,358	2,223,391	<MLD
Triglyme	3,292,358	1,238,746	<MLD
Tri-isobutylphosphate	2,592,732	5,494,952	<MLD
Trimethoprim	1,522,716	4,319,731	6,352,546
Trimethyloctylammonium	<MLD	2,858,646	<MLD
Triphenylphosphine oxide	1,152,325	8,798,276	1,937,526
Tris(1,3-dichloroisopropyl)phosphate (TDCPP)	1,522,716	1,683,425	1,334,035
Tris(1-chloro-2-propyl)phosphate (TCPP)	1,843,721	26998.32	14039.13
Tris(2-chloroethyl)phosphate (TCEP)	1,234,634	1,524,611	1,365,797
Valsartan	1,901,337	3,716,239	349.39
Vancomycin	<MLD	12990.96	<MLD
Verapamil	2,880,814	1,016,407	3,176,273
Warfarin	4,115,448	3,176,273	<MLD

Table S7: List of detected substances with the reference to Water Hazard Classes (WHC).

		List of detected substances with the reference to the WHC 1	List of detected substances with the reference to the WHC 2	List of detected substances with the reference to the WHC 3
		1H-Benzotriazole	1,3-Diphenylguanidine	2,4-Dichlorophenol
		4'-Aminoacetanilide	1-Naphthylamine	3,3'-Dichlorobenzidine
		4-Aminoantipyrine	2-(Piperazin-1-yl)ethanamine	4-Chloroaniline
		4-Aminobenzamide	2-Aminobisphenyl	Aniline Yellow
		Acetaminophen (Paracetamol)	2-methyl-4-chlorophenoxyacetic acid (MCPA)	Atrazine
		Amidosulfuron	2-Morpholinothiobenzothiazole	Azinphos methyl
		Benzenesulfonic acid	4-Chlorophenol	Azobenzene
		Benzophenone-4 (Sulfobenzone)	4-isopropylaniline	Benzidine
		Bezafibrate	4-Methylbenzylidene camphor	Carbandazim
		Bifonazol	4-Nitrophenole	Carbaryl
		Bis(2-ethylhexyl)phosphate	Allopurinol	Chlorfenvinphos
		Caffeine	Amantadine	Ciclopirox
		Cyclohexylamine	Bentazone	Climbazole
		Di-n-butyl phosphate	Benzocaine	Clotrimazole
		Glimepiride	Benzophenone-3	Clozapine
		Hexa(methoxymethyl)melamine	Benzothiazole	Cyclophosphamide
		Isophorone	Carbamazepine	Diazinon
		Lauric isopropanolamide	Chloridazon	Dichlorvos
		Nitrendipin	DEET	Diclofenac
		Nitrofurantoin	Diazepam	Dimethoate
		Pentoxifylline	Diphenylphosphine oxide	Dinoseb
		Phenazone	Dodemorph	Diuron
		p-Toluenesulfonamide	Fluoxastrobin	Edinphos
		Scopolamine-N-butyl	Furosemide	Ethyl azinphos
		Tetraglyme	Glibenclamide	Ethylenethiourea
		Theophyllin	Ketoprofen	Fenpropimorph
		Tri(butoxyethyl)phosphate	Lauramidopropylbetaine	Fenthion
		Triethylcitrate	Lauryl diethanolamide	Ifosfamide
		Triglyme	Lenacil	Indometacin
		Triisobutylphosphate	Metamitron	Isoproturon
		Tris(1-chloro-2-propyl)phosphate	N-Butylbenzenesulfonamide	Kresoxim-methyl
		Tris(2-ethylhexyl)phosphate	Pyraclostrobin	Linuron
		Vardenafil	Quinmerac	Lorazepam
			Quinmerac	Methiocarb
			Quinoline	Michler's ketone
			Saccharin	N,N-Dimethyldecylamine
			Sucralose	N,N-Dimethyldodecylamine
			Sulcotrione	N,N-Dimethyltetradecylamine
			Telmisartan	N,N-Dimethyltetradecylamine-N-oxide
			Terbuthylazine	Omethoate
			Terbuthylazine-2-hydroxy	Perfluorooctanoic acid
			Terbutryn	Pirimiphos-methyl
			Tetrabromobisphenol A	Propanil
			Thiabandazole	Pyrazophos
			Torasemide	Simazine
			Triadimenol	Spiroxamine
			Trifloxystrobin	Tebuconazole
			Trifloxystrobin NOA413161	Thiacloprid
			Triphenylphosphine oxide	Tributylamine
			Tris(1,3-dichloroisopropyl)phosphate	Trichlorfon
				Tris(2-chloroethyl)phosphate
Number and a sum concentration [ng/L] of detected substances hazardous to water	WWTP_1	22 (10816 ng/L)	21 (9156 ng/L)	18 (1763 ng/L)
	WWTP_2_-O3	23 (15743ng/L)	28 (49827 ng/L)	17 (2365 ng/L)
	WWTP_2_+O3	15 (3836 ng/L)	16 (38396 ng/L)	13 (164 ng/L)
Total concentration of detected substances hazardous to water, [ng/L]	WWTP_1	21735		
	WWTP_2_-O3	67935		
	WWTP_2_+O3	42396		