TEXTE
94/2022

Final report

Trends of pharmaceutical residues in rivers, suspended particular matter and fish – New insights by new analytical methods for active substances, their metabolites and transformation products

von:
Georg Dierkes, Lise Boulard, Arne Wick, Thomas Ternes
Bundesanstalt für Gewässerkunde, Koblenz

Herausgeber:
Bundesanstalt für Gewässerkunde

German Environment Agency

Umwelt Bundesamt

TEXTE 94/2022

RESSORTFORSCHUNGSPLANof the Federal Ministry for the Enviroment, Nature Conservation, Nuclear Safety and Consumer Protection

Project No. (FKZ) 3717 64 413 0

Report No. (UBA-FB) FB000592/1,ENG

Final report

Trends of pharmaceutical residues in rivers, suspended particular matter and fish – New insights by new analytical methods for active substances, their metabolites and transformation products

Klicken Sie hier, um Text einzugeben.

By

Georg Dierkes, Lise Boulard, Arne Wick, Thomas Ternes

Bundesanstalt für Gewässerkunde, Koblenz

On behalf of the German Environment Agency

Imprint

Publisher
Umweltbundesamt
Wörlitzer Platz 1
06844 Dessau-Roßlau
Tel.: +49 340-2103-0
Fax : +49 340-2103-2285
buergerservice@uba.de
Internet : www.umweltbundesamt.de

/umweltbundesamt.de
/umweltbundesamt

Report performed by:
Bundesanstalt für Gewässerkunde
Am Mainzer Tor 1
56068 Koblenz
Deutschland

Report completed in:
May 2021

Edited by:
Section IV 2.2 - Pharmaceuticals
Arne Hein (Fachbegleitung)

Publication as pdf:
http://www.umweltbundesamt.de/publikationen

ISSN 1862-4804

Dessau-Roßlau, September 2022

Abstract: Trends of pharmaceutical residues in rivers, suspended particular matter and fish – New insights by new analytical methods for active substances, their metabolites and transformation products

Since the last decades the increasing detections of pharmaceuticals in the aquatic environment and their risk potential for the aquatic life have become an urgent issue. Even though many pharmaceuticals are at least to some extent removed by sorption and biotransformation during conventional wastewater treatment, a quasi continues emission via wastewater treatment plants result in a so called "pseudo-persistence". Furthermore, metabolites and transformation products (TPs) can show comparable or even higher activities. Therefore, a higher prioritization of pharmaceuticals and their metabolites as well as TPs as environmental pollutants in the European Water Framework Directive and German surface waters regulation (Oberflächengewässerverordnung) would be desirable. Determination of pharmaceuticals in the aquatic environment is focused on the liquid phase. However, only a few standardized methods exist. Indeed, physicochemical parameters of some analytes let accumulation in sediment, suspended particular matter and biota seem to be likely. Hence, these matrices could be interesting alternative matrices for monitoring of pharmaceutical residues in surface waters. The aim of this project was the development, optimization, validation and benchmarking of analytical methods for quantification of selected active pharmaceutical ingredients, their metabolites and TPs in different environmental matrices (water, sediment, suspended particular matter and biota). Using these methods occurrence and distribution of the selected analytes in water, sediment, suspended particular matter and biota samples from different locations were determined. Additionally, samples from the German environmental specimen bank were analyzed to investigate possible time trends in concentrations of pharmaceuticals and to demonstrate the potential of the new methods. Finally, recommendations regarding optimal strategies for determination of pharmaceutical residues with different physicochemical properties in water systems were derived from during this project obtained new insights.

Kurzbeschreibung: Trends von Arzneimittelrückständen in Flüssen, Schwebstoffen und Fischen – Neue Erkenntnisse aus neuen analytischen Nachweiserfahren für Arzneimittelwirkstoffe, deren Metabolite und Transformationsprodukte

Während der vergangenen Dekade wurden immer mehr Arzneimittelwirkstoffe in der aquatischen Umwelt detektiert und das damit verbundene Risikopotenzial für die aquatischen Lebensgemeinschaften stellt ein drängendes Problem dar. Obwohl viele Arzneimittelwirkstoffe durch die konventionelle Abwasserreinigung zumindest teilweise durch Sorption und Biotransformation entfernt werden, führt eine quasi kontinuierliche Einleitung von Arzneimittelresten zu einer so genannten „Pseudo-Persistenz". Des Weiteren weisen manche Metabolite und Transformationsprodukte eine ähnliche oder sogar höhere Wirkung auf als die medizinischen Ausgangsstoffe. Daher wäre eine höhere Priorisierung von Arzneimittelwirkstoffen sowie deren Metabolite und Transformationsprodukte (AMT) als Umweltkontaminanten in der Europäischen Wasserrahmenrichtlinie (WRRL) und der deutschen Oberflächenwasserverordnung (OGewV) begrüßenswert. Die analytische Bestimmung erfolgt zurzeit hauptsächlich in der Wasserphase, wobei nur wenige standardisierte Methoden existieren. Die physikochemischen Eigenschaften mancher Wirkstoffe lassen eine Akkumulation an Sediment, Schwebstoffen und in Biota vermuten, so dass diese Matrices interessante Alternativen zur Wasserphase darstellen. Das Ziel des Projekts war die Entwicklung, Optimierung, Validierung und Bewertung von Quantifizierungsmethoden für AMT in verschiedenen Umweltmatrices (Wasser, Sediment, Schwebstoff und Biota). Mit Hilfe der

entwickelten Methoden wurde das Vorkommen und die Verteilung ausgewählter AMT in Wasser, Sediment, Schwebstoff und Biota von unterschiedlichen Standorten untersucht. Des Weiteren wurden mit Zeitreihen der Umweltprobenbank das Potential der neuen Methoden für die Gewässerüberwachung demonstriert. Aus den gesammelten Erkenntnissen wurden Empfehlungen für ein optimiertes Monitoring von AMT mit verschiedenen physikochemischen Eigenschaften in Oberflächengewässern abgeleitet.

Table of content

List of figures

List of tables

List of abbreviations

ASE	Accelerated solvent extraction
BfG	Bundesanstalt für Gewässerkunde
CE	Collision Energy
DP	Declustering potential
ESI	Electrospray ionization
EWG	Einwohnergleichwerte
GPC	Gel permeation chromatography
HILIC	Hydrophilic interaction liquid chromatography
HPLC	High performance liquid chromatography
IGB	Leibniz-Institut für Gewässerökologie und Binnenfischerei
LOQ	Limit of quantification
MRM	Multiple reaction monitoring
MS	mass spectrometry
MS-MS	tandem mass spectrometry
PSI	Pound-force per square inch
QuEChERS	Quick, Easy, Cheap, Effective, Rugged, Safe
RPLC	Reversed-phase liquid chromatography
SPE	Solid phase extraction
SPM	Suspended particulate matter
TOC	Total organic carbon
TP	Transformation product

Summary

Aims

The aim of the project was the development, optimization, validation and benchmarking of analytical methods for quantification of selected pharmaceuticals and their most important metabolites and TPs in different environmental matrices (water, sediment, suspended particulate matter (SPM), biota).

Content

Selection of analytes

In a first step active pharmaceutical ingredients were prioritized by consumption data and known risk potential. A list of 49 high priority pharmaceuticals has been worked out. Compound selection was based upon the following parameters: physicochemical properties (log K_{OW}; charge state at pH 6-9), production volume, ecotoxicity and occurrence in surface water. In a second step the most important metabolites and TPs of the selected pharmaceuticals were picked. In total, 110 analytes were considered for method development and environmental monitoring.

Method development

Pharmaceutical residues occur in the environment in trace concentrations (sub µg to pg/L). Therefore, high-performance sample preparation technics and high-sensitive quantification methods are required. Using liquid chromatography coupled to mass spectrometry sensitive methods for quantification of the analytes in water, SPM and biota samples were developed. Because of the large polarity and concentration range of the analytes separate methods for extreme polar and middle polar pharmaceuticals are required. Furthermore, the complex environmental matrices need special clean-up methods to reduce disturbing matrix effects. In the following brief descriptions of the different quantification approaches are given.

Water

For analysis of water samples the analytes were divided into two groups i) very till extreme polar (log D at pH=7 < 1) and ii) medium polar (log D at pH=7 >1).

For the extreme polar analytes a hydrophilic interaction liquid chromatographic (HILIC) method was developed. By unique retention mechanisms based on polar interaction this technique is able to separate even extreme polar substances. Since the sample has to be dissolved in acetonitrile for injection into the HILIC-MS/MS system a freezedrying step was established to remove the water. Detailed information about method development and validation can be found in Boulard et al. 2018 [1].

For the medium polar analytes a reversed-phase liquid chromatographic (RP-LC) separation method was developed. No sample pretreatment was necessary and samples were directly injected in to the LC-MS/MS system. Detailed information about method development and validation can be found in the final report of the previous project [2].

Suspended particulate matter (SPM)

SPM is a complex mixture of organic and inorganic material. For analysis of pharmaceuticals and their metabolites as well as TPs sorbed to SPM they have to be extracted from the matrix and co-extracted organic matrix compounds have to be eliminated by sample clean-up. For extraction a high effective pressurized liquid extraction (PLE) method was developed. After a clean-up via

solvent exchange detection was realized via direct injection-reversed phase LC-MS/MS and freeze-drying-HILIC-MS/MS as described for water samples. Overall 100 analytes were included into the method. 56 of them were pharmaceuticals and 44 metabolites and or TPs. Detailed information about method development and validation can be found in Boulard et al. 2020 [3].

Biota

Analysis of pharmaceuticals and their metabolites as well as TPs in biological matrices is very challenging, since compounds such as proteins and lipids induce strong matrix effects and are difficult to separate from analytes. Consequently, a complex sample extraction and clean-up procedure had to be developed. To cover the broad polarity range of the analytes two extraction steps with increasing polarity and non-discriminating clean-up techniques such as size exclusion were used. The developed quantification method covered 36 pharmaceuticals and 27 metabolites as well as TPs and was validated for the fish matrices filet, liver and blood plasma.

Occurrence and distribution of pharmaceuticals and their metabolites as well TPs

To get an overview about the occurrence of the selected pharmaceuticals and their metabolites as well TPs in surface water samples from water bodies affected by different amounts of wastewater were analyzed.

Water phase

Grab samples from Stechlin Lake, Rhine at Koblenz, Saar at Rehlingen and Teltow Canal were analyzed. The number of detected analytes increased with the wastewater proportion reaching up to 86 (of 110) pharmaceuticals and their metabolites as well TPs detected in the Teltow Canal. Up to 50 % of the detected target compounds were metabolites and TPs, underlining their relevance for a comprehensive monitoring of the environmental burden from pharmaceuticals. As expected concentrations considerably increased with the wastewater proportion. Highest concentrations were found in the Teltow Canal for oxipurinol, a metabolite and TP of allopurinol, with ca. 12 µg/L.

Detailed results can be found in Boulard et al. 2018 [1] and in the final report of the previous project [2].

Suspended particulate matter (SPM)

Annual composite SPM samples were analyzed to determine the pharmaceuticals and their metabolites as well TPs concentrations in the years between 2005 and 2015 along the river Rhine and in the river Saar at Rehlingen. 61 of the 100 analytes could be detected with increasing concentrations along the Rhine and higher concentrations at Rehlingen/Saar for most of the analytes. Generally speaking, the analytes showing the highest concentrations (> 10 ng/g) were either positively charged or very hydrophobic (log D >4). The concentrations of many pharmaceuticals and their metabolites as well TPs correlated well with the annual consumed quantities in Germany supporting the suggestion that communal WWTP are the main sources for pharmaceuticals and their metabolites as well TPs emission. However, some pharmaceuticals showed anomalous geographical and temporal trends indicating additional industrial discharge into the aquatic environment. Detailed results can be found in Boulard et al. 2020 [3] and in the final report of the previous project [2]. Overall the results showed that even for relatively polar pharmaceuticals SPM is a suitable matrix for reliable trend analysis as well as the identification of specific emission sources.

Biota

To get an overview about pharmaceuticals and their metabolites as well TPs burden of fish in surface waters with different wastewater loads breams from the rivers Rhine and Saar, the

Teltow Canal as well as carps received from WWTP effluent polishing ponds were analyzed. In general, a positive correlation was observed between wastewater content in water and the pharmaceutical burden in fish. Concerning distribution of pharmaceuticals and their metabolites as well TPs within different fish tissues filet, liver and blood plasma samples were investigated.

In carps fed by WWTP effluent and the Teltow Canal highly effected by wastewater (up to 100% from April to October) many of the analytes were detected at least once. In breams from the Saar and Rhine nine pharmaceuticals were detected and concentrations were lower compared to the other sampling sites. Concentration patterns of the analytes strongly differed between the sampling sites. Due to expectable comparable discharge patterns via wastewater differences must be caused by species specific uptakes or metabolization rates. The investigation of the distribution of the analytes within the fish revealed in general highest concentrations in fish liver, followed by fillet and plasma. Liver to fillet concentration ratios showed no relationship to the polarity or physicochemical properties (e.g. charge) of the analytes. Moreover, no correlation between plasma concentrations and concentrations in liver and filet could be found. In contrast to nonpolar contaminants such as hexachlorobenzene, polychlorinated biphenyls and polybrominated biphenylethers, for the investigated pharmaceuticals lipid content seems to play no crucial role in accumulation. Beside the parent pharmaceuticals several metabolites and TPs were found in the fish tissues. Concentrations of metabolites were comparable or even slightly higher than those of their parent pharmaceuticals (e.g. n-desmethyl tramadol or norlidocaine). This could be a hint for formation of these metabolites in fish. Overall, determined concentrations were consistent with previous European studies. However, 17 analytes were detected in fish tissue for the first time in this study. In particular, the newly detected metabolites/TPs were: three metabolites and TPs of carbamazepine, a metabolite of citalopram, a metabolite of diphenhydramine, a metabolite of lidocaine, three metabolites of tramadol and a TP of gabapentin. Seven parent pharmaceuticals were also detected for the first time: amisulpride, bicalutamide, chlorothiazide, flecainide, lidocaine, quetiapine and sitagliptin.

Between 2005 and 2015 time trends of the analytes were determined in bream liver and fillet for the location of Rehlingen/Saar. On the contrary to the suspended particulate matter, only limited correlations were observed between the consumption and measured concentrations. Several reasons could explain this observation i) consumption was relatively constant for the few analytes with concentration >LOQ ii) fish mobility iii) individual uptake and metabolism. Detailed results can be found in Boulard et al. 2020 [4].

Distribution of pharmaceuticals

79 of 117 analyzed pharmaceuticals and their TPs as well as metabolites could be detected at least one time in any of the three matrices. Of these 24 were found in all three matrixes, 30 in water and SPM, three in fish and SPM, 21 exclusively in water and one exclusively in fish. Substances such as sertraline and fluoxetine with high sorption affinities could not be detected in the water phase but were detected in SPM and fish tissues. In contrast some of the very polar compounds such as oxazepam and primidone were only found in the water phase. Didesmethylcitalopram was exclusively detected in fish tissues and is thus probably formed by transformation of citalopram or desmethylcitalopram in fish. Non-detections in fish may be caused by limited uptake or fast excretion, elevated detection limits or transformation to unknown metabolites.

Conclusions

The results clearly supported that the emission from domestic WWTPs are the major source for pharmaceuticals in the samples investigated. However, anomalies in the concentration pattern indicate additional point sources, most likely from production sites. Most of pharmaceuticals and their TPs as well as metabolites could be detected in all three investigated compartments. Only non-polar compounds with strong sorption affinities and very polar compounds were exclusively found in SPM/fish and water, respectively. The metabolite didesmethylcitalopram was exclusively found in fish indicating a transformation of citalopram or its human metabolite desmethylcitalopram in fish. Metabolites and TPs proved to be important compounds in environmental monitoring. Concentrations of metabolites and TPs were comparable or in some cases (e.g. O-desmethylvenlafaxine a metabolite of venlafaxine) even slightly higher than those of their parent pharmaceuticals. Since metabolites and TPs might have similar activities or in a few cases even an enhanced ecotoxicological potential they are of emerging concern and should not be neglected.

Regarding monitoring activities all three investigated matrices have different benefits and limitations. Water samples are easy to take and show little matrix effects, which facilitates analysis of dissolved trace amounts of pharmaceuticals. However, grab samples give only a snap shot of the contamination and compounds tending to adsorb to organic matter often show water concentration below LOD. Compounds with high sorption affinity such as cations (e.g. sitagliptine) are predestinated for analyzing in SPM and measuring only the water phase would underestimate their loads in surface waters. Hence, integrative sampling of SPM gives a comprehensive overview about environmental burden. Moreover, SPM was confirmed to be a suitable matrix for retrospective investigation of time trends using for example the archive of the German Environmental Specimen Bank. Due to good correlation between SPM concentrations and consumption data even prediction of future SPM concentration can be predicted on basis of changes in consumption behavior. On the contrary fish prove to be not suitable for trend analysis of pharmaceuticals. However, analysis of fish gives information about bioaccumulation and biotransformation. However, for those pharmaceuticals investigated only relatively low accumulation rates (<< 200) were observed and concentrations were quite low even in fish from WWTP fish monitoring ponds. Consequently, the risk for consumers can be estimated as low. Nevertheless, adverse effects on aquatic life cannot be excluded at chronic low-level burden. Ecotoxicological tests are needed to investigate adverse effects of pharmaceuticals on behavior, reproduction and energy budget in aquatic life.

The results of this study give first insides into distribution and fate of pharmaceuticals in the aquatic environment. By means of a comprehensive monitoring including coherent sampling of all three matrices at different sampling sites a more detailed examination of the behavior of different compound classes would be possible.

Results of this project have been published in form of three peer-reviewed articles:

Boulard et al. (2018): Utilization of large volume zwitterionic hydrophilic interaction liquid chromatography for the analysis of polar pharmaceuticals in aqueous environmental samples: Benefits and limitations (https://doi.org/10.1016/j.chroma.2017.12.023)

Boulard et al. (2020): Spatial distribution and temporal trends of pharmaceuticals sorbed to suspended particulate matter of German rivers (https://doi.org/10.1016/j.watres.2019.115366)

Boulard et al. (2020): Development of an analytical method to quantify pharmaceuticals in fish tissues by liquid chromatography-tandem mass spectrometry detection and application to environmental samples (https://doi.org/10.1016/j.chroma.2020.461612)

Zusammenfassung

Ziele

Das Ziel des Projekts war die Entwicklung, Optimierung, Validierung und Bewertung von analytischen Methoden zur Quantifizierung von ausgesuchten Arzneimittelwirkstoffen und deren wichtigsten Metaboliten sowie Transformationsprodukten (TPs) in verschiedenen Umweltmatrizes (Wasser, Sediment, Schwebstoff und Biota).

Herangehensweise

Auswahl der Analyten

Im ersten Schritt wurden Arzneimittelwirkstoffe anhand von Verbrauchsdaten und bekanntem Risikopotential ausgewählt. Es ergab sich eine Liste von 49 hochpriorisierten Arzneimittelwirkstoffen. Die Substanzauswahl erfolgte nach folgenden Kriterien: physikochemische Eigenschaften (log K_{OW}; Ladung bei pH 6-9), Produktionsmengen, Ökotoxizität und Vorkommen in Oberflächengewässern. In einem zweiten Schritt wurden für die ausgewählten Arzneimittelwirkstoffe die wichtigsten Metabolite und TPs über eine Literaturrecherche identifiziert. Insgesamt wurden 117 Analyten bei der Methodenentwicklung und dem anschließenden Umweltmonitoring berücksichtigt.

Methodenentwicklung

Arzneimittelwirkstoffe treten in der Umwelt im Spurenbereich (unterer µg bis ng/L Bereich) auf. Daher sind hocheffiziente Probenvorbereitungstechniken und hochsensitive Quantifizierungsmethoden notwendig. Durch den Einsatz der Flüssigchromatographie gekoppelt an die Massenspektrometrie konnten ausreichend sensitive Methoden für den quantitativen Nachweis der Arzneimittelwirkstoffe in Wasser, Schwebstoff und Biota entwickelt werden. Auf Grund des großen Polaritäts- und Konzentrationsbereichs der untersuchten Analyten wurden separate Methoden für extrem polare und mittelpolare Wirkstoffe entwickelt. Des Weiteren erforderten die komplexen Matrizes angepasste Probenvorbereitungsschritte zur Reduzierung von störenden Matrixeffekten. Im Folgenden werden die Quantifizierungsansätze kurz erläutert.

Wasserphase

Für die Analyse der Wasserproben wurden die Analyten in zwei Gruppen eingeteilt: i) sehr bis extrem polare (log D bei pH=7 <1) und ii) mittelpolare (log D bei pH=7 >1).

Für die extrem und sehr polaren Analyten wurde eine hydrophile Interaktion flüssigchromatographische (HILIC) Methode entwickelt. Bei dieser Art der Chromatographie basiert die Retention auf der Säule auf einzigartigen polaren Wechselwirkungen und ermöglicht die Auftrennung von sogar extrem polaren Verbindungen. Da die Proben für die Analyse in Acetonitril vorliegen müssen, wurde zunächst das Wasser durch Gefriertrocknung entfernt. Detaillierte Informationen über die Methode und die Methodenvalidierung befinden sich in Boulard et al. 2018 [1].

Für die mittelpolaren Analyten wurde eine Umkehrphasenflüssigchromatographie (RP-LC) entwickelt. Die Analyse erfolgte ohne Probenvorbereitung durch Direktinjektion ins LC-MS/MS System. Detaillierte Informationen über die Methode und die Methodenvalidierung befinden sich im Abschlussbericht des Vorläuferprojekts [2].

Schwebstoff

Schwebstoffe sind eine komplexe Mischung aus organischen und anorganischen Materialien. Vor der Analyse müssen die sorbierten Stoffe zunächst aus der Matrix extrahiert werden und co-extrahierte organische Matrixbestandteile müssen durch ein Proben Clean-Up aus dem Extrakt entfernt werden. Für die Extraktion wurde eine hocheffiziente Flüssigextraktion unter erhöhtem Druck und Temperatur (PLE) entwickelt. Nach einem Clean-Up via Lösemittelaustausch wurden die Extrakte per Direktinjektion mittels RP-LC-MS/MS bzw. nach Gefriertrocknung per HILIC-MS/MS wie für die Wasserproben beschrieben analysiert. Insgesamt wurden 100 Analyten darunter 56 Arzneimittelwirkstoffe sowie 44 Metabolite und TPs in die Methode intrigiert. Detaillierte Informationen über die Methode und die Methodenvalidierung befinden sich in Boulard et al. 2020 [3].

Biota

Die Analyse von Arzneimittelwirkstoffen sowie deren Metabolite und TPs in biologischen Matrizes stellt eine große Herausforderung dar. Matrixbestandteile wie Proteine und Lipide führen zu starken Matrixeffekten und sind schwer von den Analyten abzutrennen. Dementsprechend musste eine aufwändige Probenextraktion und -aufreinigung entwickelt werden. Zur Abdeckung des breiten Polaritätsbereichs wurde mit zwei Extraktionsschritten mit unterschiedlich polaren Lösungsmitteln und nichtdiskriminierenden Ausreinigungstechniken wie Größenausschlusschromatographie gearbeitet. Die Methode umfasste 36 Wirkstoffe sowie 27 Metabolite und TPs. Sie konnte erfolgreich für die Matrizes Fischfilet, -leber und -plasma validiert werden.

Vorkommen und Verteilung von Arzneimittelwirkstoffen und deren Metaboliten und TPs

Um einen Überblick über das Vorkommen der ausgewählten Arzneimittelwirkstoffe und deren Metabolite sowie TPs in Oberflächengewässern zu erhalten, wurden Proben aus Gewässern mit unterschiedlichem Abwasseranteil untersucht.

Wasserphase

Stichproben aus dem Stechlinsee, Rhein bei Koblenz, Saar bei Rehlingen und dem Teltow Kanal wurden untersucht. Die Anzahl der detektierten Verbindungen stieg mit dem Abwasseranteil an und erreichte bis zu 86 im Teltow Kanal. Bis zu 50 % der gefundenen Verbindungen waren Metaboliten oder TPs, was deren Bedeutung für das Ausmaß der Belastung der Umwelt mit Arzneimitteln verdeutlicht. Wie zu erwarten stiegen die gefundenen Konzentrationen ebenfalls mit dem Abwasseranteil an. Die höchste gefundene Konzentration war für das Oxipurinol, ein Metabolit des Allopurinols, im Teltow Kanal mit ca. 12 µg/L. Eine detaillierte Beschreibung der Ergebnisse befindet sich in Boulard et al. 2018 [1] und im Abschlussbericht des Vorläuferprojekts [2].

Schwebstoff

Jahresmischproben aus den Jahren 2005 bis 2105 von Rhein und Saar wurden untersucht. 61 von 100 Analyten konnten nachgewiesen werden. Die Konzentrationen stiegen für die meisten Analyten entlang des Rheins an und zeigten die höchsten Konzentrationen in der Saar. Im Allgemeinen zeigten positive geladene oder sehr hydrophobe Substanzen (log D >4) die

höchsten Konzentrationen (> 10 ng/g). Die Konzentrationen der Wirkstoffe sowie deren Metaboliten und TPs wiesen eine gute Korrelation mit den Jahresverbrauchsdaten für Deutschland auf. Dies unterstützt die Hypothese, dass kommunale Kläranlagen die Hauptquellen für Arzneimittelwirkstoffe und deren Metaboliten und TPS in Oberflächengewässern darstellen. Allerdings zeigten einige Wirkstoffe abnormale geographische und zeitliche Trends auf, die auf zusätzliche industrielle Einleitungen hindeuten. Detaillierte Informationen sind in Boulard et al. 2020 [3] und im Abschlussbericht des Vorläuferprojekts [2] zu finden. Insgesamt zeigen die Ergebnisse, dass Schwebstoffe sogar für relativ polare Arzneimittelwirkstoffe eine verlässliche Matrix für die Untersuchung von Trends und zur Identifizierung von spezifischen Quellen darstellen.

Biota

Um einen Überblick über die Belastung von Fischen mit Arzneimittelwirkstoffen zu erhalten, wurden Brassen aus den Rhein, der Saar und dem Teltowkanal sowie Karpfen aus Bioakkumulationsteichen von Kläranlagen untersucht. Bezüglich der Verteilung der Wirkstoffe sowie deren Metaboliten und TPs wurden Fischfilets, Lebern und Blutplasmaproben untersucht.

In den Karpfen, die direkt im Kläranlagenauslauf gehalten wurden, und in den Brassen aus dem Teltowkanal, der stark von Abwasser beeinflusst ist (bis zu 100 % Abwasseranteil von April bis Oktober) konnte viele der untersuchten Substanzen zumindest einmal nachgewiesen werden.

In Brassen aus der Saar und dem Rhein wurden neun Substanzen nachgewiesen und die Konzentrationen lagen unter denen der anderen beiden Probenahmestellen. Die Belastungsmuster variierten sehr stark zwischen den einzelnen Probenahmestellen, was auf Grund der zu erwartenden vergleichbaren Zusammensetzung des jeweiligen Abwassers auf speziesspezifische Aufnahme- und Metabolisierungsraten zurückzuführen ist.

Die Untersuchungen bezüglich der Verteilung im Fischgewebe zeigten, dass die höchsten Konzentrationen in der Leber gefolgt vom Filet und Plasma zu finden sind. Die Konzentrationsverhältnisse von Leber zu Filet zeigten keinen Zusammenhang bezüglich Polarität und den physikochemischen Eigenschaften (z. B. Ladung) der Wirkstoffe. Des Weiteren konnte keine Korrelation zwischen den Plasmakonzentrationen und der Konzentrationen in der Leber und dem Filet festgestellt werden. Im Gegensatz zu unpolaren Mikroschadstoffen wie Hexachlorbenzen, polychlorierte Biphenyle und polybromierte Biphenylether, scheint der Fettgehalt in den Fischen keine kritische Rolle bei der Akkumulation zu spielen.

Neben den eigentlichen Wirkstoffen konnten auch zahlreiche Metaboliten und TPs im Fischgewebe nachgewiesen werden. Die Metabolitkonzentrationen lagen im gleichen Bereich oder sogar über denen der Ausgangsverbindungen (z. B. N-Desmethyltramadol oder Norlidocain). Dies könnte auf die in-vivo Bildung dieser Metabolite im Fisch hindeuten. Insgesamt betrachtet waren die ermittelten Konzentrationen vergleichbar mit denen aus anderen europäischen Studien. Allerdings konnten 17 Analyten erstmalig in dieser Studie in Fisch nachgewiesen werden. Bei diesen Substanzen handelte es sich im Einzelnen um: 3 Metaboliten und TPs von Carbamazepin, ein Metabolit von Citalopram, ein Metabolit von Diephenhydramin, ein Metabolit von Lidocain, 3 Metaboliten von Tramadol und ein TP von Gabapentin. Sieben Wirkstoffe konnten ebenfalls erstmalig nachgewiesen werden: Amisulprid, Bicalutamid, Chlorthiazid, Flecainid, Lidocain, Quetiapin und Sitagliptin.

Es wurden Zeittrends für den Zeitraum zwischen 2005 und 2015 für den Standort Rehlingen an der Saar in Brassenlebern und -filets untersucht. Im Gegensatz zu den

Schwebstoffuntersuchungen konnten nur begrenzte Korrelationen mit den jeweiligen Verbrauchsdaten festgestellt werden. Mehre Erklärungsansätze kommen für diesen Befund in Frage: i) der Verbrauch bleibt im betrachteten Zeitraum für die wenigen Wirkstoffe mit Konzentrationen >LOQ im Fisch weitestgehend konstant, ii) Mobilität der Fische, iii) individuelle Aufnahme und Metabolisierung. Eine detaillierte Betrachtung der Ergebnisse kann in Boulard et al. 2020 [4] nachgelesen werden.

Verteilung der Arzneimittelwirkstoffe

79 der 117 untersuchten Arzneitmittelwirkstoffe und deren Mataboliten sowie TPs konnten mindestens einmal in einen der drei Matrizes nachgewiesen werden. Von diesen wurden 24 in allen drei Matrizes, 30 in Wasser und Schwebstoff, drei in Fisch und Schwebstoff, 21 exklusiv in der Wasserphase und einer exklusiv in Fisch gefunden werden. Substanzen mit hoher Sorptionsaffinität wie Sertralin oder Fluoxetin konnten nicht in der Wasserphase aber im Schwebstoff und Fischgewebe detektiert werden. Im Gegensatz dazu wurden sehr polare Verbindungen wie das Oxazepam und das Primidon nur in der Wasserphase gefunden. Didesmethylcitalopram wurde ausschließlich in Fischgewebe nachgewiesen und wurde daher vermutlich im Fisch durch Metabolisierung von Citalopram oder Desmethylcitalopram gebildet. Negativbefunde im Fisch sind ggfs. auf eine limitierte Aufnahme bzw. schnelle Ausscheidung, zu hohe Nachweisgrenzen oder die Bildung unbekannter Metabolite zurückzuführen.

Zusammenfassung

Die Ergebnisse legen Emissionen von kommunalen Kläranlagen als Hauptquelle für Arzneimittelwirkstoffe in den untersuchten Proben nahe. Allerdings deuten Anomalien in den Konzentrationsmustern auf zusätzliche Punktquellen vermutlich von Produktionsstandorten hin. Die meisten Arzneimittelwirkstoffe und deren Metaboliten sowie TPs konnten in allen drei Matrizes nachgewiesen werden. Nur unpolare Verbindungen mit starken Sorptionseigenschaften wurden ausschließlich in Schwebstoff und Fisch gefunden. Wo hingegen sehr polare Verbindungen nur in der Wasserphase nachgewiesen werden konnten. Der Metabolit Didesmethylcitalopram wurde ausschließlich in Fisch gefunden, was auf eine in-vivo Transformation von Citalopram oder seines menschlichen Metaboliten Desmetylcitalopram im Fisch hindeutet.

Metaboliten und TPs haben sich als bedeutend in Bezug auf die Belastung von Gewässern mit Arzneimitteln herausgestellt. Konzentrationen von Metaboliten und TPs waren vergleichbar oder in manchen Fällen sogar höher als die der jeweiligen Ausgangsstoffe. Da bei Metaboliten und TPs von einer ähnlichen Aktivität oder in manchen Fällen sogar von einem erhöhten ökotoxikologischen Potential auszugehen ist, ist deren Vorkommen in Gewässern besorgniserregend und sollte nicht unbeachtet bleiben.

Bezüglich eines Gewässermonitorings weisen alle drei untersuchten Matrizes Vorteile und Limitierungen auf. Wasserproben lassen sich einfach nehmen und zeigen nur geringe Matrixeffekte, was eine schnelle Analyse von Arzneimittelwirkstoffen im Spurenbereich ermöglicht. Allerdings bilden Stichproben nur eine Momentaufnahme der Belastung ab und sorbtive Verbindungen liegen häufig nur in Konzentrationen unterhalb der Nachweisgrenze in der Wasserphase vor. Stark sorbierende Stoffe wie Kationen (z. B. Sitagliptin) lassen sich am besten im Schwebstoff nachweisen, da eine Bestimmung in der Wasserphase zu einer Unterschätzung der Frachten in Oberflächengewässern führen würde. Des Weiteren liefert eine integrative Beprobung (z. B. Monatsmischproben) einen umfangreichen Überblick über die

Entwicklung der Gewässerbelastung. Dies verdeutlichen auch die retrospektivischen Untersuchungen von Zeitreihen in Schwebstoffen der Umweltprobenbank. Auf Basis der guten Korrelationen zwischen den Schwebstoffkonzentrationen und den Verbrauchsdaten ist sogar eine Vorhersage von zukünftigen Schwebstoffkonzentrationen im Zuge von Änderungen im Konsumverhalten möglich. Die Betrachtung von Trends in Fischen ergab keine Korrelation mit den Verbrauchsdaten. Stattdessen gibt die Analyse von Fischen Aufschlüsse über die Bioakkumulation und Biotransformation von Arzneimittelwirkstoffen. Für die untersuchten Arzneimittelwirkstoffe wurden nur relative niedrige Akkumulationsraten (<<200) festgestellt und dementsprechend selbst in den Fischen aus Kläranlagenteichen nur geringe Konzentrationen gefunden. Dementsprechend kann durch den Verzehr von Fisch aus den untersuchten Gewässern von einem geringen Risiko für den Verbraucher in Bezug auf die Arzneimittelwirkstoffbelastung ausgegangen werden. Nichtsdestotrotz können Beeinträchtigungen von Wasserlebewesen durch eine chronische Exposition von niedrigen Konzentrationen nicht ausgeschlossen werden. In weiteren Studien sollte der Einfluss von Arzneimittelwirkstoffen und deren Metaboliten sowie TPs auf das Verhalten, Reproduktion und den Energiehaushalt untersucht werden.

Die Ergebnisse dieser Studie geben nur erste Einblicke in die Verteilung und das Verhalten von Arzneimittelwirkstoffen in der aquatischen Umwelt. Durch ein umfassendes Monitoring, das eine zeitlich zusammenhängende Beprobung aller drei Matrizes an verschiedenen Probenahmestellen beinhaltet, wäre eine detaillierte Betrachtung des Umweltverhaltens von unterschiedlichen Substanzklassen möglich.

Die Ergebnisse des Projekts wurden in drei wissenschaftlichen Artikeln veröffentlicht:

Boulard et al. (2018): Utilization of large volume zwitterionic hydrophilic interaction liquid chromatography for the analysis of polar pharmaceuticals in aqueous environmental samples: Benefits and limitations (https://doi.org/10.1016/j.chroma.2017.12.023)

Boulard et al. (2020): Spatial distribution and temporal trends of pharmaceuticals sorbed to suspended particulate matter of German rivers (https://doi.org/10.1016/j.watres.2019.115366)

Boulard et al. (2020): Development of an analytical method to quantify pharmaceuticals in fish tissues by liquid chromatography-tandem mass spectrometry detection and application to environmental samples (https://doi.org/10.1016/j.chroma.2020.461612)

1 Introduction

The project run from March 2018 until April 2020 as a follow-up project of the project "Method development for analysis of pharmaceuticals in environmental samples" (Forschungskennzahl: 3715674130). The final report can be found at https://www.umweltbundesamt.de/publikationen/method-development-for-analysis-of-pharmaceuticals

Results of this project have been published in form of three peer-reviewed articles [1, 3, 4]. These provide technical details of the analytical methods and a detailed description of methods development.

1.1 Project background

Since the last decades the increasing pollution of the aquatic environment with pharmaceuticals and their risk potential for the aquatic life has become an urgent issue. The number of pharmaceuticals detectable in the environment is rising each day. The tremendous number of pharmaceuticals on the market and the various transformation reactions in the body as well as in the environment lead to an enormous number of potential contaminants.

After uptake and distribution pharmaceuticals are metabolized within the human body. In most cases oxidative biotransformation reactions lead to more polar metabolites, which are excreted after conjugation to glucuronic acid, sulfate or amino acids via urine. The excretion pattern varies strongly between the different pharmaceuticals and even individuals. So, there are pharmaceuticals which are excreted mostly unchanged and others which are completely metabolized such as allopurinol to its metabolite oxipurinol.

For most pharmaceuticals effluents of municipal wastewater treatment plants (WWTPs) are indicated as the major source in the aquatic environment. Parent pharmaceuticals and their metabolites are continuously discharged into the WWTPs by domestic and hospital wastewater, after they were excreted, washed off from the human skin or directly disposed. Conventional biological wastewater treatment is often not able to mineralize organic micropollutants, but rather a high variety of TPs are formed (reviewed in [5]). These TPs are discharged together with the remaining parent pharmaceuticals and metabolites by WWTPs. Metabolites and TPs might have similar activities or in a few cases even an enhanced ecotoxicological potential as reported in [6] and [7]. Thus, it is crucial to know which stable TPs are formed in biological WWTP processes and are discharged by municipal WWTPs into rivers and streams.

So far, metabolites and TPs are still scarcely considered in environmental studies and consequently there is a lack of data concerning occurrence, distribution and fate. Especially the relevance of TPs and metabolites is inadequate investigated. Distribution of chemical compounds between different environmental compartments depends on physicochemical properties and can strongly differ between parent compounds and TPs or metabolites due to changes in polarity, charge or molecular size. The sorption of hydrophobic micropollutants such as PCBs or PAHs on suspended particulate matter (SPM) is well investigated, but there are only few studies about pharmaceuticals. Beside their relative low hydrophobicity especially cationic pharmaceuticals promise high sorption potentials. SPM can function as a kind of natural passive sampling material providing a integrative image of the aquatic pollution. Consequently, SPM

could be an interesting matrix for environmental studies especially for retrospective investigations of time trends.

Fish and other aquatic life can take up pharmaceuticals from the surrounding water through the gills and dermal absorption. Another route is dietary intake of suspended particulate matter and prey. Bioavailability and species-specific metabolism and excretion behavior are important factors for internal burden in organisms. These attributes depend on physicochemical properties, too. Depending on metabolism and excretion behavior some pharmaceuticals may accumulate in the aquatic food web and finally in fish tissue. Accumulation of pharmaceuticals and associated metabolites and TPs in biota is scarily investigated and there are only a few data about their occurrence in fish. Consequently, there are no reliable data for evaluation of the resulting potential risks for top predators and human consumers.

With respect to environmental monitoring strategies not only the distribution but also other parameters such as sampling, stability or analytical effort are important criteria for the choice of the best matrix. Water, sediment/suspended particulate matter and biota provide different benefits and limitations and which is the best choice depends on the particular scientific question. However, comparative studies are scare and beside a standard method for selected pharmaceuticals in water (DIN 38407-47:2017-07) there are currently no guidelines for monitoring of pharmaceuticals in the aquatic environment. Furthermore, there is a lack of comprehensive analytical methods allowing simultaneous determination of a broad range of pharmaceuticals and their metabolites as well as TPs in SPM and fish.

The aim of this project was to close this gap of knowledge and to elaborate recommendations for the optimal monitoring strategy for pharmaceuticals in water, sediment, suspended particulate matter (SPM) and biota (e.g. fish).

1.2 Conceptual design

The aim of the project was the development, optimization, validation and benchmarking of analytical methods for quantification of selected pharmaceuticals and their most important metabolites and TPs in different environmental matrices (water, sediment, suspended particulate matter (SPM), biota).

To reach the project aims a project concept was elaborated including an in-depth review of scientific literature, innovative method development, groundbreaking biodegradation experiments and extensive monitoring campaigns. In a first step a selection of metabolites and TPs of prioritized pharmaceuticals was done (ref. chapter 2). For these highly sensitive analytical methods for quantification in the matrices water, suspended particulate matter (SPM) and different fish tissues (fillet, liver and blood) were developed and validated (ref. chapter 3). The newly developed analysis methods were utilized to evaluate the occurrence and distribution of the analytes in environmental samples from different aquatic systems. Sampling sites were selected to cover a broad range of affection by urban wastewater and industrial discharge (ref. chapter 4). Finally, an inter-matrix comparison was done and recommendations for monitoring activities of pharmaceuticals in aquatic systems were derived from study data (ref. chapter 5).

2 Selection of metabolites and TPs

In the previous project a categorization and prioritization of pharmaceuticals has been done [2]. A list of 60 high priority pharmaceuticals has been worked out. Compound selection was based upon the following parameters: physicochemical properties (log K_{OW}; charge state at pH 6-9), production volume, ecotoxicity and occurrence in surface water. The same list of pharmaceuticals was selected for this project. For these pharmaceuticals the most important metabolites and transformations products were identified by meaning of a comprehensive literature search focussing on human metabolism and biodegradation studies as well as photo degradation experiments. In a second step the metabolites and TPs were prioritized regarding their percentage in the mass balance of the particular pharmaceutical, their stability and ecotoxicological potential. A limitation was the availability of commercial reference standards and stable isotope labeled surrogate standards for compensation of matrix effects. The finally selected analytes are listed in Table 1. In total 117 (60 pharmaceuticals and 57 metabolites and or transformation products) analytes were considered for further analysis.

Table 1: Pharmaceuticals analyzed in the different matrices within this project

analyte	category	water	SPM	fish
Aliskiren	P	X	X	
Amisulpride	P	X	X	X
Atenolol	P	X	X	
Atenolol acid	M, TP	X	X	
Hydroxyatenolol	M	X	X	
Bezafibrate	P	X	X	X
Bicalutamide	P	X	X	X
Candesartan	P	X	X	X
Carbamazepine	P	X	X	X
2-Hydroxycarbamazepine	M	X	X	X
3-Hydroxycarbamazepine	M	X	X	X
10-hydroxy-10,11-dihydroxycarbamazepine	M	X	X	X
10,11-dihydroxy-10,11-dihydrocarbamazepine	M	X	X	X
Acridone	M, TP	X	X	X
9-Acridine carboxylic acid	TP	X	X	
Cetirizine	P	X	X	X

analyte	category	water	SPM	fish
Citalopram	P	X	X	X
Desmethylcitalopram	M	X	X	X
Didemethylcitalopram	M	X	X	X
Chlorothiazide	P	X	X	X
Clarithromycin	P	X	X	
Clopidogrel	P	X	X	X
Clopidogrel carboxylic acid	M	X	X	X
Diclofenac	P	X	X	X
4'-hydroxy-diclofenac	M, TP	X	X	
Diclofenac carboxylic acid	TP	X	X	
Diclofenac lactam	TP	X	X	
Diphenhydramine	P	X	X	X
N-Desmethyl diphenhydramine	M, TP	X	X	X
Duloxetine	P		X	
Fexofenadine	P	X	X	X
Flecainide	P		X	X
Flecainide-meta-O-dealkylated	M	X	X	X
Fluconazole	P	X	X	X
Fluoxetine	P	X	X	X
Norfluoxetine	M	X	X	X
Furosemide	P	X	X	X
Gabapentin	P	X	X	
Gabapentin lactam	TP	X	X	X
Hydrochlorothiazide	P	X	X	X
Ibuprofen	P	X		
2-Hydroxyibuprofen	M	X		
Carboxyibuprofen	M	X		
Irbesartan	P	X	X	

analyte	category	water	SPM	fish
Lamotrigine	P	X	X	X
Levetiracetam	P	X	X	
Levetiracetam acid	M	X	X	
Lidocaine	P	X	X	X
Nor lidocaine	M	X	X	X
Metoprolol	P	X	X	X
Hydroxy metoprolol	M, TP	X	X	
O-Desmethyl Metoprolol	M, TP	X	X	X
Naproxen	P	X	X	
O-Desmethyl-Naproxen	M	X	X	
Olmesartan	P	X	X	
Oxazepam	P	X	X	X
Phenytoin	P	X	X	X
Pregabalin	P	X	X	
Pregabalin lactam	TP			X
Primidone	P	X	X	X
Quetiapine	P		X	X
7-hydroxy-quetiapine	M		X	
Quetiapine sulfoxide	M		X	X
Ritalinic acid	TP	X	X	
Roxithromycin	P	X	X	
Sertraline	P	X	X	X
N-Desmethyl sertraline	M	X	X	
Sertraline ketone	M		X	
Sildenafil	P	X	X	
Sitagliptin	P	X	X	X
Sotalol	P	X	X	
Sulfamethoxazole	P	X	X	X

analyte	category	water	SPM	fish
N-Acetyl sulfamethoxazole	M	X	X	X
Sulpiride	P	X	X	X
Tadalafil	P	X	X	
Telmisartan	P	X	X	X
Torasemide	P	X	X	X
Hydroxytorasemide	M	X	X	X
Tramadol	P	X	X	X
Dehydrotramadol	M	X	X	
O-Desmethyl-Tramadol	M	X	X	X
N-Desmethyl-Tramadol	M	X	X	X
N,O-Didesmethyl-Tramadol	M	X	X	X
Trimethoprim	P	X	X	X
3-Desmethyl trimethoprim	M	X	X	X
5-(3,4,5-Trimethoxybenzoyl)-2,4-pyrimidinediamine	TP	X	X	X
Valsartan	P	X	X	X
Valeryl-4-hydroxyvalsartan	M	X	X	X
Valsartanic acid	TP	X	X	X
Venlafaxine	P	X	X	X
N-Desmethyl venlafaxine	M	X	X	X
O-Desmethyl venlafaxine	M	X	X	X
N,O-Desmethyl venlafaxine	M	X	X	X
Xipamide	P	X	X	X
4-Acetamidoantipyrine	M	X	X	
4-Formylaminoantipyrine	M	X		
4-Methylaminoantipyrine	M	X		
Abacavir	P	X	X	
Abacavir carboxylate	M	X		

analyte	category	water	SPM	fish
Aciclovir	P	X	X	
Bisoprolol	P	X	X	
Clindamycin	P	X	X	
Clindamycin sulfoxide	M	X	X	
Diatrizoate	P	X		
Emtricitabine	P	X	X	
Emtricitabine carboxylate	TP	X		
Emtricitabine S-oxide	TP	X		
Lamivudine	P	X	X	
Metformin	P	X	X	
Guanyl urea	TP	X	X	
N-acetyl mesalazine	M	X		
Oxipurinol	M, TP	X		
Paracetamol	P	X		
Ranitidine	P	X		
Desmethyl ranitidine	M	X		
Ranitidine N-oxide	M	X		
Ranitidine S-oxide	M	X		

P= pharmaceutical, M=metabolite, TP= transformation product

3 Analytical methods

For quantification of the top priority pharmaceuticals and their metabolites and TPs in environmental samples highly sensitive and selective analytical methods are required. Liquid chromatographic methods with tandem mass spectrometric detection (LC-MS-MS) are state of the art. Complex matrices such as sediment/suspended particulate matter and biota samples demand extensive sample preparation for separation of interfering matrix compounds. The analytical methods described in the final report of the precursor project were complemented by the metabolites and transformations products or optimized and finalized, if necessary.

3.1 Water phase

Two analytical methods were developed to determine the selected 110 analytes in the water phase i) a RPLC-ESI-MS/MS method for determination of medium polar to apolar PTMs and ii) a HILIC-ESI-MS/MS method for the analysis of very polar analytes.

The reverse phase method was adapted from the method described by [8], using direct injection of water samples. Briefly, 20 µL of a 0.01 mg/L mixture of internal standards were spiked to 1 mL water sample. The separation was performed using an Agilent Elipse Plus C18 column (150 x 2.1, 3.5 µm) equipped with a Zorbax SB-C8 column guard (2.1 x 12.5 mm). The flow rate was set to 300 µL/min. Eluent A was 0.1% acetic acid and eluent B was acetonitrile. The following solvent gradient was applied: 0 to 1 min, 0 % B, 1 to 2 min 0 to 20 % B, 2 to 16 min, 20 to 100 % B, 16 to 19 min, 100 % B and 19 to 25 min, 0 to 100 % B. The injection volume was 80 µL and the column temperature was set to 25 °C.

For HILIC analysis, the samples have to be injected in a diluent with a high proportion of an organic solvent (typically acetonitrile or methanol). Solvent exchange was proceeded by freeze-drying, with subsequent re-dilution of the samples in acetonitrile/Milli-Q (90/10, v/v). Chromatographic separation was performed on a zwitterionic HILIC Nucleodur (250 x 3 mm, 3 µm, Macherey-Nagel, Düren, Germany) equipped with an EC HILIC Nucleodur column guard (4 x 3 mm, 3 µm, Macherey-Nagel). The flow rate was set to 500 µL/min. Eluent A was 10 mM ammonium formate with 0.1 % formic acid and eluent B, 7.5 mM ammonium formate in acetonitrile/Milli-Q, (90/10, v/v) with 0.1 % formic acid. The following solvent gradient was applied: 0 to 3 min, 100% B, 3 to 17 min 100 to 75% B, 17 to 22 min, 75% B and 22.1 to 33 min 100% B. The injection volume was 70 µL and the column temperature was set to 25 °C. A detailed description of method development and optimization can be found in chapter 3 of the final report of the previous project [2] and in Boulard et al. [1].

LOQs ranged from 0.5 to 200 ng/L for surface water sample with a median LOQ at 5 ng/L.

3.2 Suspended particulate matter

For analysis of suspended particulate matter (SPM) the analytes have to be extracted from the solid sample matrix and dissolved in an appropriate solvent. Extraction of SPM was performed by pressurized liquid extraction with an ASE 350 (Thermofisher scientific, Darmstadt, Germany). 0.5 g freeze-dried SPM were thoroughly mixed up with quartz sand and poured in a 10 mL stainless steel extraction cell. 6 ng (60 µL of 0.1 mg/L solution) of internal standard solution were added directly in the cells. A first extraction cycle was performed with methanol/Milli-Q, 50/50, v/v (15 min static time, 20 s purge time, 0% flush volume) and two

subsequent cycles with methanol/2% formic acid, 50/50, v/v (15 min static time, 120 s purge time, 150% flush volume). The extraction was carried out at 100°C and 100 bars and the heating time was 6 min. The extracts were purified by solvent exchange. In this aim, the extracts were filled up to 30 mL with methanol/Milli-Q (50/50). 10 mL were transferred and evaporated to dryness under a gentle stream of N_2 in a TurboVap (Biotage, Uppsala, Sweden). The residues were thoroughly re-dissolved in 100 µL Milli-Q. Subsequently, 900 µL acetonitrile were added to precipitate the impurities. The samples were centrifuged for 10 min at 6000 rpm with a Hettich Mikro 220R (Tuttlingen, Germany).

For the RPLC measurements, 100 µL supernatant were diluted in 900 µL Milli-Q, whereas for HILIC measurements, 200 µL supernatant were diluted in 800 µL acetonitrile/Milli-Q, 90/10, v/v. Subsequently, the samples were analyzed according to the methods described in 3.1. A detailed description of method development and optimization can be found in Boulard et al. [3].

LOQs ranged from 0.09 to 11.7 ng/g d.w. for SPM sample with a median LOQ at 0.65 ng/g d.w..

3.3 Fish tissue

Analysis of active pharmaceutical ingredients, their metabolites and TPs in biological matrices is a challenging task, since biomolecules such as lipids or proteins disturb chromatographic separation and mass spectrometric detection. Thus, the analytes have to be isolated from the biological matrix before the analysis. To cover the broad polarity range of the analytes non-discriminating clean-up techniques such as size exclusion, instead of adsorption columns were used. The developed analytical method is schematically represented in Figure 1. The extraction and clean-up procedure are appropriate for sample pretreatment of fillet, liver and blood. A detailed description of method development and optimization and the quality of the established method can be found in Boulard et al. [4].

Glucuronide cleavage. For plasma samples, 200 µL samples were pipetted in 2 mL of 10 mM acetate sodium buffer (adjusted to pH 4.7 with acetic acid) and 100 µL of 0.01 mg/L internal standards mix were added to the samples. Homogenized lyophilized fish tissue (50 mg for fish liver or 100 mg for fish fillet) was weighted into a 15 mL polypropylene Falcon tubes containing 500 mg of garnet matrix A (MP Biomedicals, Illkirch-Graffenstaden, France) and 150 mg of lysing matrix D (MP Biomedicals). 2 mL of 10 mM acetate sodium buffer (adjusted to pH 4.7 with acetic acid), 100 µL of 0.01 mg/L internal standards mix were added to the samples. Subsequently, cell disruption was performed in a FastPrep-24™ 5G (MP Biomedicals) equipped with a CoolTeenPrep™ adapter at 4.0 m/s for 40 s. Afterwards, 20 µL β-glucuronidase (10,000 units) were added and the samples were agitated overnight (at least 14 h) at 37 °C and 150 rpm in an orbital incubator SI500 (Stuart, Staffordshire, United Kingdom).

Sample extraction. The following day, 5 mL n-heptane were added to the samples and they were extracted by cell disruption at 4 m/s for 40 s in a FastPrep-24TM 5G. Subsequently, the extracts were centrifuged at 6000 rpm (3420 g) for 5 min in a Hettich Mikro 200R (Tuttlingen, Germany). The organic phase was separated, the samples were extracted a second time according to the same procedure and the organic phases were combined. Afterwards, the samples were extracted with 4 mL ice cold acetonitrile by the same cell disruption procedure,

centrifuged for 15 min at 6000 rpm and 4 °C before removal of the supernatant. The residues were extracted a second time, centrifuged for 5 min at 6000 rpm and the supernatants were gathered. Both extracts (n-heptane and acetonitrile) were then evaporated to 2 mL. To ensure dissolution of all analytes, the n-heptane extracts were vortexed and ultra-sonicated for 10 minutes before silica gel SPE.

Silica gel SPE. Silica gel cartridges (6 mL, 1000 mg, Chromabond, Machery-Nagel, Düren, Germany) were activated by drying at 85 °C for 3 hours prior to utilization. They were conditioned with 3 × 2 mL n-heptane before loading the n-heptane extracts. Elution was performed in three times. The first fraction was eluted with 3 x 2 mL ethyl acetate/cyclohexane (1/9, v/v), the second with 3 × 2 mL methanol/acetone (4/6, v/v) and the third with 3 × 2 mL methanol, 0.5 $\%_v$ NH_3 (fraction 3). To minimize analyte losses, the sample vessel was rinsed with each of the elution solvents. All fractions were collected separately and fraction 2 was directly eluted in the vessel containing the acetonitrile extract. Fraction 1 was discarded. Fraction 2 (already containing the acetonitrile extract) and 3 were evaporated to 1 mL and combined. 10 µL formic acid were added to avoid the sorption of the positively charged analytes. This extract was processed differently for liver and fillet extracts (see paragraph Preparation of liver/fillet samples for the RAM).

Preparation of liver samples for the RAM. The extracts were evaporated to dryness and re-dissolved in 100 µL Milli-Q. 900 µL acetonitrile were added to precipitate the impurities. The samples were centrifuged at 18000 rpm (30800 g) for 10 min and 900 µL supernatant was transferred into an evaporation vial. The extracts were evaporated to exact 100 µL. To ensure the re-dissolution of the most apolar substances, 100 µL acetonitrile were added to the samples, they were vortexed for 30 seconds and ultra-sonicated for 10 min. Subsequently, 700 µL Milli-Q were added and the samples were centrifuged at 18000 rpm for 10 minutes. The supernatants were transferred in vials for injection in the RAM.

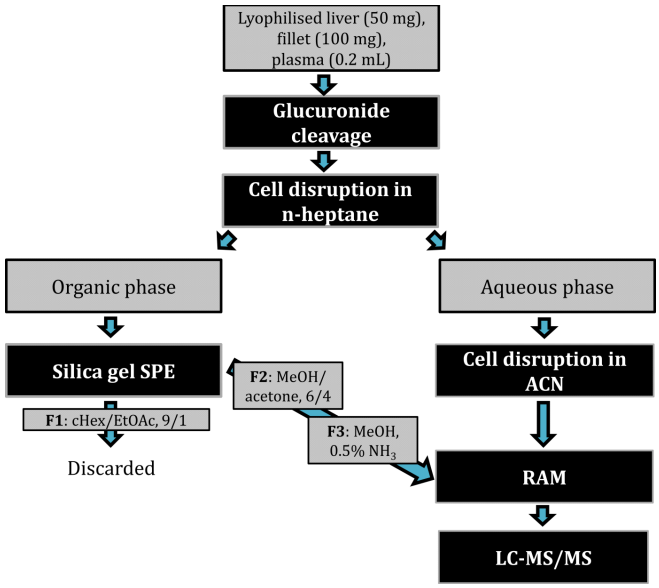
Preparation of fillet and plasma samples for the RAM. The extracts were evaporated to 100 µL and 100 µL acetonitrile were added to ensure the re-dissolution of the most apolar analytes. The samples were vortexed for 30 s and ultra-sonicated for 10 min. Subsequently, 800 µL Milli-Q were added, the samples were vortexed again and centrifuged at 18000 rpm. The supernatants were transferred in vials for injection in the RAM.

RAM. The RAM was performed with an Agilent 1260 system equipped with a G1364C fraction collector. A Lichrospher RP-8ADS (25 µm, 20 × 4 mm) was utilized with 0.1$\%_v$ formic acid as eluent A and acetonitrile as eluent B. The flow rate was set to 1 mL/min. The following gradient was performed: 0-3 min: 2% B, 3-3.5 min: 2 to 60% B, 3.5-8.5 min: 60% B, 8.5-9 min: 60 to 98% B, 14-14.5 min: 98 to 2% B, 14.5-20 min: 2% B. The eluat was collected between 3 and 13 min. Injection volume was 500 µL. Subsequently, 10 µL formic acid were added to the collected fractions and they were evaporated to 1 mL before measurement with LC-MS.

LC-MS analysis. The extracts were analyzed according to the RPLC method described in 3.1.

In fillet, LOQs ranged from 0.1 (clopidogrel) to 19 (O-desmethylmetoprol) ng/g d.w. (median: 1.4 ng/g d.w.), in liver from 0.46 (bicalutamide) to 48 (N-acetylsulfamethaxole) ng/g d.w (median: 4.73 ng/g d.w.). and in plasma from 0.05 (bicalutamide) to 5.5 (furosemide) ng/mL (median: 0.38 ng/mL.).

Figure 1: Optimized analytical method sample preparation scheme. MeOH: methanol, ACN: acetonitrile, cHex: cyclohexane, EtOAc: ethylacetate. (Source: own figure, adapted from Boulard et al. 2020 [4], BfG)



A comprehensive validation of the method was done including the following criteria: accuracy, reproducibility, instrumental precision (repeatability and inter-day precision), sensitivity and matrix effects. Over the whole polarity range the method showed got performance in all parameters for most of the analytes. Sensitivity of the method was comparable to those of methods described in literature (Table 2). The validation results qualify the method for trace analysis of pharmaceuticals in fish filet, liver and plasma. For detailed information refer to Boulard et al. [4]. Due to the use of a non-discriminating clean-up additional pharmaceuticals as well as other micropollutants can be easily integrated into this method.

Table 2: Comparison of method LOQs with literature data

Analytes	Matrix	Determined LOQ [ng/g]/ [ng/mL] for plasma	Literature LOQ [ng/g]/ [ng/mL] for plasma
Bezafibrate	Fillet	0.49	7.42-18.38[a], 0.41-0.67[b], 0.033[c]
	Liver	0.94	0.069-0.097[b], 0.27[d], 0.26[c]
	Plasma	0.18	0.20-0.41[b], 0.062[d], 0.05[e], 0.0051[c]
Carbamazepine	Fillet	0.73 (0.22)	1.92-1.97[a], 0.3[f], 0.1[g], 0.04[h], 0.035[i], 0.22-0.38[b], 1.2[j], 0.092-0.18[k], 0.012[c],
	Liver	1.64 (0.62)	0.3[f], 0.25[h], 0.065[i], 0.24-0.33[b], 0.18[d],

Analytes	Matrix	Determined LOQ [ng/g]/ [ng/mL] for plasma	Literature LOQ [ng/g]/ [ng/mL] for plasma
			0.048[c], 0.94[l], 0.55[l], 0.19[m], 0.54[n], 0.16[o]
	Plasma	0.17 (0.04)	0.020[b], 0.18-0.36[f], 0.05[l], 0.010[a], 0.16[m]
2-Hydroxycarbamazepine	Fillet	0.45	0.1[c], 0.2[d], 0.063-0.25[g]
	Liver	1.96	0.8[f], 0.83[h]
	Plasma	0.15	n.a.
10,11-Dihydroxy-10,11-dihydrocarbamazepine	Fillet	1.4	0.43-0.70[b]
	Liver	10.58	0.86-1.2[b]
	Plasma	0.23	n.a.
Cetirizine	Fillet	4.5	0.13-0.21[b]
	Liver	16.47	0.049-0.068[b]
	Plasma	0.44	0.098-0.20[b]
Citalopram	Fillet	1.2	0.6[f], 0.4[g], 0.16[h], 0.084[i], 0.24-0.40[b], 0.062-0.24[r], 1.4-2.4[s]
	Liver	4.73	1.7[f], 0.29[h], 0.249[i], 0.19-0.27[b], 0.090-0.8[r], 0.9-2.8[s]
	Plasma	0.26	0.095-0.19[b], 0.40-1.9[r], 1.4-2.4[s], 0.25[t], 0.08-0.13[p]
Desmethylcitalopram	Fillet	1.2	0.15-0.25[b]
	Liver	2.7	0.23-0.32[b]
	Plasma	0.38	0.098-0.20[b]
Clopidogrel	Fillet	0.1	0.1[f], 0.1[g], 0.26[h], 0.16-0.37[k]
	Liver	0.65	0.6[f], 0.87[h]
	Plasma	0.073	n.a.
Diclofenac	Fillet	2.4	79.14-303.36[a], 0.4[f], 0.2[g], 0.62[h], 0.345[i], 0.20-0.33[b], 0.03[j], 6.9-9.1[k], 0.35[u],
	Liver	2.52	0.042[c], 2.31[o] 0.4[f], 2.16[h], 1.12[i], 8.4-12[b], 0.83[d], 0.21[c], 2.31[o]
	Plasma	0.41	0.68-1.4[b], 0.15[d], 0.1[e], 1[v], 0.012[c], 2.31[q]

Analytes	Matrix	Determined LOQ [ng/g]/ [ng/mL] for plasma	Literature LOQ [ng/g]/ [ng/mL] for plasma
Diphenhydramine	Fillet	0.29	0.048[i], 0.2[j], 0.025[c], 0.07[l], 0.04[m], 0.05[n], 0.11[o]
	Liver	0.67	0.130[i], 0.12[d], 0.028[c], 6.0[l], 0.11[o]
	Plasma	0.22	0.028[d], 0.12-0.24[p], 0.010[c], 0.13[q]
Fexofenadine	Fillet	1.6	0.034-0.053[b]
	Liver	14.2	0.28-0.39[b]
	Plasma	0.28	0.25-0.5[b], 0.5[e]
Fluconazole	Fillet	0.43	0.13[w]
	Liver	1.32	0.61[w]
	Plasma	0.21	n.a.
Fluoxetine	Fillet	4.7 (2.59)	0.3-3.4[s], 0.07[x], 0.05[y], 5.3[l], 0.78[m], 6.73[n], 0.36[o]
	Liver	7.49 (6.95)	1.0-6.4[s], 0.05[y], 5.7[l], 0.36[o]
	Plasma	0.47 (0.24)	1[e], 0.3-3.4[s], 0.25[t], 0.3-0.6[p], 0.85[q]
Norfluoxetine	Fillet	5.5	0.040[i], 0.14[x], 0.05[y], 3.0[l], 0.83[m], 2.9[n], 0.71[o]
	Liver	34.94	0.122[i], 0.05[y], 6.7[l], 0.71[o]
	Plasma	0.83	2.50[t], 0.365-0.6[p], 0.99[q]
Furosemide	Fillet	3.2	n.a.
	Liver	17.75	n.a.
	Plasma	5.5	8-16[p]
Hydrochlorothiazide	Fillet	1.3	0.05[f], 0.2[g], 0.57[h], 0.21-0.239[k]
	Liver	3.31	0.2[f], 0.35[h]
	Plasma	0.33	4-4.8[p]
Metoprolol	Fillet	3	23.76-26.19[a], 0.6[f], 0.7[g], 0.60[h], 0.17-0.23[b], 0.14-0.60[k], 2.5[n]
	Liver	9.34	2.0[f], 1.19[h], 0.028-0.041[b],
	Plasma	1.3	0.055-0.10[b], 0.3-0.6[p]
Oxazepam	Fillet	1.3	0.19-0.33[b], 0.5[z]
	Liver	3.28	0.76-1.1[b]
	Liver	3.28	0.76-1.1[f]

Analytes	Matrix	Determined LOQ [ng/g]/ [ng/mL] for plasma	Literature LOQ [ng/g]/ [ng/mL] for plasma
	Plasma	0.48	0.20-0.45[b], 0.5[e], 0.8-1.33[p]
Primidone	Fillet	2	0.06[u]
	Liver	7.35	n.a.
	Plasma	0.58	n.a.
Sertraline	Fillet	9.4	2.6[f], 1.1[g], 0.61[h], 0.092[i], 0.094-0.15[b], 0.32-1.2[r], 1.3-5.6[s], 0.05[y], 0.017[c]
	Liver	9.58	2.1[l], 0.53[m], 3.57[n], 0.99[o] 22.1[f], 3.28[h], 0.182[i], 0.075-0.11[b], 0.48[d], 0.020-0.18[r], 1.0-5.2[s], 0.05[y], 0.11[c]
	Plasma	0.91	9.6[l], 0.99[o] 0.22-0.45[b], 0.19[d], 0.5[e], 0.5-0.86[r], 1.3-5.6[s], 0.25[t], 0.08-0.16[p], 0.022[c], 0.99[q]
Sulfamethoxazole	Fillet	4.8	0.662[i], 0.13-0.20[b], 0.03[j], 0.05[u], 2.9[l], 0.72[m], 2.29[n], 1.87[o]
	Liver	16.84	4.51[i], 0.33-0.61[b], 3.8[l], 1.87[o]
	Plasma	1.1	0.24-0.47[b], 1[v], 0.12-0.24[p], 1.9[q]
N-Acetyl sulfamethoxazole	Fillet	14	4.46[i], 0.062-0.11[b]
	Liver	48.17	21.4[i], 11-20[b]
	Plasma	1.6	0.79-1.5[b]
Telmisartan	Fillet	0.59	0.14-0.23[b]
	Liver	0.96	0.28-0.39[b]
	Plasma	0.086	0.23-0.46[b], 1[e]
Tramadol	Fillet	0.49	0.71-1.2[b], 0.085-0.14[r]
	Liver	1.39	0.33-0.54[b], 0.78-2.5[r]
	Plasma	0.16	0.11-0.22[b], 0.1[e], 0.40-0.64[r]
Trimethoprim	Fillet	0.82	9.41-28.30[a], 0.162[i], 0.090-0.14[b], 0.03[j], 3.5[l], 1.09, 2.15[n], 0.45[o]
	Liver	2.01	0.434[i], 0.090-0.15[b], 2.3[l], 0.45[o]
	Plasma	0.16	0.058-0.11[b], 1[v], 0.3-0.6[p], 2.8[q]
Valsartan	Fillet	5.3	0.13-0.22[b]

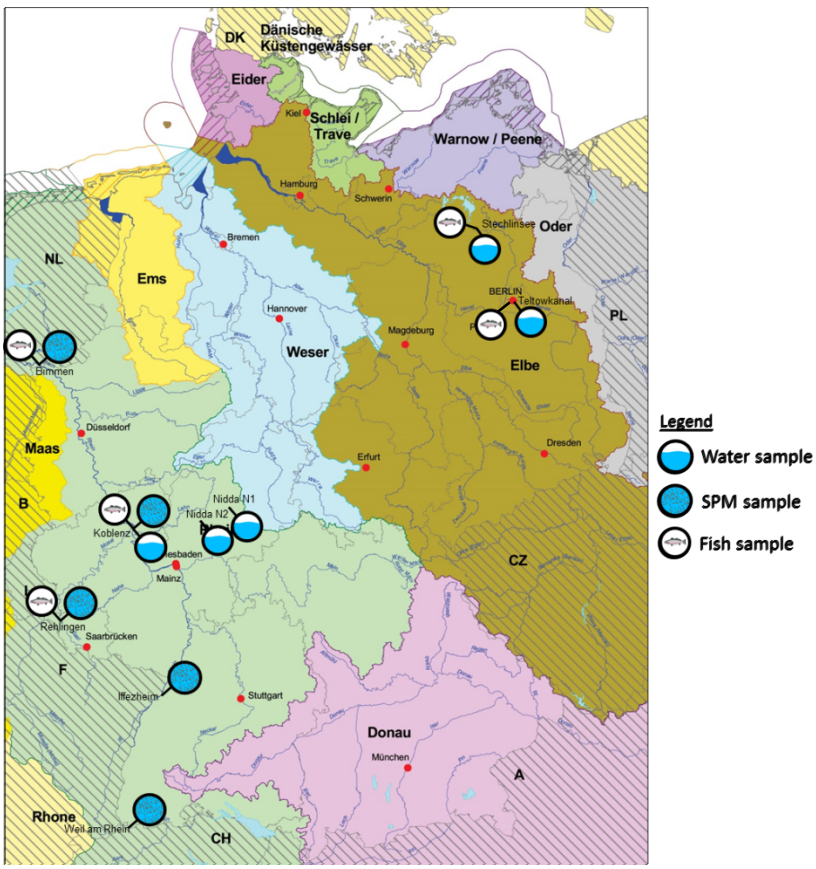
Analytes	Matrix	Determined LOQ [ng/g]/ [ng/mL] for plasma	Literature LOQ [ng/g]/ [ng/mL] for plasma
	Liver	36.6	1.3-1.8[b]
	Plasma	1.7	0.80-1.6[b], 0.8-1.6[p]
Venlafaxine	Fillet	2.4 (0.97)	1.04-1.82[a], 0.09[f], 0.1[g], 0.55[h], 0.260[i], 0.16-0.27[b], 0.40-1.5[r], 1.3-2.9[s], 0.27-1.25[k]
	Liver	1.68 (0.94)	0.5, 1.33[h], 1.10[i], 0.18-0.26[b], 0.32-1.1[r], 1.0-3.4[s]
	Plasma	0.76 (0.37)	0.10-0.19[b], 0.34-1.7[r], 1.3-2.9[s], 0.25[t], 0.13-0.16[p]
O-Desmethyl venlafaxine	Fillet	5.65 (1.1)	0.249[i], 0.18-0.25[b]
	Liver	12.39 (4.63)	1.50[i], 0.15-0.23[b]
	Plasma	1.0 (0.27)	0.083-0.15[b]
	Liver	12.39 (4.63)	0.15-0.23[f]
	Plasma	1.0 (0.27)	0.083-0.15[f]

[a] [9], [b] [10], [c] [11], [d] [12], [e] [13], [f] [14], [g] [15], [h] [16], [i] [17], [j] [18], [k] [19], [l] [20], [m] [21], [n] [22], [o] [23], [p] [24], [q] [25], [r] [26], [s] [27], [t] [28], [u] [29], [v] [30], [w] [31], [x] [32], [y] [33], [z] [34]

4 Environmental monitoring

To evaluate the occurrence of pharmaceuticals in surface water affected by different amounts of wastewater, SPM and fish samples from Lake Stechlin, Saar (Rehlingen), Rhine (Koblenz), and Teltow Canal were analyzed. The Lake Stechlin is not affected by wastewater and only marginal affected by other pollution routes. In contrast, the Teltow Canal is highly affected by effluent of wastewater treatment plants and industrial discharge. At Koblenz, the Rhine has an average wastewater proportion estimated to 5% [35] whereas at Rehlingen wastewater proportion is estimated to 15%. Due to different issues at the sampling sites Teltow Canal and Lake Stechlin no SPM samples were available.

Figure 2: Sampling sites for the three matrices. Different colors show the river catchment areas according to German Wasserrahmenrichtlinie. (Source: Umweltbundesamt; map is based upon Länderarbeitsgemeinschaft Wasser (LAWA) and Bundesamt für Kartographie und Geodäsie (BKG))

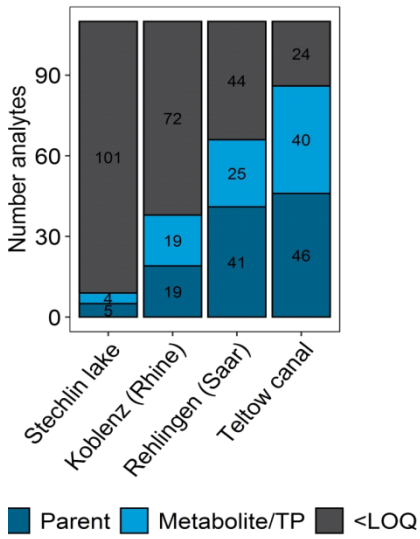


4.1 Water samples

Grab samples were taken at Stechlin Lake, Rhine (Koblenz), Saar (Rehlingen) and Teltow Canal in 2018. The number of detected pharmaceuticals and their metabolites as well TPs is represented in Figure 3. Number of detections was relatively high with up to 86 of 110 analytes detected in Teltow Canal. The number of detected analytes increases with the wastewater proportion and a high proportion of the detected analytes were either metabolites or TPs (up to 50% in Koblenz).

The detailed concentrations of all analytes can be found in the final report of the previous project [2] and in Boulard et al. [1].

Figure 3: Number of detected analytes in surface water samples according to the sampling locations (Source: own figure, BfG)



Boxplots of the corresponding concentrations are represented in Figure 4 and 5. Concentrations show a very broad dispersion with extreme high concentrations up to 12 µg/L for a few analytes and one order of magnitude lower median concentrations. In general, an increase of the concentrations with the wastewater proportion could be recognized. In Teltow Canal, maximal concentrations were measured for oxipurinol, metabolite of allopurinol, guanylurea, TP of metformin, valsartan acid, a metabolite of valsartan, and 4-formylaminoantipyrine, metabolite of metamizole sodium. At the other sampling sites, metformin and guanylurea showed the highest concentrations.

In many cases metabolite concentrations were in the same range as those of their parent pharmaceuticals. In accordance with the results of Hermes et al. [8], higher concentrations for the metabolite clopidogrel acid (108 ng/L at Teltow Canal) than its parent clopidogrel (7 ng/L) could be measured. O-desmethylvenlafaxine showed concentrations threefold higher than its parent (530 ng/L against179 ng/L). Similar trends can be observed for the relationship between TPs and parent pharmaceuticals. In the case of emtricitabine for example a concentration of 71 ng/L is accompanied by concentration of its TPs emtricitabine carboxylate and emtricitabine S-oxide of 1090 ng/L and 310 ng/L, respectively.

In conclusion, in the water phase a high number of pharmaceuticals could be detected in particularly in surface water with a high wastewater proportion. Some of the very polar pharmaceuticals showed very high concentrations, confirming the importance to include them into monitoring, even if a special analytical method beside utilization of reverse phase is needed. A high number of the detected analytes were metabolites and/or TPs and their concentrations were often in the same range or superior to that of their parent pharmaceuticals showing the importance to integrate them into monitoring. Beside an annual consumption of 1.6 t and 8 t fluoxetine and sertraline could not be detected in the water samples. Due to high sorption affinities water concentrations of these pharmaceuticals seems to be below LOQ.

Figure 4: Boxplots of the water concentrations at the investigated sampling locations (Source: own figure, BfG)

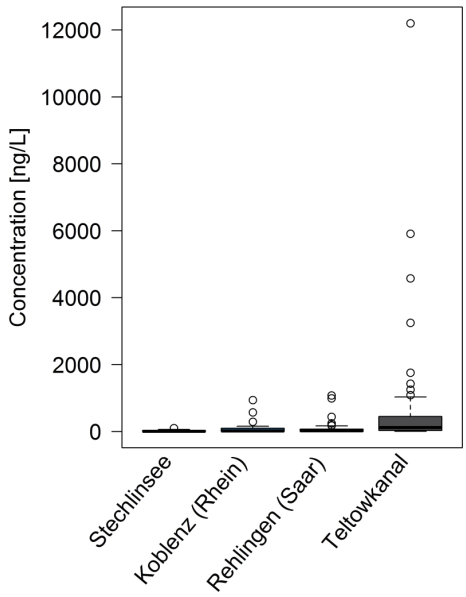
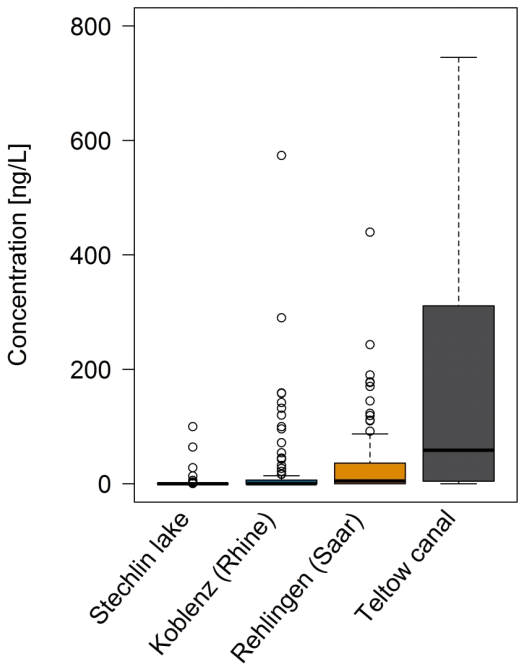


Figure 5: Zoom between 0 and 800 ng/L of Figure 4 (Source: own figure, BfG)

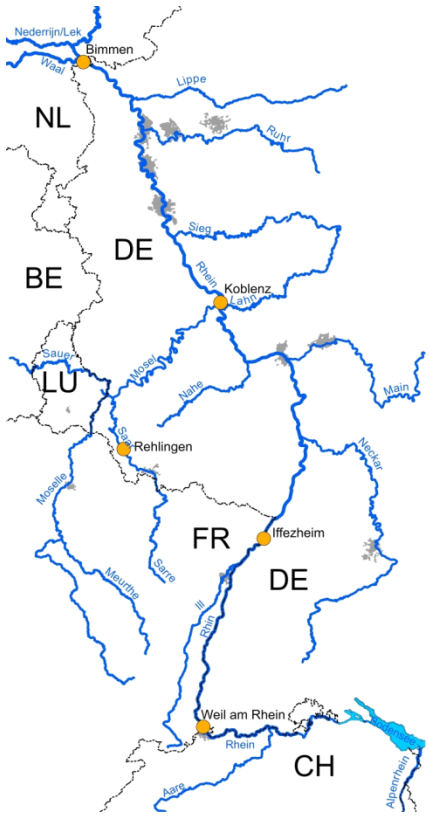


4.2 Suspended particulate matter

Although several studies confirmed a wide distribution of pharmaceuticals in rivers and streams, only a limited knowledge is available about the partitioning of pharmaceuticals between the water phase and SPM. To close this knowledge gap 99 pharmaceuticals and their

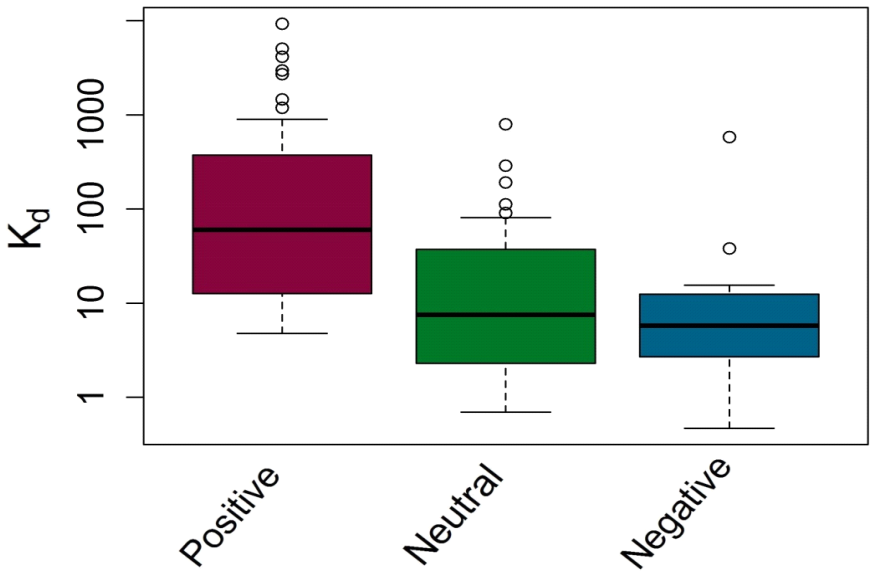
metabolites as well TPs were analyzed in SPM samples from four different sites at the river Rhine, Weil (km 173), Iffezheim (km 333), Koblenz (km 590) and Bimmen (km 863) and at one site at the river Saar: Rehlingen (km 54) (Figure 6). The samples were obtained from the German Environmental Specimen Bank located in Schmallenberg, Germany. Sampling and preparation are described in details by Schulze et al. [36]. Briefly, the samples were collected with sedimentation boxes, emptied each month and the SPM were subsequently shock-frozen in liquid nitrogen. At the end of the year, the samples of each month were combined to annual composite samples, freeze-dried and homogenized before storage above liquid nitrogen with a temperature below -150 °C.

Figure 6: Sampling sites for SPM analysis (Source: own figure, adapted from Boulard et al. 2020 [3], BfG)



To get a better understanding of the sorption behavior of pharmaceuticals and their metabolites as well TPs and to estimate the relevance of SPM for the transport of them in water bodies, sorption experiments were carried out. Freundlich isotherms were determined for 90 analytes. All showed a linear sorption behavior. Distribution coefficients (Kd) ranged from 0.64 L/kg to 9300 L/kg. For 18 compounds, Kd values were found to be above 100 L/kg. In general, positively charged analytes showed higher Kd values than the neutral and negatively charged substances (Figure 7). A list with the actual values can be found in Boulard et al. [3]. Despite their high polarity and water solubility the tested substances show significant sorption to SPM. This makes SPM to an interesting matrix for an integrated monitoring of pharmaceuticals.

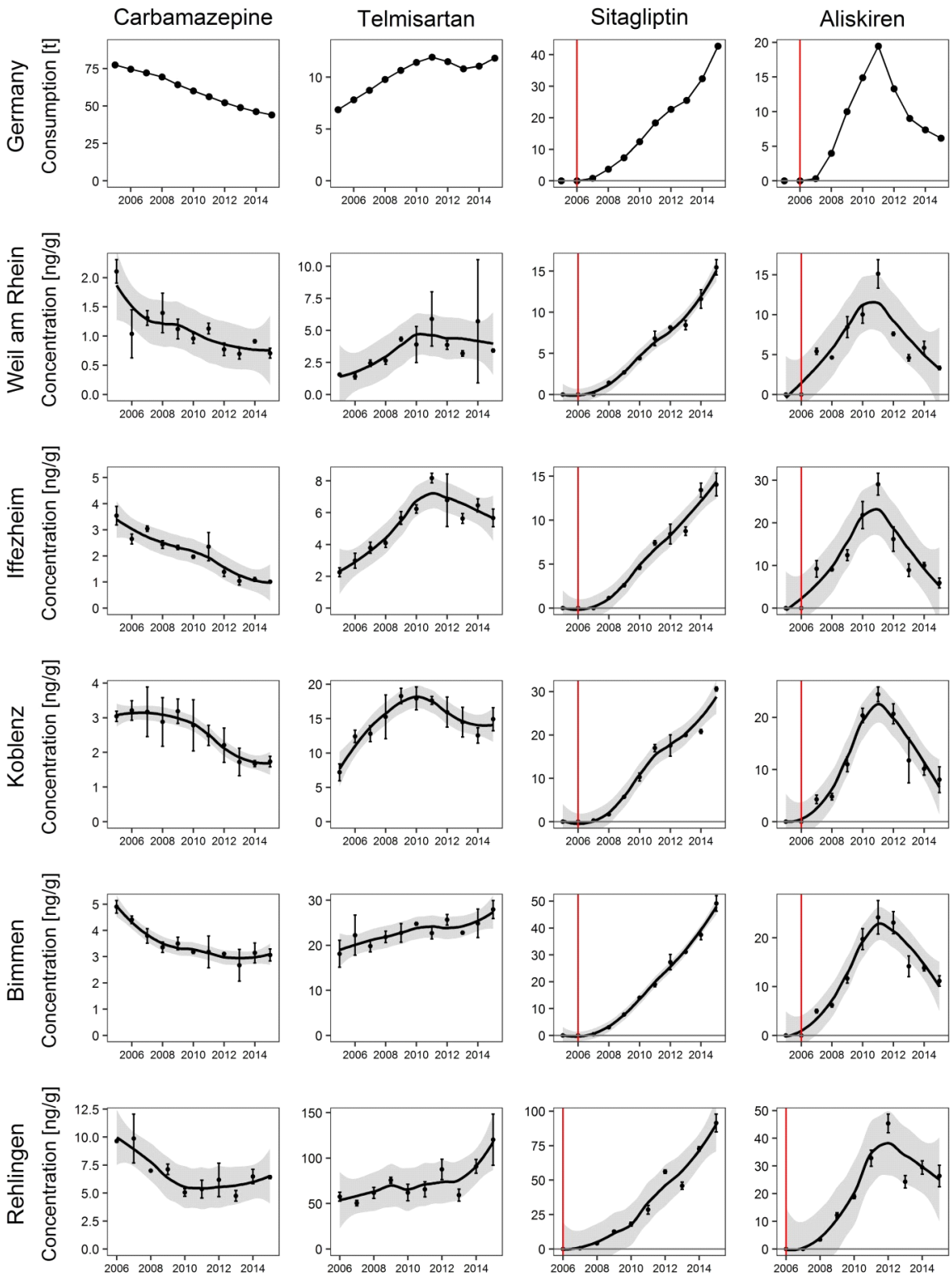
Figure 7: Kd of analytes according to the charge of the analytes at pH 7 (Source: own figure, BfG)



To evaluate the potential of SPM analysis in environmental monitoring annual composite samples of the years between 2005 and 2015 at four sites of the river Rhine: Weil, Iffezheim, Koblenz and Bimmen as well as between 2006 and 2015 at one site at the river Saar, at Rehlingen were analyzed. In these SPM samples, up to 61 of the 100 analytes were detected with concentrations up to 190 ng/g for guanylurea, a TP of the antidiabetic metformin. Generally speaking, the analytes showing the highest concentrations (> 10 ng/g) were either positively charged or very hydrophobic (log D >4). The few exceptions (acesulfame, leveritacetam acid and valsartan) correspond to substances whose consumption is very high (>100 t pro year ([3])). For most analytes, increasing concentrations were found along the length of the Rhine and higher concentrations were measured in Rehlingen/Saar which is consistent with the corresponding wastewater contents. The concentrations of many analytes correlated well with the annual consumed quantities in Germany (Figure 8). This was in particular the case for SPM from Koblenz and Bimmen, where respectively 52% and 63% of the analytes detected every year show a good correlation ($p < 0.05$, Pearson coefficients > 0.6) with the German consumption.

All other results from the trend analysis in SPM can be found in the Supporting Information of Boulard et al. [3] (Link: https://ars.els-cdn.com/content/image/1-s2.0-S0043135419311406-mmc1.pdf)

Figure 8: Consumption and temporal concentration trends of carbamazepine, telmisartan, sitagliptin and aliskiren in SPM from the sampling sites Weil am Rhein (Rhine, km 173), Iffezheim (Rhine, km 333), Koblenz (Rhine, km 590), Bimmen (Rhine, km 863), Rehlingen (Saar, km 54) (Source: own figure, adapted from Boulard et al. 2020 [3], BfG).



The ubiquitary distribution and the course of the concentration trends suggest discharge from urban WWTPs as main source for most of the investigated pharmaceuticals. However, some pharmaceuticals and their metabolites as well TPs showed anomalous concentration trends

indicating additional sources. Normalization of the data with the antiepileptic drug carbamazepine as an intrinsic tracer for municipal wastewater revealed anomalous trends for four analytes: guanylurea, fluoxetine, venlafaxine and bisoprolol. Geographical and temporal trends of these pharmaceuticals suggest an additional industrial discharge. Fluoxetine for example show higher SPM concentrations in Weil compared to Koblenz, which could not be solely explained by consumption but only by an additional industrial discharge into the Rhine upstream of Weil. Further investigations and a specific monitoring should clearly identify these sources.

To conclude, SPM is an appropriate matrix for investigation of the pollution status of water systems with micropollutants even for relatively polar compounds such as pharmaceuticlas and their metabolites as well TPs. Especially the sorption of positively charged compounds is considerable due to the electrostatic interactions even for rather polar compounds such as guanylurea. Temporal trends between 2005 and 2015 revealed the ubiquitous presence of pharmaceuticals in SPM of the river Rhine and the river Saar. The good correlation found for SPM concentrations and consumption data give proof of the stability, homogeneity and representativeness of annual composite samples. In some cases, linear relationships between consumption and SPM concentration allow prediction of future SPM concentration based on consumption changes. The results also demonstrate that elimination efficiencies of WWTPs, the main sources of pharmaceuticals, were not improved during the last decade. Consequently, elevated consumption of pharmaceutical will lead to higher pharmaceutical burden in water bodies in the future if no further treatment steps (advanced treatment by ozonation or activated carbon) will be implemented. Furthermore, anomalies in concentration pattern and trends can give hints regarding local industrial discharges or other sources. This underlines the suitability of SPM analysis as a valuable tool for surveys and trend monitoring. A monitoring limited to analysis of dissolved concentrations in filtered water samples would lead to an underestimation of the loads and the environmental burden of pharmaceuticals wit high sorption affinities. In the case of fluoxetine and sertraline environmental burdens were actually overlooked by the used water analysis.

4.3 Fish

Monitoring in fish included bream composite samples from the rivers Saar and Rhine, eleven breams from the Teltow Canal as well as carps issued from 5 different WWTP fish monitoring ponds.

In carps fed by WWTP effluent up to 32 of 63 analytes could be detected in at least one fish sample (Figure 9). This indicates a broad uptake of pharmaceuticals by carps. In breams from the Teltow Canal 20 analytes were detected at least once, all of them were also found in the carps at similar concentrations. This can be explained by the elevated wastewater proportion of the Teltow Canal, which can reach levels up to 100% from April to October. In breams from the Saar and Rhine nine pharmaceuticals were detected and concentrations were lower compared to the other sampling sites. Striking differences in the concentration patterns of the analytes were observed between carps and breams from the different sampling sites. For example, diclofenac and desmethylcitalopram showed the highest concentrations of all analytes in carps from WWTP fish monitoring ponds, while these analytes showed medium concentrations in breams from Teltow Canal and were not detectable in breams from Saar and Rhine. In contrast, norlidocaine and sitagliptin showed highest concentrations in breams from Teltow Canal but

were a minor contaminant in carps from WWTP fish monitoring ponds. Due to expectable comparable discharge patterns via wastewater differences must be caused by species specific uptakes or metabolization rates[37]. Overall, there is a general positive correlation between the wastewater contamination of the aquatic system and the detected residues in fish.

The distribution of the analytes within the fish was investigated by analyzing liver, fillet and blood plasma separately. In general, liver samples showed highest concentrations, followed by fillet and plasma. As an exception clopidogrel was measured in significant slightly higher concentrations in fillet. For certain analytes, a correlation could be observed between concentrations in liver and fillet ($p < 0.05$) over all sites. Liver to fillet concentration ratios showed no relationship to the polarity or physicochemical properties (e.g. charge) of the analytes. In blood plasma only the anti-inflammatory diclofenac, the antiplatelet clopidogrel and the antiarrhythmic agent flecainide could be found. Surprisingly the antihypertensive telmisartan which show the highest concentrations in liver could not be detected in plasma, while diclofenac could not be detected in liver and filet. In general, no correlation between plasma concentrations and concentrations in liver and filet could be observed. However, only plasma samples from fish with relatively low pharmaceutical concentrations were analyzed allowing statements about distribution only for 9 analytes. Substance specific uptake and pharmacokinetic seem to prevent general conclusions and predictions.

The correlation between the fillet concentrations and the lipid content was evaluated for the 11 individual bream samples from the Teltow Canal due to their similarity concerning exposure, age and environmental conditions. In contrast to nonpolar contaminants such as hexachlorobenzene, polychlorinated biphenyls and polybrominated biphenylethers, for the investigated pharmaceuticals lipid content seems to play no crucial role in accumulation. Individual uptake rates and metabolism rates seem to be more important.

Beside the parent pharmaceuticals several metabolites and TPs were found in the fish tissues. Concentrations of metabolites were comparable or even slightly higher than those of their parent pharmaceuticals. For example, n-desmethyl tramadol, a human metabolite of the analgesic tramadol, showed concentrations in the same range as tramadol and norlidocaine, a human metabolite of the local anesthetic lidocaine, was detected in Teltow Canal fish with concentrations of a factor 2 to 9 higher than of lidocaine. In fillet of breams from Rhine and Saar, norlidocaine was found at concentrations up to 3.0 ± 0.9 ng/g d.w., while its parent pharmaceutical lidocaine was not detected at all. This indicate an active metabolization of pharmaceuticals leading to the same or similar metabolites as in human.

Determined concentrations were consistent with previous European studies [[16], [10], [21], [26]]. However, 17 analytes were detected in fish tissue for the first time in this study. In particular, the newly detected metabolites/TPs were: three metabolites and TPs of carbamazepine, a metabolite of citalopram, a metabolite of diphenhydramine, a metabolite of lidocaine, three metabolites of tramadol and a TP of gabapentin. Seven parent pharmaceuticals were also detected for the first time: amisulpride, bicalutamide, chlorothiazide, flecainide, lidocaine, quetiapine and sitagliptin.

Figure 9: Overview of the number of detected parent pharmaceuticals and corresponding metabolites/TP at the different sampling locations. (Source: own figure, adapted from Boulard et al. 2020 [4], BfG)

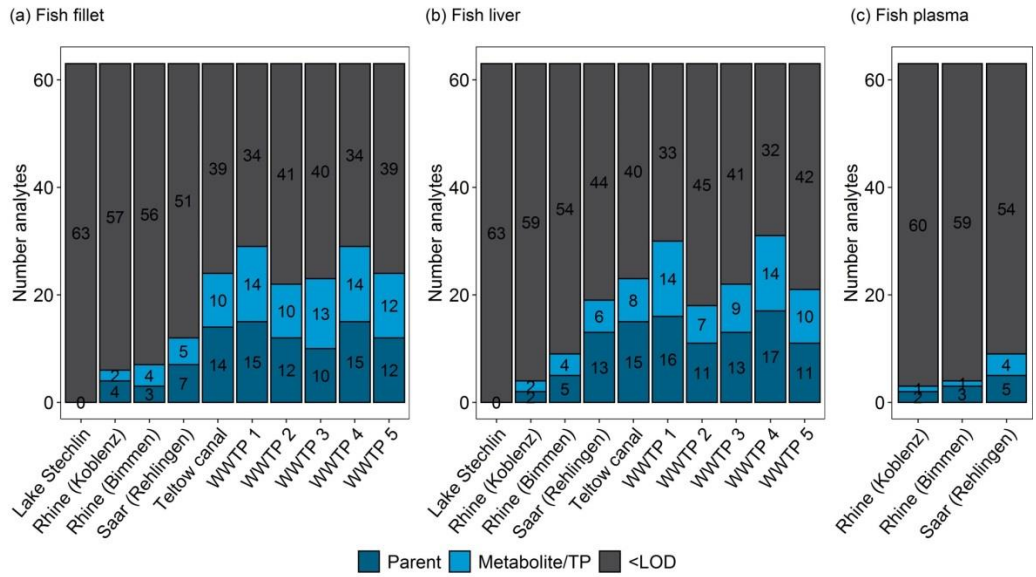
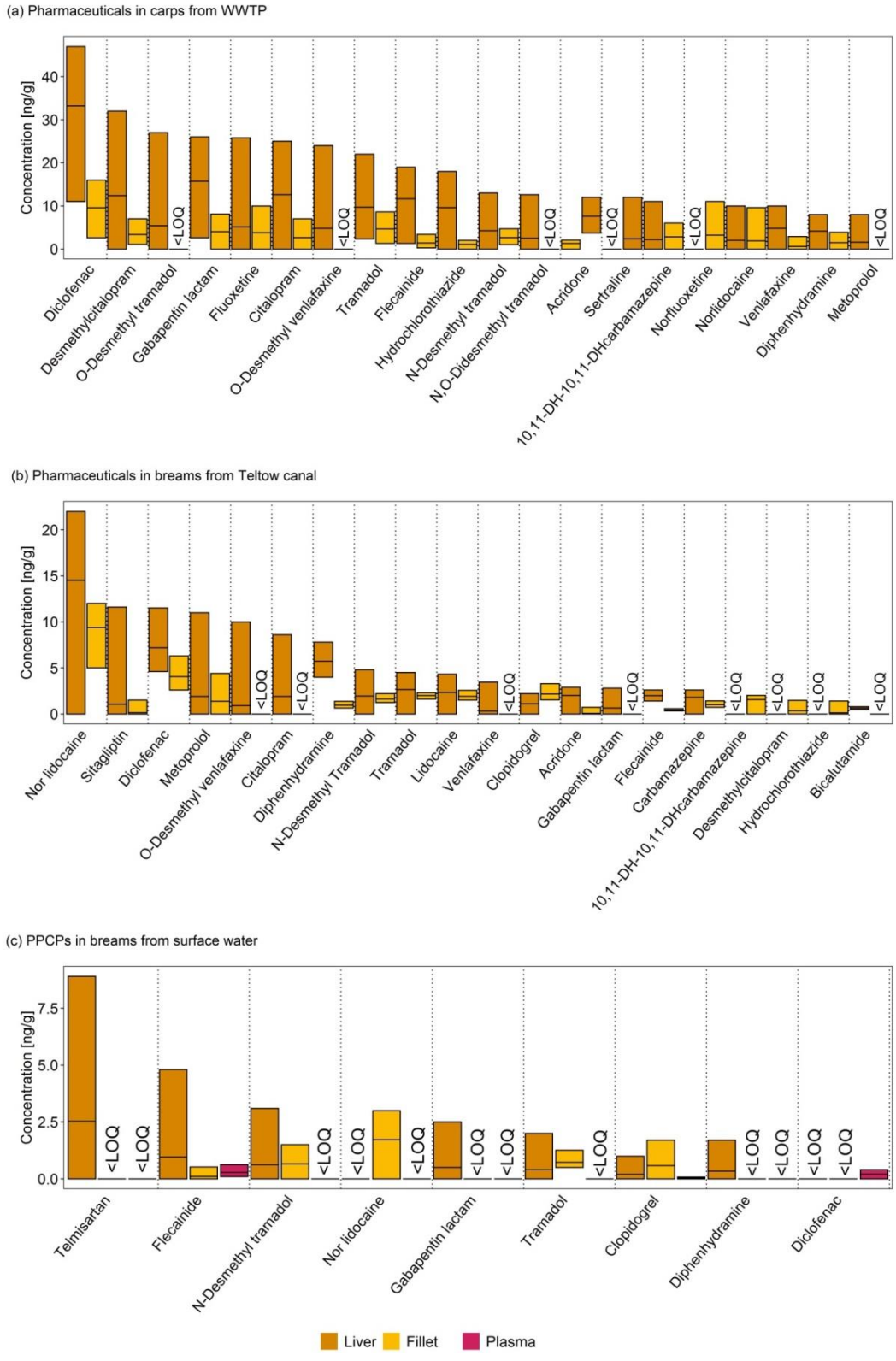


Figure 10: Minimal, mean and maximal concentrations of pharmaceuticals in fish captured from surface water and WWTP fish monitoring ponds. For WWTPs samples, due to the high number of analytes detected only the 20 analytes with the highest concentrations are shown. Concentrations in ng/g dry weight. (Source: own figure, adapted from Boulard et al. 2020 [4], BfG)



In particular, four of these analytes (flecainide, gabapentin lactam, norlidocaine and N-desmethyl tramadol) were identified in (relative low wastewater proportion) river fish and are thus particularly relevant for further investigations.

Flecainide is a rather polar (log D at pH 7: 0.66) and positively charged antiarrhythmic agent. It was detected in fish fillet from Rehlingen at 0.52 ± 0.06 ng/g d.w., in liver at 4.8 ± 0.7 ng/g d.w. and in fish plasma at 0.63 ± 0.08 ng/mL. In Teltow Canal, its concentrations ranged from 0.32 ± 0.07 to 0.58 ± 0.08 ng/g d.w. in fillet and from 1.4 ± 0.3 to 2.6 ± 0.6 ng/g d.w. in liver. Flecainide was also detected in all WWTP samples at concentrations between 0.29 ± 0.06 and 3.4 ± 0.3 ng/g d.w. in fillet and between 1.3 ± 0.6 and 19 ± 7 ng/g d.w. in fish liver.

Gabapentin lactam, a TP of the antiepileptic drug gabapentin [38], was also detected for the first time in fish. A concentration of 2.5 ng/g d.w. was quantified in fish liver from Rehlingen in 2015, while it was not detected in bream liver from Koblenz (2015, 2016) and Bimmen (2015). In Teltow Canal concentrations from <1.51 to 2.8 ± 0.3 ng/g d.w. were determined in liver while the concentration in the water phase was 200 ng/L. Gabapentin lactam could be detected in all WWTP common carp samples with a maximal concentration of 26 ± 2 ng/g d.w. in liver and 8.1 ± 0.4 ng/g d.w. in fillet. Gabapentin lactam has been reported to be more stable than gabapentin with regard to biotic as well as abiotic degradation [38].

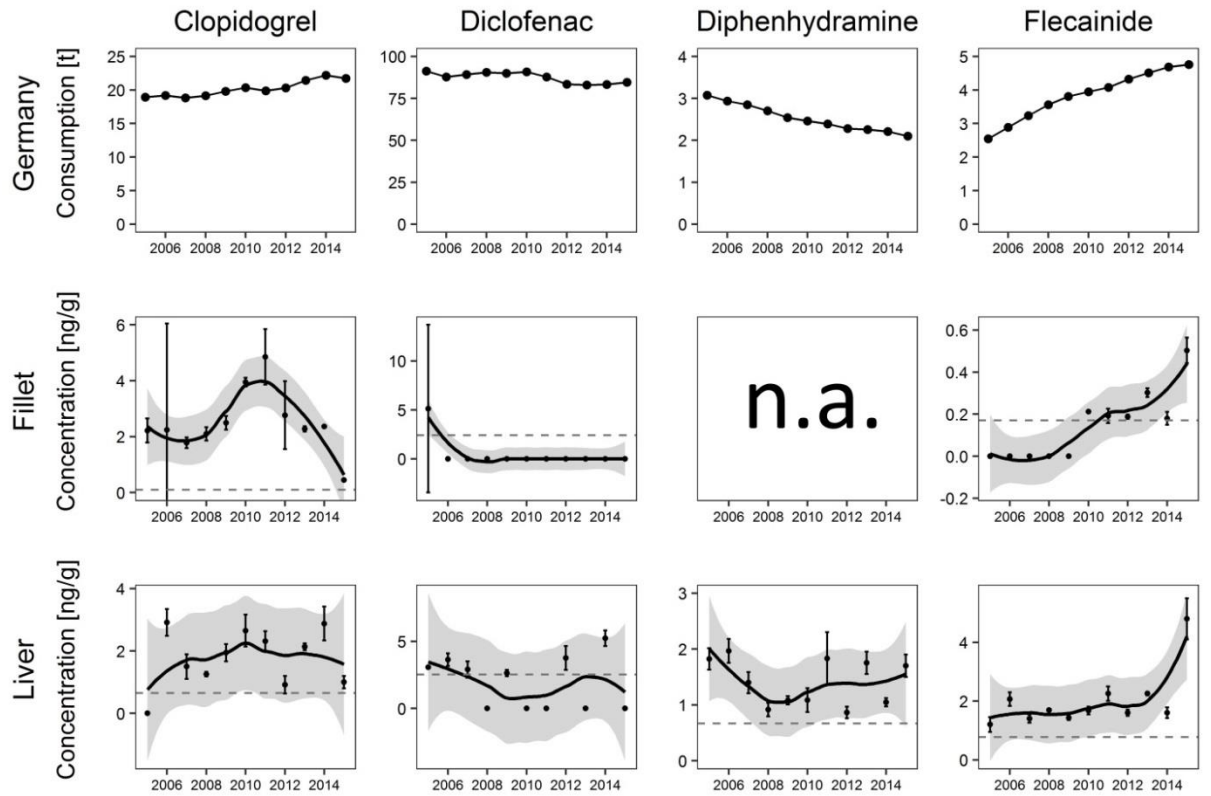
Norlidocaine, a human metabolite of the local anesthetic lidocaine, was detected in river bream fillets from Koblenz, Bimmen and Rehlingen at concentrations up to 3.0 ± 0.9 ng/g d.w., while its parent pharmaceutical lidocaine was not found in fish from Rehlingen above LOQ. Norlidocaine was detected in most Teltow Canal samples at concentrations ranging from 5 ± 1 to 12 ± 2 ng/g d.w. in fillet and from <6.74 to 22 ± 2 ng/g in liver whereas lidocaine concentrations were a factor 2 to 9 lower. Norlidocaine was also detected in all WWTP fillets with concentrations up to 9.6 ± 0.5 ng/g d.w. and in fish liver from WWTP 1 at 10 ± 3 ng/g. Lidocaine could only be quantified in the WWTP 1 samples and its concentrations were a factor three lower than its metabolite (9.6 ± 0.5 ng/g d.w. against 3.9 ± 0.1 ng/g d.w.).

N-Desmethyl tramadol is a human metabolite of the analgesic tramadol. In bream, it was detected at concentrations up to 1.5 ± 0.5 ng/g d.w. in fillet and up to 3.1 ± 0.4 ng/g d.w. in liver. In the same samples, tramadol concentrations ranged up to 1.26 ± 0.06 in fillet and to 2.0 ± 0.8 ng/g d.w. in liver. In Teltow Canal samples, N-desmethyl tramadol concentrations ranged from 1.24 ± 0.08 to 2.2 ± 0.4 in fillet and from <2.37 to 4.8 ± 0.6 ng/g d.w. in liver whereas tramadol concentrations ranged from 1.6 ± 0.2 to 2.3 ± 0.2 ng/g d.w. in fillet and from <1.39 to 4.5 ± 0.3 ng/g d.w. in liver. N-desmethyl tramadol was also detected in WWTP carps at concentrations up to 2.9 ± 0.3 ng/g d.w. in fillet and up to 13 ± 2 ng/g d.w. in liver, whereas tramadol concentrations ranged from 1.3 to 8.6 ± 0.7 ng/g d.w. in fillet and from 2.01 ± 0.01 to 22 ± 3 ng/g d.w. in liver.

Between 2005 and 2015 time trends of the analytes were determined in bream liver and fillet for the location of Rehlingen/Saar. Due to the limited number of pharmaceuticals with concentrations > LOQ only a limited number of time trends could be established. They are shown in Figure 10. On the contrary to the suspended particulate matter, only limited correlations were observed between the consumption and measured concentrations. Several reasons could explain this observation i) consumption was relatively constant for the quantified

analytes ii) fish mobility iii) individual uptake and metabolism can strongly vary according to the organism. This last point is confirmed by the non-correlation between the fillet and the liver concentrations. Furthermore, increased concentrations of pharmaceuticals may promote enzymatic activities in fish resulting in increased metabolization rates and excretion.

Figure 11: Consumption and temporal concentration trends of clopidogrel, diclofenac, diphenhydramine and flecainide in fish fillet and liver from the sampling site Rehlingen (Saar, km 54). (Source: own figure, adapted from Boulard et al. [3]), BfG



All other results from the fish analysis can be found in the Supporting Information of Boulard et al. 2020B (Link: https://ars.els-cdn.com/content/image/1-s2.0-S0021967320308864-mmc1.pdf)

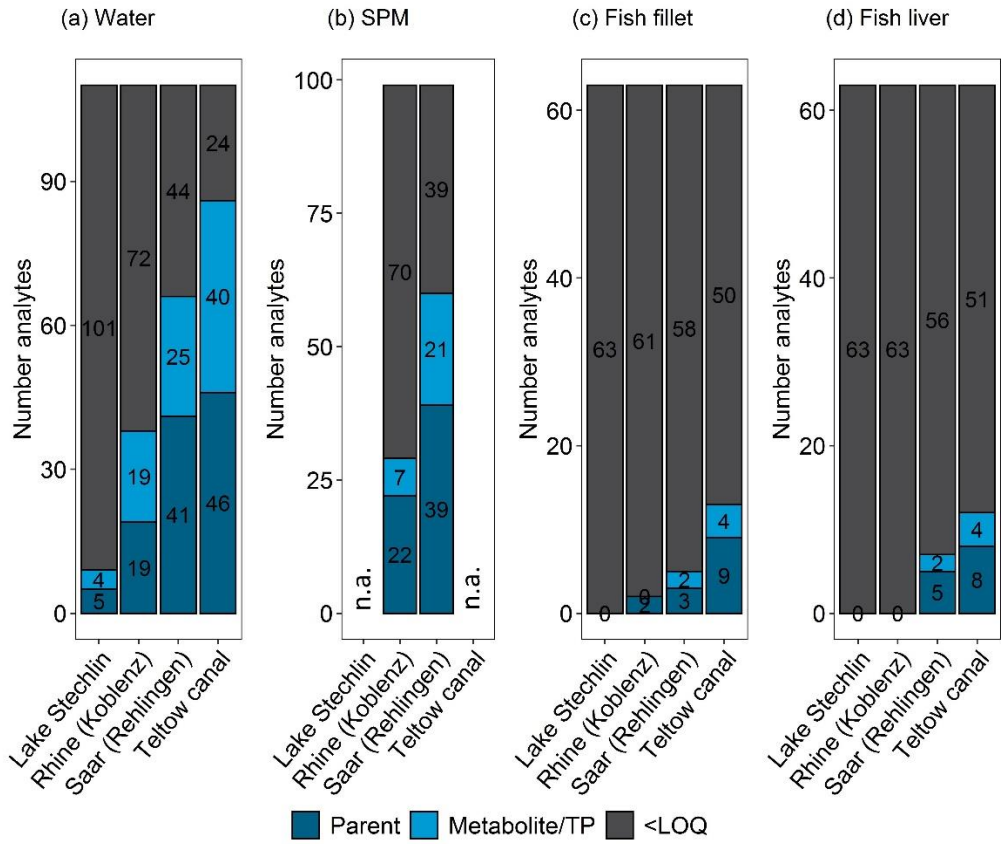
To conclude, pharmaceuticals and their TPs and metabolites are taken up by fish and can be found even in fish from aquatic systems with low wastewater affection. Distribution between compartments seems to be compound specific, but highest concentration can in general be found in liver. In blood plasma only a very limited number of pharmaceuticals could be detected. Thus, due to the challenge to collect sufficient amounts of plasma material for chemical analysis, plasma seems to not to be a favorable matrix for monitoring of pharmaceuticals in fish. In contrast to lipophilic contaminants enrichment of pharmaceuticals in fish tissue seems to be independent from fat content. Furthermore, metabolites play an important role, if the metabolites are taken up by the fish from the environment or formed by themselves has to be investigated in further studies. However, pharmaceutical concentrations in fish filet from the investigated rivers were quite low indicating bioaccumulation factors several orders of magnitude lower than those of substances known for their high accumulation potential such as PAHs or PBDEs. Therefore, no risk for consumers should be expected since total amounts in a portion of 200 g filet lay in the range of a few µg even for the highest contaminations.

5 Conclusion

5.1 Comparison between the matrices

The respective number of detections is an important criterion in order to evaluate the relevance of an environmental matrix in the framework of pharmaceutical analysis (Figure 12). In the water phase, as well as in the suspended particulate matter, more of the half of the analytes was detected in Rehlingen (Saar), whereas in fish samples, the number of detections was much lower with only 5 analytes detected in fish fillet and 7 in fish liver. In all matrices, metabolites and TP represent a high proportion of the detection.

Figure 12: Overview of the number of detected parent pharmaceuticals and corresponding metabolites/TPs at the different sampling locations and for the different investigated matrix (Source: own figure, BfG)



5.1.1 Water and SPM

In aquatic systems, the water phase is several order of magnitude superior to the amount of solid particles. In the Rhine, for example, SPM concentrations reache only 10-25 mg/L. So even for compounds with high Kd-values the major part is located in the water phase and not sorbed to particles. Consequently, for calculation of loads water concentrations preponderate. Furthermore, eco-toxicological parameter such as predicted no effect concentrations (PNECs) are generally based upon water concentrations. Water analysis is easier and generally a much higher throughput compared to SPM analysis is possible since no extraction and sample clean-up are required.

However, for long-term trend analysis an integrative sampling is essential. Sedimentation boxes enable continuous sampling over a long period, while a continuous sampling of the water is very challenging. Moreover, the methodology for the conservation of the SPM samples over a long period is well established, while this is not the case for water samples. Finally, SPM acts like a natural passive sampling material enriching compounds from the water phase. In certain cases, compounds could be detected in SPM, but were frequently below LOQ in the water phase. For example, water concentrations of sertraline were seldom above the LOQ of 20 ng/L, but it could be detected in SPM at all sampling locations.

Thus, in the framework of pharmaceutical determination in the environment, SPM analysis and water analysis are complementary.

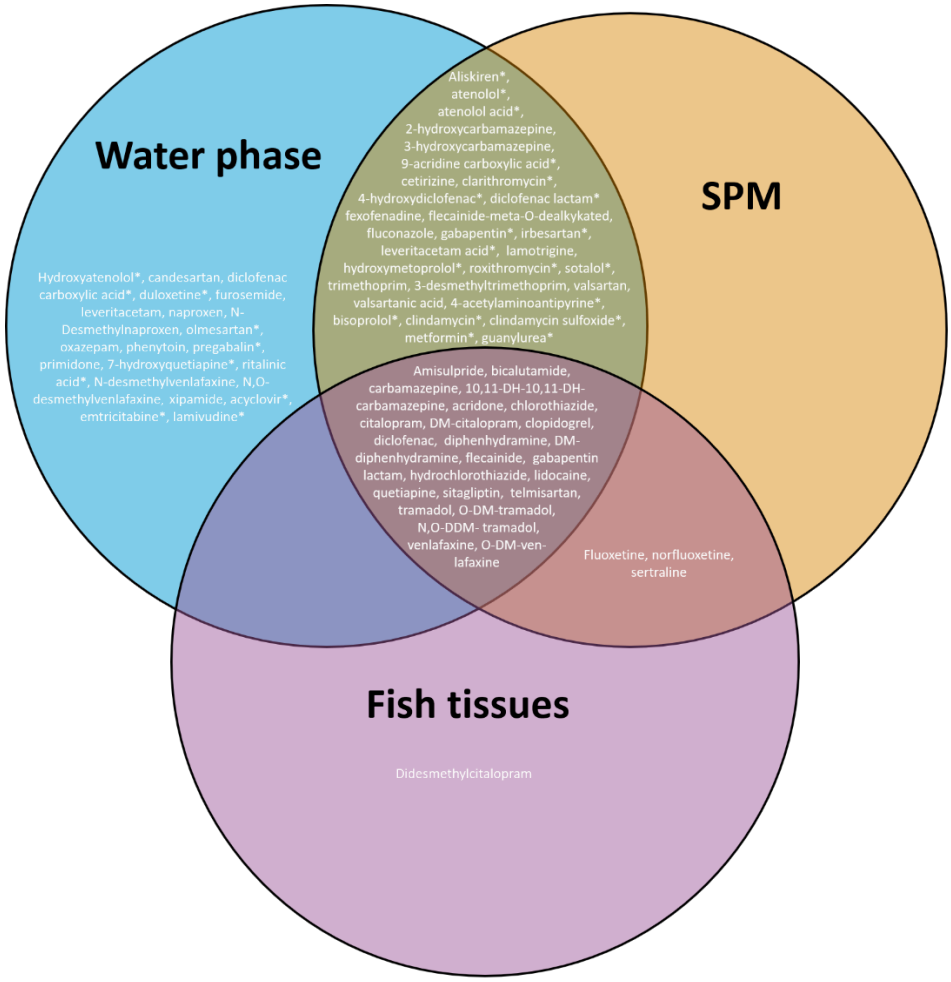
5.1.2 Fish and SPM

In order to examine, the relative relevance of fish and SPM matrix in the framework of pharmaceutical analysis in the environment, the respective partition coefficient to water of the detected analytes were compared, e.g. field derived Kd for SPM and field derived bioaccumulation factors (BAF) for fish (Table 2). All determined bioaccumulation factors were lower than their corresponding Kd except for clopidogrel whose Kd and BAF were similar. In consequence, in order to maximize the number of detections, it is more relevant to analyze SPM than fish. Nonetheless analysis of pharmaceuticals in fish and biota in general is essential for ecotoxicological studies and to evaluate the risk for consumers and top predators.

5.2 Occurrence and fate analysis of the pharmaceutical residues

79 of 117 analyzed pharmaceuticals and their TPs as well as metabolites could be detected at least one time in any of the three matrixes. Of these 24 were found in all three matrixes, 30 in water and SPM, three in fish and SPM, 21 exclusively in water and one exclusively in fish (ref. Figure 13). Fluoxetine, norfluoxetine and sertraline have high sorption affinities (Kd of 4100 ± 300 L/kg, 3000 ±200 L/kg and 2710 ± 50 L/kg, respectively) related to their positive charge at environmental pH and nonpolar structure (log D at pH 7 of 1.504, 1.160 and 2.668, respectively). In consequence, they could not be detected in the water phase but were detected in SPM and fish tissues. In contrast the very polar compounds were only found in the water phase. Didesmethylcitalopram was exclusively detected in fish tissues and is thus probably formed by transformation of citalopram or desmethylcitalopram in fish. Non-detections in fish may be caused by elevated detection limits as it is the case for lamotrigine. Furthermore, transformation to unknown metabolites could responsible for the absence of some pharmaceuticals in fish. However, due to overall low concentrations in most cases scarcely above the LOQ and the fact that only at the sampling site Koblenz sample from all three matrices were analyzed conclusions about occurrence and fate of pharmaceuticals are very limited.

Figure 13: Venn diagram visualizing distribution of pharmaceuticals and their metabolites as well TPs within the three matrices water, SPM and fish (Source: own figure, BfG)



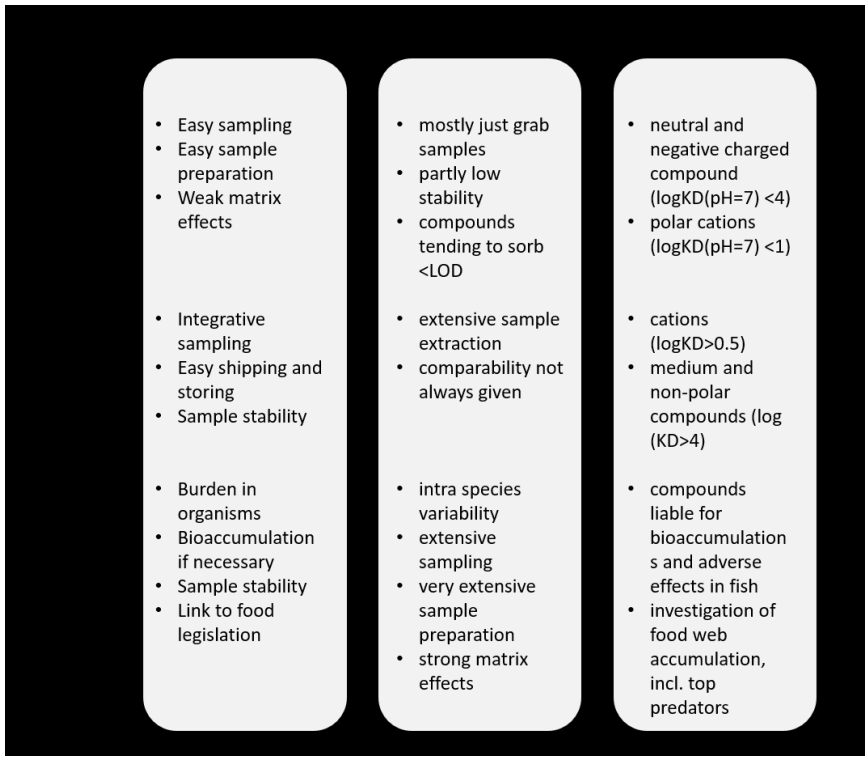
***analytes could not be analyzed with the used method in fish**

5.3 Recommendations for environmental monitoring

This study confirmed that the occurrence of even relatively polar pharmaceuticals and their human metabolites is not restricted to the water phase but are also ubiquitously present in SPM and certain compounds also in fish from German rivers. Wastewater discharge is the major source, but the study reveals evidence of relevant additional industrial emissions. Monitoring of pharmaceuticals in the aquatic environment could be done in all three investigated matrices, but each has its benefits and limitations. Water samples are easy to take and show little matrix effects even when using direct injection with minimal sample preparation. However, grab samples give only a snapshot of the contamination. Automatic taking of composite water samples is quite complex and sample stability has to be ensured by conservation measurements. Additionally, compounds tending to sorb often show water concentration below LOD. Such compounds, e.g. cations are predestinated for analyzing in SPM. For a risk assessment analysis of both water and SPM is even mandatory. Integrative sampling of SPM gives a comprehensive overview about environmental burden and due to high sample stability retrospective investigation of time trends are possible. Complex mechanisms such as bioaccumulation and

biotransformation hamper direct prediction of actual micropollutant burden in biota by water or SPM concentrations. Consequently, analysis of biota such as fish is inevitable to discover potential effects. However, for the investigated pharmaceuticals no elevated accumulation could be shown and concentrations were quite low even in fish from WWTP fish monitoring ponds. Differences in distribution between tissues were substance specific. In general, no preferred tissue could be determined, since liver showed higher concentrations but also higher matrix effects. Fish serum seems to be rather improper for monitoring. Extensive sampling and sample preparation as well as high LODs caused by strong matrix effects limit the applicability of fish in environmental monitoring. Furthermore, intra species variation may require a sufficiently large number of fish to derive clear trends and accumulation patterns. For all three matrices the importance of metabolites and TPs could be shown and it is highly recommended to include major metabolites and TPs into monitoring activities.

Figure 14: Benefits, limitations and recommended applications of the investigated matrices (Source: own figure, BfG)



5.4 Outlook/Future issues

Based on the results of this study the following future issues and questions arise

i. (eco)toxicological risks of TPs and metabolites of pharmaceuticals

Several of the investigated pharmaceuticals and their TPs and metabolites could be detected in in the aquatic environment. Especially findings in biota rises concern about (eco)toxicological effects. Beside acute toxic effects adverse effects on populations regarding reproduction and energy budget should be investigated.

ii. uptake routes and bioaccumulation/-magnification of pharmaceuticals in fish

Uptake routes for pharmaceuticals in fish are still unclear. Whether the compounds are accumulated from the water phase or contaminated food lead to biomagnification, has to be investigated by comprehensive studies of all environmental compartments and the whole food web.

iii. metabolism of pharmaceuticals in fish

Due to critical differences in enzyme activity biotransformation of pharmaceuticals in fish can strongly differ from human or mammalian metabolism. For identification of specific TPs and interpretation of pharmaceutical burden in fish metabolism studies should be carried out in fish for emerging pharmaceuticals.

iv. byproducts and educts of synthesis as marker for industrial discharge

Beside metabolites byproducts and educts of the industrial synthesis of pharmaceuticals could be interesting analytes for environmental monitoring. They could be specific marker for industrial discharge and consequently a helpful tool for identifying and location of sources for pharmaceuticals in aquatic systems beside WWTP effluents.

6 List of references

1. Boulard, L., G. Dierkes, and T. Ternes, *Utilization of large volume zwitterionic hydrophilic interaction liquid chromatography for the analysis of polar pharmaceuticals in aqueous environmental samples: Benefits and limitations.* Journal of Chromatography A, 2018. **1535**: p. 27-43.
2. Ternes T, D.G., Boulard L, Weizel A, *Method development for analysis of pharmaceuticals in environmental samples.* Umweltbundesamt, 2019.
3. Boulard, L., et al., *Spatial distribution and temporal trends of pharmaceuticals sorbed to suspended particulate matter of German rivers.* Water Research, 2020. **171**: p. 115366.
4. Boulard, L., et al., *Development of an analytical method to quantify pharmaceuticals in fish tissues by liquid chromatography-tandem mass spectrometry detection and application to environmental samples.* Journal of Chromatography A, 2020. **1633**: p. 461612.
5. Evgenidou, E.N., I.K. Konstantinou, and D.A. Lambropoulou, *Occurrence and removal of transformation products of PPCPs and illicit drugs in wastewaters: a review.* Sci Total Environ, 2015. **505**: p. 905-26.
6. Celiz, M.D., J. Tso, and D.S. Aga, *Pharmaceutical metabolites in the environment: analytical challenges and ecological risks.* Environ Toxicol Chem, 2009. **28**(12): p. 2473-84.
7. Cwiertny, D.M., et al., *Environmental designer drugs: when transformation may not eliminate risk.* Environ Sci Technol, 2014. **48**(20): p. 11737-45.
8. Hermes, N., et al., *Quantification of more than 150 micropollutants including transformation products in aqueous samples by liquid chromatography-tandem mass spectrometry using scheduled multiple reaction monitoring.* J Chromatogr A, 2018. **1531**: p. 64-73.
9. Peña-Herrera, J.M., et al., *Development and validation of an analytical method for determination of pharmaceuticals in fish muscle based on QuEChERS extraction and SWATH acquisition using LC-QTOF-MS/MS system.* Talanta, 2019. **199**: p. 370-379.
10. Grabicova, K., et al., *Development of a robust extraction procedure for the HPLC-ESI-HRPS determination of multi-residual pharmaceuticals in biota samples.* Analytica Chimica Acta, 2018. **1022**: p. 53-60.
11. Tanoue, R., et al., *Uptake and Tissue Distribution of Pharmaceuticals and Personal Care Products in Wild Fish from Treated-Wastewater-Impacted Streams.* Environmental Science & Technology, 2015. **49**(19): p. 11649-11658.
12. Tanoue, R., et al., *Simultaneous determination of polar pharmaceuticals and personal care products in biological organs and tissues.* Journal of Chromatography A, 2014. **1355**: p. 193-205.
13. Fick, J., et al., *Therapeutic Levels of Levonorgestrel Detected in Blood Plasma of Fish: Results from Screening Rainbow Trout Exposed to Treated Sewage Effluents.* Environmental Science & Technology, 2010. **44**(7): p. 2661-2666.
14. Moreno-González, R., et al., *Do pharmaceuticals bioaccumulate in marine molluscs and fish from a coastal lagoon?* Environmental Research, 2016. **146**: p. 282-298.
15. Huerta, B., et al., *Presence of pharmaceuticals in fish collected from urban rivers in the U.S. EPA 2008–2009 National Rivers and Streams Assessment.* Science of The Total Environment, 2018. **634**: p. 542-549.
16. Huerta, B., et al., *Analysis of multi-class pharmaceuticals in fish tissues by ultra-high-performance liquid chromatography tandem mass spectrometry.* Journal of Chromatography A, 2013. **1288**: p. 63-72.
17. Arnnok, P., et al., *Selective Uptake and Bioaccumulation of Antidepressants in Fish from Effluent-Impacted Niagara River.* Environmental Science & Technology, 2017. **51**(18): p. 10652-10662.
18. Xie, H., et al., *Pharmaceuticals and personal care products in water, sediments, aquatic organisms, and fish feeds in the Pearl River Delta: Occurrence, distribution, potential*

sources, and health risk assessment. Science of The Total Environment, 2019. **659**: p. 230-239.
19. Rojo, M., et al., *Human pharmaceuticals in three major fish species from the Uruguay River (South America) with different feeding habits.* Environmental Pollution, 2019. **252**: p. 146-154.
20. Du, B., et al., *Evaluation of an isotope dilution liquid chromatography tandem mass spectrometry method for pharmaceuticals in fish.* Journal of Chromatography A, 2012. **1253**: p. 177-183.
21. Subedi, B., et al., *Occurrence of Pharmaceuticals and Personal Care Products in German Fish Tissue: A National Study.* Environmental Science & Technology, 2012. **46**(16): p. 9047-9054.
22. Ramirez, A.J., et al., *Analysis of Pharmaceuticals in Fish Using Liquid Chromatography-Tandem Mass Spectrometry.* Analytical Chemistry, 2007. **79**(8): p. 3155-3163.
23. Ondarza, P.M., et al., *Pharmaceuticals, illicit drugs and their metabolites in fish from Argentina: Implications for protected areas influenced by urbanization.* Science of The Total Environment, 2019. **649**: p. 1029-1037.
24. Muir, D., et al., *Bioaccumulation of pharmaceuticals and personal care product chemicals in fish exposed to wastewater effluent in an urban wetland.* 2017. **7**(1): p. 1-11.
25. Bean, T.G., et al., *Pharmaceuticals in water, fish and osprey nestlings in Delaware River and Bay.* Environmental Pollution, 2018. **232**: p. 533-545.
26. Grabicova, K., et al., *Bioaccumulation of psychoactive pharmaceuticals in fish in an effluent dominated stream.* Water Research, 2017. **124**: p. 654-662.
27. Grabicova, K., et al., *Tissue-specific bioconcentration of antidepressants in fish exposed to effluent from a municipal sewage treatment plant.* Science of The Total Environment, 2014. **488-489**: p. 46-50.
28. Gelsleichter, J. and N.J. Szabo, *Uptake of human pharmaceuticals in bull sharks (Carcharhinus leucas) inhabiting a wastewater-impacted river.* Science of The Total Environment, 2013. **456-457**: p. 196-201.
29. Omar, T.F.T., et al., *Occurrence and level of emerging organic contaminant in fish and mollusk from Klang River estuary, Malaysia and assessment on human health risk.* Environmental Pollution, 2019. **248**: p. 763-773.
30. Larabie, S.P., M. Houde, and C.J.J.o.C.A. Gagnon, *Determination of the bioavailability of selected pharmaceutical residues in fish plasma using liquid chromatography coupled to tandem mass spectrometry.* 2017. **1522**: p. 48-55.
31. Yao, L., et al., *Simultaneous determination of 24 personal care products in fish muscle and liver tissues using QuEChERS extraction coupled with ultra pressure liquid chromatography-tandem mass spectrometry and gas chromatography-mass spectrometer analyses.* Analytical and Bioanalytical Chemistry, 2016. **408**(28): p. 8177-8193.
32. Chu, S. and C.D. Metcalfe, *Analysis of paroxetine, fluoxetine and norfluoxetine in fish tissues using pressurized liquid extraction, mixed mode solid phase extraction cleanup and liquid chromatography–tandem mass spectrometry.* Journal of Chromatography A, 2007. **1163**(1): p. 112-118.
33. Brooks, B.W., et al., *Determination of select antidepressants in fish from an effluent-dominated stream.* Environ Toxicol Chem, 2005. **24**(2): p. 464-9.
34. Brodin, T., et al., *Dilute Concentrations of a Psychiatric Drug Alter Behavior of Fish from Natural Populations.* 2013. **339**(6121): p. 814-815.
35. Schlüsener, M.P., et al., *Occurrence of venlafaxine, other antidepressants and selected metabolites in the Rhine catchment in the face of climate change.* Environ Pollut, 2015. **196**: p. 247-56.
36. Schulze, T., et al., *The German Environmental Specimen Bank.* Journal of Soils and Sediments, 2007. **7**(6): p. 361-367.
37. Tanoue, R., et al., *Uptake and Metabolism of Human Pharmaceuticals by Fish: A Case Study with the Opioid Analgesic Tramadol.* Environmental Science & Technology, 2017. **51**(21): p. 12825-12835.

38. Henning, N., et al., *Biotransformation of gabapentin in surface water matrices under different redox conditions and the occurrence of one major TP in the aquatic environment.* Water Res, 2018. **137**: p. 290-300.