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Die Anwendbarkeit des Konzepts *Benign by Design* auf pharmazeutische Wirkstoffe im Allgemeinen und Sulfonamid-Antibiotika im Speziellen

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Zusammenfassung

Das Vorkommen von Pharmazeutika in der Umwelt und damit einhergehende Risiken für die planetare Gesundheit sind ein langbekanntes und zunehmend untersuchtes Umweltproblem. Beispielsweise tragen Antibiotika in der Umwelt zur Entwicklung antimikrobieller Resistenzen bei. In den letzten Jahren ist diese Problematik stärker in den gesellschaftspolitischen Fokus gerückt. Die Förderung des Konzepts *Benign by Design* (BbD) ist ein bedeutender Bestandteil der Nachhaltigkeitsstrategien unter dem Europäischen *Green Deal.* BbD steht für eine wirksame Prävention von Pharmazeutika in der Umwelt, indem bereits während der pharmazeutischen Forschung und Entwicklung (FuE) ihr Umwelteintrag berücksichtigt wird. Ziel ist es, Pharmazeutika zu entwickeln, die schnell und vollständig in der Umwelt mineralisieren. Allerdings ist die Machbarkeit von BbD noch nicht ausreichend untersucht und verstanden. Dies führte zu dem primären Ziel der vorliegenden Arbeit: Ein Beitrag zur Anwendung von BbD im pharmazeutischen FuE-Prozess. Dafür wurde a) ein Konzept zur Implementierung von BbD in den FuE-Prozess mithilfe einer Literaturarbeit und einer Interviewstudie entwickelt und b) im Rahmen einer Fallstudie ein besseres Verständnis gewonnen, wie BbD auf Sulfonamid-Antibiotika (SUA) angewendet werden kann. SUA wurden ausgewählt, da Umweltauswirkungen bekannt sind, somit Bedarf an BbD besteht, und die Vielzahl an SUA-Vertretern Spielraum beim Re-Design bietet.

Im ersten Teil dieser Arbeit wurden das BbD-Konzept und der herkömmliche FuE-Prozess im Detail beleuchtet und gegenübergestellt. Hier lag der Fokus insbesondere auf i) Beispielen von in der Umwelt abbaubaren Wirkstoffen, ii) der Gegenüberstellung von pharmazeutisch relevanten Eigenschaften mit der biologischen Abbaubarkeit in der Umwelt, iii) den Merkmalen des FuE-Prozesses und iv) verfügbaren *in vitro* und *in silico* Teststrategien inklusive Designregeln. Darauf basierend wurde ein Konzept zur Anwendung von BbD im FuE-Prozess entwickelt. Es wurden finanzielle, regulatorische und soziale Anreize zur Unterstützung industrieller Forschung im Bereich von BbD, beispielsweise zur Entwicklung geeigneter Methoden, identifiziert (Publikation 1, P1).

Aufbauend auf dem in P1 entwickelten Konzept wurde eine Interviewstudie mit Expert:innen aus der Wirkstoffentwicklung durchgeführt, um weitere Einblicke in den FuE-Prozess und die Berücksichtigung von Umweltaspekten zu erhalten. Die Interviews haben gezeigt, dass die Strukturen und Abläufe des FuE-Prozesses sehr flexibel und anpassungsfähig sind. Insbesondere die Multiparameter Optimierung ist sehr iterativ und dynamisch. Es bestehen somit keine prozessualen Hindernisse für die frühzeitige Berücksichtigung neuartiger Parameter, inklusive solcher zum Vorkommen und Verhalten in der Umwelt. Herausforderungen bestehen allerdings vor allem im Zusammenhang mit dem derzeitigen Wissensstand zu Umweltparametern und der Verfügbarkeit von geeigneten *in silico* und *in vitro* Hochdurchsatz-Methoden z. B. zum Testen der Bioabbaubarkeit in der

Umwelt. Daher bedarf es einer konstruktiven Zusammenarbeit insbesondere zwischen FuE- und Umweltexpert:innen (P2).

In der Fallstudie zur Anwendbarkeit von BbD auf SUA wurde zunächst der aktuelle Wissensstand über Transformationsprodukte (TP) zu SUA in einer Übersichtsarbeit erörtert. Daten zur Abbaubarkeit und Bioaktivität von TP standen im Fokus, da diese Daten zu SUA ähnlichen Strukturen dem Re-Design von SUA nutzen sollten. Allerdings lagen nur für einen geringen Teil der in der Literatur beschriebenen SUA-TP Daten zum Vorkommen, zu physikochemischen Eigenschaften, zum Abbau und zur (Öko-)Toxizität vor (4 %, 4 %, 31 % bzw. 35 % der 607 SUA-TP). Zudem stammten die Daten häufig aus Mischungstests mit verringerter Aussagekraft für die Einzelsubstanzen. Dennoch fanden sich erste Anhaltspunkte für BbD, beispielsweise zur antibakteriellen Aktivität von *para*-modifizierten SUA-TP. Darüber hinaus verdeutlicht die Vielzahl an vorgeschlagenen TP-Strukturen die Bedeutung von BbD. Schnelle und vollständige Mineralisierung in die Umwelt – das Ziel von BbD – vermeidet die Bildung persistenter TP, welche andernfalls zu komplexen Stoffgemischen in der Umwelt beitrügen (P3).

Aufbauend auf der Übersichtsarbeit (P3) wurden experimentelle Studien durchgeführt. TP von sechs unterschiedlichen SUA wurden mittels Photolyse generiert (nicht zielgerichteter Designansatz) und als Mischungen mithilfe des Leuchtbakterientests (LBT) und manometrischen Respirationstests (MRT, OECD 301F) hinsichtlich ihrer antibakteriellen Wirksamkeit und leichten Bioabbaubarkeit untersucht. Einige Mischungen verursachten im LBT eine bakterielle Leuchthemmung, welche auf das Vorhandensein bioaktiver SUA-TP, z. B. auf Isomere der Ausgangssubstanzen, zurückzuführen war. Im MRT wurden keine SUA-Derivate, welche in der Umwelt mineralisieren könnten, identifiziert. Die einzeln getesteten hydroxylierten Sulfanilamidderivate zeigten ebenfalls keine verbesserte Bioabbaubarkeit. Daher führten diese *in vitro* Studien zu keinem SUA-Derivat, welches für BbD von Interesse sein könnte. Dennoch wurde das Verständnis über SUA-TP und den nicht zielgerichteten BbD-Ansatz bedeutsam verbessert. Bioaktive TP-Mischungen (LBT) und Eliminierungsraten einzelner SUA-TP (MRT) konnten bestimmt und damit Datenlücken gefüllt werden. Stärken, Schwächen und potentielle Weiterentwicklungen des nicht zielgerichteten BbD-Ansatzes wurden aufgezeigt (P4).

Die konzeptionellen Studien (P1, P2) und die SUA-Fallstudie (P3, P4) trugen entscheidend zur Anwendung von BbD bei. Erstere demonstrierten die prozessuale Machbarkeit von BbD in pharmazeutischen Unternehmen und identifizierte wichtige Schritte auf dem Weg zu dessen Umsetzung. Dazu zählen das Verständnis von BbD als neuartiger Ansatz und die Erhebungen von umweltbezogenen Bioabbaudaten als Voraussetzung für den nächsten Schritt – die Entwicklung geeigneter Screening-Methoden. Die SUA-Fallstudie lieferte einen signifikanten Beitrag zum Verständnis von einem BbD-Ansatz und zur Entwicklung von in der Umwelt abbaubaren SUA. Aufgrund identifizierter Herausforderungen, wie z. B. die Interpretation von Ergebnissen aus Mischungstests, könnte künftige Forschung bei der Untersuchung von Alternativen zum nicht zielgerichteten Design ansetzen. Bei zukünftigen Arbeiten zu BbD wird eine interdisziplinäre Zusammenarbeit zwischen Umweltwissenschaftler:innen und Expert:innen der Pharmazie oder Wirkstoffentwicklung von großem Vorteil sein.

Abstract

The presence of pharmaceuticals in the environment, with their associated risks to planetary health, is an increasingly recognised and studied environmental problem. For example, antibiotics in the environment contribute to the development of antimicrobial resistance. In recent years, this problem has become more of a socio-political focus. Promoting the concept *Benign by Design* (BbD) is an important part of the European Green Deal's sustainability strategies. Following BbD means to effectively prevent the problem of pharmaceuticals in the environment by considering the end-of-life stage of the compound already during the research and development (R&D) process. The goal is to design pharmaceuticals that mineralize rapidly and completely in the environment.

The primary objective of this thesis was to research the feasibility of applying BbD to pharmaceuticals, a topic which has not been sufficiently investigated and understood. To this end, we developed a concept for the implementation of BbD in the R&D process through a literature review and an interview study. Furthermore, we performed a case study on sulfonamide antibiotics (SUAs) to promote BbD and contribute to the design of SUAs that mineralise in the environment. SUAs were selected as environmental impacts are known and there are numerous representatives offering a range of redesign options.

In the first part of this thesis, we analysed and compared the BbD concept and the common pharmaceutical R&D. The focus here was particularly on i) examples of pharmaceuticals that are better biodegradable than parent structures, ii) the comparison between biodegradability in the environment and pharmaceutically relevant properties, iii) features of the R&D process, and iv) available *in vitro* and *in silico* test strategies including design rules. The scientific discussion resulted in a BbD concept tailored to pharmaceutical R&D. We identified regulatory, financial and social incentives that could support industrial research of BbD, e.g. for developing suitable methods (Publication 1).

Building on the concept developed in Publication 1, we conducted an interview study with R&D experts to gain further insights into drug discovery and development and the consideration of environmental aspects. The interviews showed that the flows and structures of the R&D process are very flexible and adaptable. Especially the multi-parameter optimisation is highly iterative and dynamic. Therefore, no process related barriers exist to consider novel parameters at an early stage, including those related to environmental occurrence and behaviour. However, there are other challenges to actually include environmental parameters, particularly regarding the current state of knowledge of such parameters and the availability of suitable *in silico* and *in vitro* high-throughput test method, e.g. for biodegradability in the environment. Therefore, there is a need for constructive collaboration between

pharmaceutical companies, authorities and the scientific community, including the exchange between R&D experts and environmental scientists (Publication 2).

In the case study on the applicability of BbD to SUAs, the current state of knowledge on transformation products (TPs) for SUAs was first discussed in a review. The focus was on data on the degradability and bioactivity of TPs, as these data on SUA-like structures are likely valuable for redesigning SUAs. However, data on the occurrence, physicochemical properties, degradation and (eco)toxicity were only available for a small proportion of the SUA-TPs described in the literature (4%, 4%, 31% and 35% of 607 structures, respectively). In addition, the data often originated from tests of mixtures with weaker significance for the individual substances. Nevertheless, initial indications of BbD were found, for example, on the antibacterial activity of *para*-modified SUA-TPs. In addition, the large number of proposed structures of TPs emphasises the importance of BbD. Fast and complete environmental mineralization, the aim of BbD, avoids the formation of persistent TP, which would otherwise contribute to complex mixtures of substances in the environment (Publication 3).

Based on the review (P3), we conducted experimental studies. We generated transformation products of six different SUAs by photolysis (non-targeted design approach). We then analysed them as mixtures using the luminescent bacteria test (LBT) and manometric respiration tests (MRT, OECD 301F) for antibacterial efficacy and ready biodegradability, respectively. In the LBT, some mixtures caused bacterial luminescence inhibition due to the presence of certain SUA-TPs, e.g. isomers of the parent compounds. In the MRT, we did not identify any SUA derivative that could mineralise in the environment. The individually tested hydroxylated sulfanilamide derivatives also showed no improved biodegradability. Therefore, these *in vitro* studies did not lead to any SUA derivative that could be relevant for BbD. Nevertheless, we improved the understanding of SUA-TPs and the non-targeted BbD approach. Bioactive TP mixtures and elimination rates of SUA-TPs were determined and, thus, data gaps were filled. Strengths, weaknesses and potential further developments of the non-targeted BbD approach were identified (Publication 4).

The conceptual studies (P1, P2) and the case study on SUAs (P3, P4) contribute significantly to the application of BbD. The conceptual studies demonstrated the procedural feasibility of BbD in pharmaceutical companies and identified various incentives for research and development on BbD and relevant steps for its implementation. These include an understanding BbD as a novel approach and collecting environmental biodegradation data as a prerequisite for the next step, developing suitable screening methods. The case study made a significant contribution to the understanding of a BbD approach and the development of environmentally degradable SUAs. Due to the identified challenges in interpreting results from mixture tests, future research could start with investigating alternatives to the non-targeted design approach. In future work on BbD, interdisciplinary cooperation between environmental scientists and experts in pharmacy or drug development will be fundamental.

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Abkürzungsverzeichnis

ADME	Absorption-Distribution-Metabolismus-Elimination
AS	Arbeitsschwerpunkt
BbD	*Benign by Design*
DMTA	*Design-Make-Test-Analyse*
EMA	Europäische Arzneimittel-Agentur (*European Medicines Agency*)
EU	Europäische Union
FuE	Forschung und Entwicklung = Wirkstoffentwicklung
HPLC	Hochleistungsflüssigkeitschromatographie
LBT	Leuchtbakterientest
MRT	Manometrischer Respirationstest
MS/MS	Tandem-Massenspektrometrie
OECD	Organisation für wirtschaftliche Zusammenarbeit und Entwicklung (*Organisation for Economic Co-operation and Development*)
P1 - P4	Publikation 1 - Publikation 4
PiE	Pharmazeutika in der Umwelt (*Pharmaceuticals in the Environment*)
QSBR	Quantitative Struktur-Bioabbau-Beziehung (*Quantitative Structure-Biodegradation Relationship*)
REACH	*Registration, Evaluation, Authorisation and Restriction of Chemicals*
SAICM	Strategischer Ansatz für das internationale Chemikalienmanagement
SIX	Sulfisoxazol
SMP	Sulfamethoxypyridazin
SMT	Sulfamethizol
SMX	Sulfamethoxazol
SQX	Sulfaquinoxalin-Na
STZ	Sulfathiazol
SUA	Sulfonamid-Antibiotika
TP	Transformationsprodukt(e)
UN	Vereinte Nationen (*United Nations*)
UV	Ultraviolett
WHO	Weltgesundheitsorganisation (*World Health Organization*)

1 Einleitung

1.1 Gesellschaftspolitische Entwicklungen zu Pharmazeutika in der Umwelt

Das Vorkommen von Pharmazeutika in der Umwelt (*Pharmaceuticals in the Environment*, PiE) und damit einhergehende Risiken für Ökosysteme und die menschliche Gesundheit sind ein langbekanntes und zunehmend untersuchtes Umweltproblem (Holdgate, 1981; Triebskorn et al., 2007; Kümmerer, 2008, Kümmerer, 2010b; Tyler und Goodhead, 2010; Brodin et al., 2013; Fent, 2015; Vincze et al., 2015; aus der Beek et al., 2016; Miller et al., 2018; OECD, 2019; Wilkinson et al., 2022). In den letzten Jahren hat diese Thematik auf nationaler, aber vor allem auch auf internationaler und globaler Ebene an Bedeutung gewonnen, um Umweltschutzmaßnahmen insbesondere zum Schutz der aquatischen Umwelt voranzutreiben.

Global wurden beispielsweise die 17 Ziele für nachhaltige Entwicklung der Vereinten Nationen formuliert (UN, 2015). Insbesondere ‚Gesundheit und Wohlergehen' (Ziel 3) und ‚sauberes Wasser' (Ziel 6) stehen in Verbindung mit PiE. Des Weiteren wurden Maßnahmen zu PiE unter dem Strategischen Ansatz zum Internationalen Chemikalienmanagement (SAICM) des Umweltprogramms der UN entwickelt (aus der Beek et al., 2015) und Leitlinien für klimaresistente und ökologisch nachhaltige Gesundheitseinrichtungen von der Weltgesundheitsorganisation (WHO) verfasst (Corvalan et al., 2020). Auch Nicht-Regierungsorganisationen, wie beispielsweise *Health Care Without Harm*, sind seit langem sehr aktiv zu dem Thema PiE (Health Care Without Harm, 2023). Auf internationaler Ebene waren der Europäische *Green Deal* und der resultierende *Zero Pollution Action Plan* große Meilensteine zur grünen Transformation der Wirtschaft und Gesellschaften der Europäischen Union (EU; Europäische Kommission, 2021). Die EU-Chemikalienstrategie für Nachhaltigkeit (Europäische Kommission, 2020a) und der strategische Ansatz der EU zu Arzneimitteln in der Umwelt (Europäische Kommission, 2019) sind Kerninitiativen des Europäischen *Green Deals* und liefern große Aktionspakete und damit Forschungs- und Handlungsbedarf auf diversen Ebenen, um die ambitionierten Umweltziele zu erreichen.

EU-Strategien fördern Gesetzesänderungen, erkennbar z. B. i) in dem von der Europäischen Kommission angenommenen Vorschlag zur Änderung der Wasserrahmenrichtlinie, der Grundwasserrichtlinie und der Richtlinie über Umweltqualitätsnormen (Europäische Kommission, 2022) und ii) in dem angenommenen Vorschlag für eine neue Richtlinie und eine neue Verordnung zur Reform der EU-Gesetzgebung zu Humanarzneimitteln (Europäische Kommission, 2023). Ersteres dient unter anderem der Aktualisierung der Listen für Schadstoffe in Oberflächen- und Grundwasser, für welche Qualitätsnormen eingehalten und Überwachungsdaten häufiger zur Verfügung gestellt werden müssen. Durch

die Aktualisierung würden auch weitere Pharmazeutika dazukommen, wie z. B. bestimmte Östrogene, darunter 17α-Ethinylestradiol, und Diclofenac. Zweiteres schreibt der Minderung von Umweltgefahren und -risiken eine größere Rolle bei der Entscheidung über die Zulassung von Arzneimitteln zu. Das im Rahmen der Zulassung verlangte Dossier der Umweltrisikobewertung hat allerdings auch mit der Reform keine Auswirkung auf die Zulassung von Humanarzneimitteln, wie es im Gegensatz dazu bei Tierarzneimitteln der Fall ist (Europäische Kommission, 2004, 2023). Europäische Umweltämter veröffentlichten kürzlich Vorschläge zur Verbesserung der Umweltrisikobewertung von Humanarzneimitteln (Gildemeister et al., 2023) und zur Regulierung von obligatorischen Maßnahmen zur Risikominderung seitens europäischer Umweltämter. Kurz- und langzeitige Maßnahmen werden vorgeschlagen, unter anderem die eingeschränkte Bewerbung von Arzneimitteln und die Entwicklung von grüneren Wirkstoffen (Moermond et al., 2023). Anfang 2024 wurde die erste Revision der Leitlinie zur Umweltrisikobewertung veröffentlicht (Datum des Inkrafttretens: 1. September 2024). Eine bedeutsame Änderung ist beispielsweise, dass nun für jegliche Generika auch bei einem nicht zu erwartenden Konsumanstieg des Wirkstoffs über die Umweltrisikobewertung zu berichten ist. Die Leitlinie regt aber zum Bereitstellen und Einreichen von bereits durchgeführten Umweltstudien unter Zustimmung des Dateneigentümers an, damit die Anzahl an Studien auf ein nötiges Maß begrenzt wird.

Folgende politische Entwicklungen begünstigen ebenfalls die Reduzierung von PiE: die EU-Taxonomie für nachhaltige Aktivitäten (EU, 2020), der Vorschlag zur Beschränkung der Herstellung, des Inverkehrbringens und der Verwendung von per- und polyfluorierten Alkylverbindungen im Rahmen der REACH-Verordnung (Europäische Chemikalienagentur, 2023) und der Vorschlag zur Überarbeitung der EU-Richtlinie zur Behandlung von kommunalem Abwasser mit erweiterter Umweltverantwortung für Hersteller von Arzneimitteln und Kosmetika (EU, 2023).

Diese Vielfalt an Aktivitäten mit diversen Lösungsansätzen zu PiE beteiligt zahlreiche Akteure entlang des Lebenszyklus eines Arzneimittels von der Entwicklung bis hin zum Verbleib in der Umwelt. Das Konzept *Benign by Design* zur Minimierung von PiE nach dem *Begin-of-Pipe* Ansatz gilt hierbei als besonders zielführend. Im Gegensatz zu *End-of-Pipe* Maßnahmen, wie die Abwasserbehandlung, handelt es sich um eine präventive Maßnahme frühzeitig während der Entwicklung (Kümmerer, 2007). *Benign by Design* ist ein wesentlicher Bestandteil der derzeitigen Aktionspläne, z. B. bei der Förderung von umweltfreundlicheren Chemikalien und Arzneimitteln (Chemikalienstrategie, Arzneimittelstrategie). Allerdings ist die Machbarkeit von *Benign by Design* im Rahmen der Wirkstoffentwicklung noch nicht ausreichend untersucht (Deloitte et al., 2018). Dies ist wiederum Voraussetzung für eine erfolgreiche Anwendung in der Wirkstoffentwicklung.

1.2 *Benign by Design* als Teil der Grünen Chemie und Pharmazie

Die zwölf Prinzipien der Grünen Chemie von Anastas und Warner (1998) dienen der Entwicklung sicherer und umweltfreundlicherer Chemikalien, Verfahren und Produkte. Sie können bei der Entwicklung und großtechnischen Produktion von Arzneimitteln angewandt werden, um umweltfreundlichere Arzneimittel, inklusive ihrer Wirkstoffe und weiterer Inhaltsstoffe (z. B. Hilfsstoffe), zu entwickeln und diese auf grünere Weise herzustellen. Die meisten Prinzipien der Grünen Chemie zielen auf eine sichere und umweltfreundlichere Synthese ab. Diese werden von der Industrie bereits teilweise berücksichtigt (Becker et al., 2022; De Soete et al., 2017; Diorazio et al., 2021). Anwendungsbeispiele aus der industriellen Arzneimittelherstellung sind der Lösungsmittel-Leitfaden, der Umweltquotient, die Prozessmassenintensität, die Lebenszyklusbewertung und Modelle der Grünen Chemie (ACS GCI Pharmaceutical Roundtable; Ang et al., 2021).

Die sogenannte Grüne Pharmazie ist ein Konzept zur Vermeidung bzw. signifikanten Reduktion von negativen Auswirkungen durch Arzneimittel auf die Umwelt und menschliche Gesundheit, welche beispielsweise in Verbindung mit der Herstellung oder dem Eintrag und Verbleib in der Umwelt stehen. Zur Reduzierung von PiE bestehen diverse Möglichkeiten für den Gesundheitssektor (Daughton, 2003; Kümmerer, 2010d; Toma und Crişan, 2018). Dabei ist die Anwendung der Prinzipien der Grünen Pharmazie bereits während der Arzneimittelentwicklung von großer Bedeutung, um möglichen negativen Umweltauswirkungen bereits frühzeitig vorzubeugen (Kümmerer, 2010d).

Die oben beschriebenen Prinzipien der Grünen Chemie gelten für eine grünere Arzneimittelherstellung gleichermaßen. Darüber hinaus führt die Funktion von Arzneimitteln zu Besonderheiten der Grünen Pharmazie. Ein wichtiger Punkt ist ihre essentielle Rolle für die Gesundheit von Mensch und Tier im Fall einer medizinischen Notwendigkeit. Entscheidungen über die Marktzulassung müssen begründet und verhältnismäßig sein, um diese nicht gegenstandslos zu behindern. Gleiches würde für etwaige bindende Kriterien zur Umweltfreundlichkeit gelten (Gildemeister et al., 2023; Moermond et al., 2023). Eine weitere Besonderheit bei Arzneimitteln ist ihre offene Anwendung. Ähnlich wie bei Kosmetika und Pestiziden bedeutet dies, dass a) Arzneimittelrückstände nach der Ausscheidung durch Mensch und Tier in die Umwelt gelangen, da sie nicht im geschlossenen System geführt werden können (Kümmerer et al., 2020) und b) es sich bei ihren Wirkstoffen um bioaktive Substanzen handelt, die zudem über genügende chemische Stabilität während der Herstellung, des Transports und der Lagerung für eine begründete Anzahl an Jahren und über ausreichende metabolische Stabilität während der Anwendung verfügen müssen. Daraus resultieren mögliche Auswirkungen auf die Umwelt, v. a. hinsichtlich Persistenz und Ökotoxizität (Schwarz et al., 2021). Aus diesen Gründen kann dem zehnten Prinzip der Grünen Chemie, *Design for Degradation,* eine besondere Relevanz in der Grünen Pharmazie zugeschrieben werden. Allerdings bestehen Wissenslücken hinsichtlich der Machbarkeit dieses Ansatzes insbesondere in der industriellen Praxis (Deloitte et al., 2018).

Die Entwicklung von Wirkstoffen, die in der Umwelt vollständig zu anorganischen Verbindungen (z. B. Wasser, Kohlenstoffdioxid, Sulfat oder Nitrat; d. h. Mineralisierung) abgebaut werden können, ist ein wirksames Designkonzept zur Vermeidung von PiE. Dabei handelt es sich um das Konzept *Benign by Design* (BbD), welches auf dem Prinzip *Design for Degradation* basiert (Anastas und Warner, 1998). BbD ist die gezielte Entwicklung einer chemischen Verbindung mit optimalen Eigenschaften für ihre Anwendung (bei pharmazeutischen Wirkstoffen z. B. die pharmakologische Wirksamkeit und Sicherheit) und für schnelle und vollständige Mineralisierung, nachdem die Verbindung in die Kläranlage oder direkt in die Umwelt gelangt ist. Schätzungen zufolge werden weltweit über 80 % aller Abwässer ohne Behandlung in die Umwelt eingeleitet (UN, 2017). Daher strebt BbD nach einer schnellen und vollständigen Mineralisierung in der Umwelt, sodass keine Behandlung in der Kläranlage erforderlich ist (Kümmerer, 2007, 2010c).

Die pharmakologische Wirksamkeit könnte der vollständigen Mineralisierung in der Umwelt entgegenstehen, da eine gewisse metabolische und chemische Stabilität, beispielsweise hinsichtlich Hydrolyse und Oxidation, benötigt wird, die mögliche Abbauprozesse in der Umwelt behindert (Boethling et al., 2007; Seo et al., 2009). BbD nutzt allerdings aus, dass die Stabilität und Abbaubarkeit neben den Eigenschaften des Moleküls stark von den Umgebungsbedingungen abhängen. Folglich variiert das Abbaupotenzial entlang des Lebenszyklus der Verbindung mit unterschiedlichen Umgebungsbedingungen. Relevante Umgebungsparameter für (Bio)Abbauprozesse sind beispielsweise Temperatur, pH-Wert, Licht- und Sauerstoff- sowie Nährstoffverfügbarkeit und die Zusammensetzung der Mikroorganismen (Kümmerer, 2010c). Folglich widersprechen sich die benötigte Stabilität bis zum Erzielen der pharmazeutischen Wirkung und die vollständige Mineralisierung in der Umwelt unter der Berücksichtigung dieser unterschiedlichen Umgebungsbedingungen und Kinetiken nicht zwangsläufig. Das zeigt auch das patentierte Antibiotikum CIP-Hemi: Während es bei leicht saurem pH-Wert ($pH \leq 6$) zur Beschleunigung der Hydrolyse des Hemiaminals kommt, läuft diese unter physiologischen Bedingungen ($pH \approx 7{,}4$) deutlich langsamer ab (Kümmerer et al., 2019c; Leder et al., 2021). Ein weiteres Beispiel für das nicht zwangsläufige Widersprechen sind die bereits in großen Mengen vermarkteten Wirkstoffe wie die Valproinsäure und Beta-Laktam-Antibiotika, welche in der Umwelt vollständig abbaubar sind, obwohl sie nicht gezielt dafür entwickelt wurden (Kümmerer, 2010c).

Basierend auf dem Stand der Forschung haben Lorenz et al. (2021) folgende vier BbD Ansätze für eine Projekt angepasste Anwendung definiert: zielgerichtetes und nicht zielgerichtetes *de novo* Design sowie zielgerichtetes und nicht zielgerichtetes Re-Design. Während beim *de novo* Design eine Neuentwicklung stattfindet (z. B. eines Moleküls oder einer Funktion), werden beim Re-Design bereits vorhandene und wirksame Moleküle leicht modifiziert. Das Re-Design von Wirkstoffen bietet den Vorteil, dass bereits funktionelle Eigenschaften, wie z. B. die pharmazeutisch relevanten Eigenschaften, gegeben sind, und der Fokus somit auf die Verbesserung der Bioabbaubarkeit gelegt werden kann. Anwendungsbeispiele zu *Benign by Re-Design* sind nötig, um i) Möglichkeiten und Herausforderungen dieses Konzepts aufzuzeigen, ii) im besten Fall grünere Alternativen vorzuschlagen und iii)

Erkenntnisse, z. B. zu Test- und Bewertungsstrategien, zu gewinnen, welche auch dem *de novo* Design dienen können. Zu dem zielgerichteten und nicht zielgerichteten Re-Design gibt es bereits erste Beispiele in der Literatur, wie z. B. CIP-Hemi, ein abbaubares Fluorchinolon, und 4-Hydroxypropranolol, ein bioabbaubarer Betablocker (Kümmerer, 2019). Gleichzeitig besteht weiterer Forschungsbedarf zu den genannten Punkten i) bis iii), um die Machbarkeit von BbD zu prüfen bzw. aufzuzeigen (De Soete et al., 2017). Dies inkludiert die Berücksichtigung der herkömmlichen industriellen Wirkstoffentwicklung. Nach den Fluorchinolonen und Betablockern wurden die Sulfonamid-Antibiotika als ein weiteres geeignetes Fallbeispiel für *Benign by Re-Design* identifiziert, und dies aus drei Gründen: es sind Auswirkungen durch ihren Umwelteintrag bekannt; die Datenlage dieser intensiv untersuchten Antibiotikaklasse liefert Anhaltspunkte für BbD; die Vielzahl an Sulfonamid-Vertretern bietet Handlungsspielraum beim Re-Design.

1.3 Sulfonamid-Antibiotika

Sulfonamid-Antibiotika (SUA) sind eine seit den 70er Jahren prominente Antibiotikagruppe, sowohl in der Human- als auch in der Veterinärmedizin. Mit gut 500 Tonnen Wirkstoffgewicht, die 2018 in 31 europäischen Ländern verkauft wurden, machen SUA nach den Tetracyclinen und Penicillinen den drittgrößten Anteil am Umsatz von Veterinärantibiotika aus (Europäische Arzneimittel-Agentur). Bei Humanarzneimitteln ist der Verbrauch im Vergleich zu anderen Antibiotikaklassen, wie z. B. zur Gruppe der Beta-Laktame und Penicilline sowie Makrolide, Lincosamide und Streptogramine, von geringerer Bedeutung. Beispielsweise lag der europäische Konsum (als Grundversorgung und im Krankenhaus) für SUA im Jahr 2021 bei 0,57 definierte Tagesdosen je 1.000 Einwohner im Vergleich zu 16,5 definierte Tagesdosen für Antibiotika insgesamt (Europäisches Zentrum für die Prävention und die Kontrolle von Krankheiten, ECDC, 2022). Vertreter, die je nach Infektionskeim in der Human- oder Veterinärmedizin eingesetzt werden, sind beispielsweise Sulfamethoxazol, Sulfadiazin und Sulfamerazin (**Abbildung 1**). SUA besitzen ein Sulfanilamid-Pharmakophor. Sie unterscheiden sich in ihrem spezifischen Rest. Durch das Eingreifen in die essentielle Folsäuresynthese von Mikroorganismen wirken SUA als Bakteriostatikum. Sie inhibieren als Strukturanaloge der *p*-Aminobenzoesäure kompetitiv die Dihydropteroat-Synthase. SUA wirken gegen zahlreiche grampositive und gramnegative Bakterienstämme, darunter *Streptococcus pneumoniae, Chlamydia trachomatis* und *Escherichia coli*. Die minimale Hemmkonzentration liegt je nach Keim im unteren $mg \cdot L^{-1}$ Bereich (William und Petri, 2016).

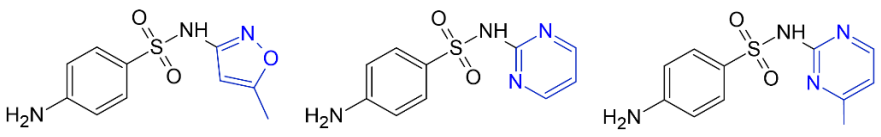

Abbildung 1: *Drei Vertreter aus der Gruppe der Sulfonamid-Antibiotika.*
Schwarz: Sulfanilamid-Pharmakophor. Blau: spezifische Reste (5-Methyl-1,2-Oxazol, Pyrimidin, 4-Methylpyrimidin).

Mittlerweile wurden einige Sulfonamid-Resistenzen nachgewiesen, z. B. bei *Neisseria meningitidis, Shigella und Escherichia coli*, sodass ihre Bedeutung in der Humanmedizin gesunken ist (William und Petri, 2016). Um die Bildung von Resistenzen gegen SUA zu verlangsamen, werden sie auch in fester Kombination mit dem antibakteriellen Wirkstoff Trimethoprim verabreicht. Die antibakterielle Wirkung wird durch einen synergistischen Effekt signifikant verbessert, indem Trimethoprim die Dihydrofolat-Reduktase des gramnegativen oder grampositiven Keims hemmt, und somit an einer weiteren Stelle in die Folsäuresynthese eingreift (Smilack, 1999). Das bekannte Kombinationspräparat Cotrimoxazol besteht aus Sulfamethoxazol und Trimethoprim und wird z. B. bei Harnwegsinfektionen eingesetzt.

Wirkstoffrückstände, einschließlich ihrer Metaboliten, gelangen primär infolge der Ausscheidung durch Patient:innen und behandelte Tiere in die Umwelt (Ebert et al., 2015; Michael et al., 2013). Ein möglicher Eintragspfad ins Oberflächenwasser sind Abwässer aus Kläranlagen aufgrund von unzureichender Entfernung. Über kontaminierte Gülle, die zur Düngung genutzt wird, können SUA-Rückstände direkt auf Felder ausgebracht werden. Letzteres stellt aufgrund des hohen Einsatzes in der Veterinärmedizin einen relevanten Eintragspfad dar (Spielmeyer, 2018; Wohde et al., 2016). In der aquatischen Umwelt kann die Rücktransformation des Metaboliten *N4*-Acetyl-Sulfamethoxazol zur Ausgangssubstanz Sulfamethoxazol (SMX) stattfinden (Radke et al., 2009). Dies verdeutlicht, dass die Gesamtbelastung an Wirkstoffen, Metaboliten und auch Transformationsprodukten zur Umweltrelevanz beiträgt. Gleichzeitig unterstreicht diese Diversität an Verbindungen nach dem Umwelteintrag von SUA die Bedeutung von BbD. Sie liegt hier in dem Ziel von BbD, nämlich in der vollständigen Mineralisierung in der Umwelt zur Verhinderung solcher komplexen Substanzgemische, deren Umweltrisiken schwieriger zu beurteilen sind.

Die kürzlich durchgeführte Studie von Wilkinson et al. (2022) über die weltweite Verunreinigung von Flüssen durch pharmazeutische Wirkstoffe zeigte, dass SMX zu den Kontaminanten mit den höchsten Konzentrationen der 61 untersuchten pharmazeutischen Wirkstoffen zählt (Mittelwert: 534 $ng \cdot L^{-1}$, Median: 436 $ng \cdot L^{-1}$, Häufigkeit: 39.2 %). An 140 Überwachungsstandorten überschritt die SMX-Konzentration die vorhergesagte unbedenkliche Konzentration von 200 $ng \cdot L^{-1}$, was somit ein Umweltrisiko anzeigt. Die Umweltkonzentrationen von SUA sind durch einen kontinuierlichen Eintrag in die Umwelt und ihre Persistenz zu erklären (Mahmoud et al., 2013; Nationales Zentrum für Biotechnologieinformationen, 2023a, 2023b).

SUA-Rückstände können sich auf die Umwelt und die menschliche Gesundheit auswirken. Beispiele sind der Einfluss auf die Zusammensetzung eines Microbioms (Cycoń et al., 2019), Phytotoxizität (Christou et al., 2018) und die Entwicklung und Selektion antimikrobieller Resistenzen insbesondere in Kläranlagen (Baran et al., 2011; Ezzariai et al., 2018; Felis et al., 2020). Letzteres ist eine ernsthafte globale Bedrohung für die menschliche Gesundheit (WHO, 2023). Neben Antibiotikaresistenz-Strategien (EU, 2022; WHO, 2001) ist auch *Benign by (Re-)Design* eine Möglichkeit zur Verringerung der Umweltkonzentrationen. Es wurde bisher allerdings noch nicht untersucht, ob Derivate von SUA als BbD Kandidat in Frage kommen, beziehungsweise wenn ja, mit welchen Strukturmerkmalen.

2 Aufbau und Ziel der Arbeit

Die Auswirkungen des Eintrags von Pharmazeutika in der Umwelt und geschilderte gesellschaftspolitische Entwicklungen zu dieser Problematik führen zum übergeordneten Ziel dieser Arbeit. Sie soll einen Beitrag zur praktischen Anwendung von BbD im Wirkstoffentwicklungsprozess leisten, um Akteure bei der Entwicklung von umweltfreundlicheren Wirkstoffen zu unterstützen (übergeordnetes Ziel, **Abbildung 2**). Zum einen wurden dafür in konzeptionellen Studien die Abläufe der herkömmlichen Wirkstoffentwicklung (Forschung und Entwicklung, FuE) durch eine Literaturarbeit und Interviews mit Expert:innen der Wirkstoffentwicklung untersucht. Ziel war es, Merkmale zu identifizieren, die für die Anwendung von BbD relevant sind, um ein Konzept zur Implementierung von BbD in den Wirkstoffentwicklungsprozess bereitzustellen (Teilziel 1). Zum anderen wurde in einer Fallstudie zu BbD die Machbarkeit des Re-Designs von SUA geprüft, um zur Entwicklung umweltfreundlicherer SUA beizutragen (Teilziel 2) und die Anwendung von BbD im Allgemeinen zu unterstützen (Teilziel 3). Daten aus der Literatur und eigenen *in vitro* Experimenten zur antibakteriellen Aktivität und Bioabbaubarkeit von SUA-Derivaten wurden gesammelt und auf Eignung für BbD geprüft. Für das experimentelle Vorgehen konnte auf bereits veröffentlichte BbD Studien aufgebaut werden (Rastogi et al., 2014, 2015a, 2015b).

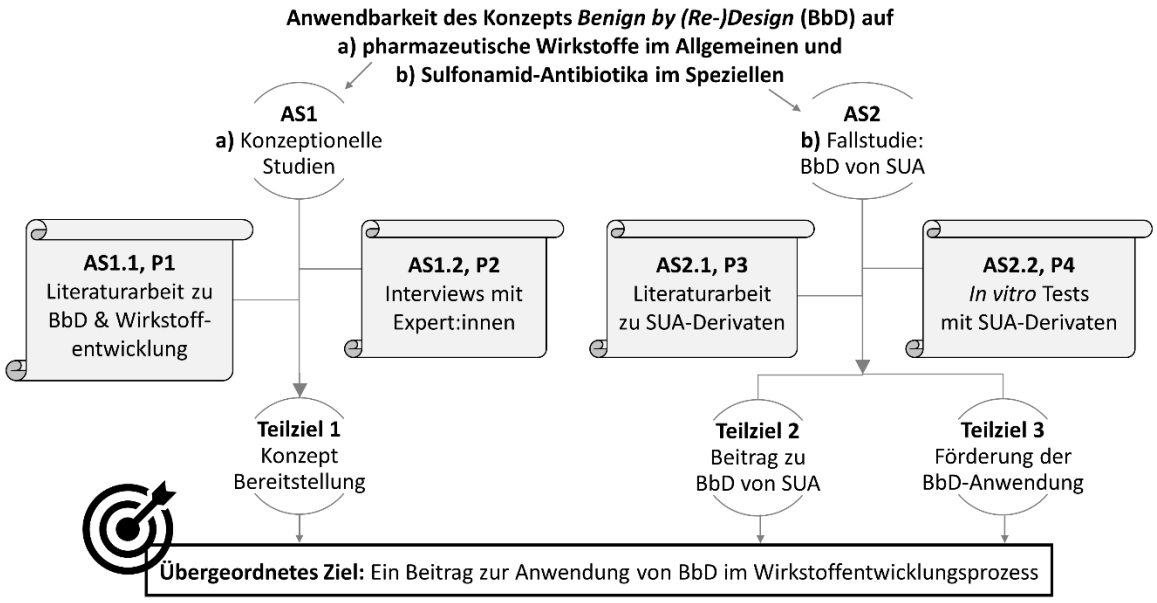

Abbildung 2: *Aufbau und Ziel der Arbeit. Vier Arbeitsschwerpunkte (AS1.1 - AS2.2) und resultierende Publikationen (P1 - P4) zum Erreichen der drei Teilziele und des übergeordneten Ziels.*

Aus dem übergeordneten Ziel und den Teilzielen ergeben sich für die konzeptionellen Studien und die SUA-Fallstudie der Dissertation je zwei Arbeitsschwerpunkte (AS1.1 - 1.2, AS2.1 - 2.2) mit konkreten Forschungsfragen, dessen Ergebnisse in je zwei Fachartikeln (Publikation P1 - 2, P3 - 4; **Tabelle 1**) veröffentlicht wurden (siehe auch **Abbildung 2**):

AS1.1, P1) Wo und unter welchen Bedingungen kann BbD im FuE-Prozess Anwendung finden? Wie verhalten sich Stoffeigenschaften, die für die pharmazeutische Anwendung verlangt werden, zu Stoffeigenschaften, die für eine bessere Abbaubarkeit in der Umwelt nötig wären?

AS1.2, P2) Welche Sichtweise haben Expert:innen aus FuE, z. B. Medizinsche Chemiker:innen, auf die Berücksichtigung des Umweltverhaltens von Wirkstoffen im FuE-Prozess? Wie ist auf dieser Grundlage die prozessuale Machbarkeit von BbD zu bewerten? Wie lassen sich etwaige Hürden überwinden?

AS2.1, P3) Was ist über SUA-Derivate wie Transformationsprodukte (TP) insbesondere hinsichtlich ihrer Abbaubarkeit in der Umwelt und antibiotischen Wirksamkeit bekannt? Welche Lehren können zur Umweltrelevanz von SUA-TP gezogen werden? Können die Daten für das Re-Design von SUA genutzt werden?

AS2.2, P4) Entstehen Derivate bei der Photolyse ausgewählter SUA, die aufgrund ihres Verhaltens in Studien zur Abbaubarkeit und Bioaktivität der generierten Mischungen für BbD von Interesse sind? Welche Lehren können aus diesen Studien für BbD gezogen werden? Verbessert zum Beispiel die Hydroxylierung des SUA-Grundkörpers die biologische Abbaubarkeit?

In Kapitel 3 und 4 werden diese vier Hauptarbeitsbereiche im Detail erläutert und anschließend global diskutiert. Um biotische Prozesse in der Umwelt deutlich von solchen im Menschen (bzw. höheren Organismen) abzugrenzen, wird im Folgenden ersteres als Transformation oder Bioabbau (zu TP) bzw. Mineralisierung (zu anorganischen Endprodukten) und zweiteres ausschließlich als Metabolismus (zu Metaboliten) bezeichnet (Kümmerer, 2010a). Die Bioabbaurate bzw. Mineralisierungsrate als Maß für die Vollständigkeit des Bioabbaus zu anorganischen Endprodukten ist von der Rate der Primäreliminierung bzw. des Primärabbaus als Maß für die Konzentrationsabnahme der Ausgangssubstanz zu unterscheiden.

In dieser Arbeit zu BbD von pharmazeutischen Wirkstoffen geht es folglich um einen wichtigen Baustein der Grünen Pharmazie: die biologische Abbaubarkeit von Wirkstoffen. Für eine umfassendere Abschätzung der Umweltfreundlichkeit wäre der gesamte Lebenszyklus des Wirkstoffs, also z. B. auch der Herstellungsprozess, nach den zwölf Prinzipien der Grünen Chemie zu bewerten. Zur Bewertung

eines gesamten Arzneimittels wären alle Inhaltsstoffe, also z. B. auch die eingesetzten Hilfsstoffe, zu berücksichtigen. Zur weiteren Einordnung dieser Arbeit ist sie von Arbeiten der Nachhaltigen Pharmazie abzugrenzen. Für die Nachhaltigkeitsbewertung eines Wirkstoffs oder Arzneimittels (bzw. einer Chemikalie oder eines Produkts im Allgemeinen) wären zahlreiche Umweltkriterien heranzuziehen. Darüber hinaus sind auch Kriterien aus den Bereichen Soziales und Wirtschaft zu berücksichtigen. Diese und weitere Arbeiten zur Grünen Pharmazie und Nachhaltigen Pharmazie sind von großer Bedeutung für die Transformation der Pharmazie, damit ‚Nachhaltige Pharmazie' in Zukunft nur noch als ‚Pharmazie' bezeichnet werden kann, nämlich wenn entsprechende Kriterien zum Standard geworden sind.

Tabelle 1: *Arbeitsschwerpunkte und resultierende Publikationen.*

Arbeitsschwerpunkt		**Titel der Publikation**
AS1 Konzeptionelle Studien: BbD in FuE	**AS1.1, P1** Anwendung von BbD in FuE	Puhlmann, N.; Mols, R.; Olsson, O.; Slootweg, J. C.; Kümmerer, K. (2021): Towards the design of active pharmaceutical ingredients mineralizing readily in the environment. In: Green Chemistry 23 (14): 5006–5023. DOI: 10.1039/D1GC01048D.
	AS1.2, P2 Sichtweise von Expert:innen aus FuE	Puhlmann, N.; Vidaurre, R.; Kümmerer, K. (2024): Designing greener active pharmaceutical ingredients: Insights from pharmaceutical industry into drug discovery and development. In: European Journal of Pharmaceutical Sciences 192: 106614. DOI: 10.1016/j.ejps.2023.106614.
AS2 Fallstudie: SUA Re-Design	**AS2.1, P3** Literaturdaten zu SUA-TP	Puhlmann, N.; Olsson, O.; Kümmerer, K. (2022): Transformation products of sulfonamides in aquatic systems: Lessons learned from available environmental fate and behaviour data. In: Science of The Total Environment 830: 154744. DOI: 10.1016/j.scitotenv.2022.154744.
	AS2.2, P4 Bewertung von Photo-TP als SUA Alternative	Puhlmann, N.; Olsson, O.; Kümmerer, K. (2024): How data on transformation products can support the redesign of sulfonamides towards better biodegradability in the environment. In: Science of The Total Environment 921: 171027. DOI: 10.1016/j.scitotenv.2024.171027.

3 Die Anwendung von *Benign by Design* in der pharmazeutischen Wirkstoffentwicklung

3.1 Ein Konzept - BbD in der pharmazeutischen Wirkstoffentwicklung

3.1.1 Problemstellung und Methode

Um die Problematik von PiE zu bekämpfen, ist die Implementierung von BbD ein vielversprechender, langfristiger *begin-of-pipe* Ansatz (Klatte et al., 2017). Allerdings werden bisher kurzfristige Maßnahmen z. B. zum Wissensaustausch, zur Sensibilisierung und Bildung von der pharmazeutischen Industrie bevorzugt. Der Grund dafür liegt vermutlich in den Bedenken gegenüber langfristigen Maßnahmen wie BbD hinsichtlich hoher Kosten und technischen Herausforderungen. Entsprechend wurden in der Industrie bisher keine bedeutenden Schritte unternommen, das Konzept BbD während der Wirkstoffentwicklung zu berücksichtigen (Deloitte et al., 2018). Neue biologisch abbaubare Wirkstoffe könnten aber als neue Geschäftsmöglichkeit wirtschaftliche Vorteile bringen. Diese Möglichkeit hängt primär von der Machbarkeit des BbD-Konzepts ab, welche wiederum durch die Entscheidungsprozesse während der Wirkstoffentwicklung bedingt ist.

Die Implementierung des BbD-Konzepts in die industrielle Wirkstoffentwicklung wurde bis zum Zeitraum des ersten Arbeitsschwerpunkts (Publikation 1) noch nicht wissenschaftlich diskutiert. Eine solche Auseinandersetzung ist wiederum Voraussetzung für die praktische Anwendung. Daher war das übergeordnete Ziel des ersten Arbeitsschwerpunkts, zu einem besseren Verständnis des BbD-Konzepts beizutragen und dadurch Aktivitäten zu BbD in der industriellen Wirkstoffentwicklung zu stimulieren. Darüber hinaus soll diese Arbeit pharmazeutische Unternehmen unterstützen, sich auf zukünftige gesetzliche Regelungen als Konsequenz oben genannter EU-Strategien vorzubereiten.

Zum Erreichen dieser Ziele wurden generische Verfahren und Prinzipien der Wirkstoffentwicklung anhand öffentlich verfügbarer Literatur zusammengefasst. Studien zum BbD-Konzept wurden ausgewertet. Verfügbare sowie noch zu entwickelnde Testmethoden für die biologische Abbaubarkeit in der Umwelt wurden zusammengetragen. Basierend auf diesen Informationen wurde herausgearbeitet, wo und mit welchen Mitteln das BbD-Konzept in die generische Wirkstoffentwicklung einbezogen werden kann.

3.1.2 Ergebnisse und Diskussion

Verfahren und Prinzipien der industriellen Wirkstoffentwicklung sind in der Literatur ausführlich beschrieben (Blass, 2015; Hughes et al., 2011; Lombardino und Lowe, 2004; Messinger et al., 2016). Die Target[1]-basierte Wirkstoffentwicklung der pharmazeutischen Industrie ist unterteilt in die i) Target Identifizierung, ii) Hit[2] Selektion, iii) Optimierung von Hits zu Leitstrukturen, iv) Optimierung dieser und v) Selektion der vielversprechendsten Kandidaten für die weitere Arzneimittelentwicklung in präklinischen und klinischen Studien. In Publikation 1 wurden die einzelnen Phasen vorgestellt. Um spätere Aspekte zu verstehen, wird die Optimierungsphase im Folgenden kurz beschrieben.

Die Optimierungsphase ist eine Herausforderung für Medizinsche Chemiker:innen aufgrund der vielen Parameter, die berücksichtigt werden müssen. Gleichzeitig ist diese sogenannte Multiparameter Optimierung auch von entscheidender Bedeutung, um zu dem erwünschten Eigenschaftsprofil des Wirkstoffs zu gelangen. Optimiert werden zum Beispiel die Aktivität, das Profil zu Absorption-Distribution-Metabolismus-Elimination (ADME), die Toxikologie, die Target Selektivität und die Bioverfügbarkeit (Bowes et al., 2012; Parry, 2019; Plowright et al., 2012). Die Entwicklung geeigneter Kandidaten muss sehr flexibel sein, um auf gewonnene Erkenntnisse während der Optimierung reagieren zu können. Daher ist die Optimierungsphase als ein sich häufig wiederholender Zyklus konzipiert, der sogenannte *Design-Make-Test-Analyse* (DMTA) Zyklus (Plowright et al., 2012; Wesolowski und Brown, 2016).

In Publikation 1 wurden die Prinzipien von BbD für Pharmazeutika erklärt. Von besonderer Relevanz ist beispielsweise das Verständnis darüber, dass kleine Veränderungen der Molekülstruktur die Bioabbaubarkeit in der Umwelt verändern können, während andere pharmakologisch relevante Eigenschaften, wie z. B. die Wirksamkeit, erhalten bleiben. Nach Änderung der Molekülstruktur können die Eigenschaften mithilfe von *in silico* Modellen vorhergesagt werden, müssen dann aber durch *in vitro* Methoden verifiziert werden. In Publikation 1 wurde nach der Vorstellung von Testmethoden für BbD hervorgehoben, welche Attribute für *in silico* und *in vitro* Methoden bei der Anwendung im Wirkstoffdesign nötig sind. Dazu zählt beispielsweise die Anwendbarkeit eines *in silico* Modells auf pharmakologisch aktive Strukturen, was insbesondere von der Anwendungsdomäne abhängt. Derzeit verfügbare Modelle zur Vorhersage der Bioabbaubarkeit, z. B. Biowin1-7 (US EPA), CASE Ultra (Multicase) und Model Applier (Leadscope), sind häufig nicht auf Wirkstoffe anwendbar und damit nicht geeignet, da die Modelle auf Daten zu strukturell einfacheren Bulkchemikalien basieren. Ein wichtiges Testattribut für *in vitro* Screenings ist z. B. ein hoher Durchsatz zum Testen von Tausenden

[1] Ein Target ist ein Molekül im Körper, in der Regel ein Protein, das mit einem bestimmten Krankheitsprozess in direkter Verbindung steht. Die Bindung eines Wirkstoffs an das Target könnte eine gewünschte therapeutische Wirkung erzielen (Amaratunga et al. (2007)).

[2] Sogenannte Hits sind Strukturen mit bestätigter Target-Aktivität.

von Strukturen innerhalb kurzer Zeit. Erste entwickelte Testmethoden zur Bioabbaubarkeit geben noch keine genügende Aussage über die Mineralisierungsrate (siehe z. B. Martin et al., 2017), was wiederum von großer Bedeutung für BbD ist. Die Charakterisierung der Testattribute zeigte folglich, dass derzeit verfügbare *in silico* und *in vitro* Methoden noch nicht zur Anwendung in den ersten Phasen der Wirkstoffentwicklung, beispielsweise während der Optimierung von Hits, geeignet sind.

Zur Veranschaulichung von BbD wurden die vier BbD-Ansätze, nämlich das zielgerichtete und nicht zielgerichtete *de novo* Design sowie das zielgerichtete und nicht zielgerichtete Re-Design (siehe auch Lorenz et al., 2021), mit Beispielen untermauert wie etwa das nicht zielgerichtete Re-Design von Betablockern (Rastogi et al., 2014, 2015a, 2015b) oder das zielgerichtete Re-Design von Ciprofloxacin (Leder et al., 2021) mit dem Resultat zweier Patente für Chinolon-Antibiotika mit einem Hemiaminal-Linker sowie Cipro-Prolin and Cipro-P2C (Kümmerer et al., 2019c; Kümmerer et al., 2019b). Die vorgestellten Beispiele (Tabelle 4, P1) zeigen, dass es grundsätzlich möglich ist, einen Wirkstoff zu entwickeln, der in der Umwelt besser abgebaut wird oder sogar leicht zu anorganischen Verbindungen (H_2O, CO_2, NO_3^-, etc.) mineralisiert, gleichzeitig aber auch die nötigen pharmazeutischen Eigenschaften aufweist.

In der übergeordneten Diskussion von Publikation 1 zur Implementierung von BbD in die generische Wirkstoffentwicklung wurde argumentiert, dass die Berücksichtigung von der Bioabbaubarkeit in der Umwelt am wirksamsten während der Multiparameter Optimierung ist (**Abbildung 3** nach Abbildung 6, P1). Es wurde hergeleitet, wie eine solche Berücksichtigung während der einzelnen Phasen des DMTA-Zyklus erfolgen müsste. Dabei spielen u. a. Designregeln zugunsten der Bioabbaubarkeit (Tabelle 5, P1) eine entscheidende Rolle. Sie sollten während der Optimierungsphase angewandt werden, um durch gezielte Strukturmodifizierung die biologische Abbaubarkeit in der Umwelt positiv zu beeinflussen. Solche Designregeln könnten auch bereits bei der Hit Selektion herangezogen werden, um solche Moleküle frühzeitig zu kennzeichnen, welche funktionelle Gruppen tragen, die den Bioabbau begünstigen beziehungsweise behindern.

Ein wesentliches Argument für die Implementierung von BbD während der Optimierung pharmazeutischer Parameter sind die Auswirkungen der gezielten Strukturmodifizierung auf die Bioabbaubarkeit in der Umwelt. Der Grund ist der Zusammenhang zwischen Strukturmerkmalen und intrinsischen Eigenschaften. Dies wurde in Publikation 1 anhand der Optimierungsparameter ADME, Toxizität und Stabilität erörtert. Die pharmazeutischen Eigenschaften können mit der biologischen Abhängigkeit in Konflikt stehen, aber auch kompatibel sein. Beispielsweise sind die Eigenschaften zur oralen Aufnahme und Verteilung des Wirkstoffs bis zum Erreichen des Wirkorts mit der Wirkstoffaufnahme in die Bakterienzelle kompatibel. Letzteres ist Voraussetzung für den biologischen Abbau in der Bakterienzelle.

Die Berücksichtigung der sogenannten *Rule of Five* von Lipinski et al. (2001)[3] sorgt für eine geeignete Balance zwischen der Hydrophilie zugunsten der Plasmalöslichkeit und der Lipophilie zugunsten der Membranpermeabilität. Auch die Aufnahme in eine Bakterienzelle wird dadurch begünstigt (Smułek und Kaczorek, 2022), sodass die Bioabbaubarkeit in der Umwelt durch die Anwendung von Lipinskis Regel positiv beeinflusst wird. Wenn die Moleküleigenschaften, beispielsweise eine Molekülgröße von > 500 Da, die Aufnahme in die Bakterienzelle behindern, könnten aber auch von Bakterien sezernierte Exoenzyme einen biologischen Abbau außerhalb der Bakterienzellen ermöglichen (Krueger et al., 2015; Mahmoudi et al., 2023).

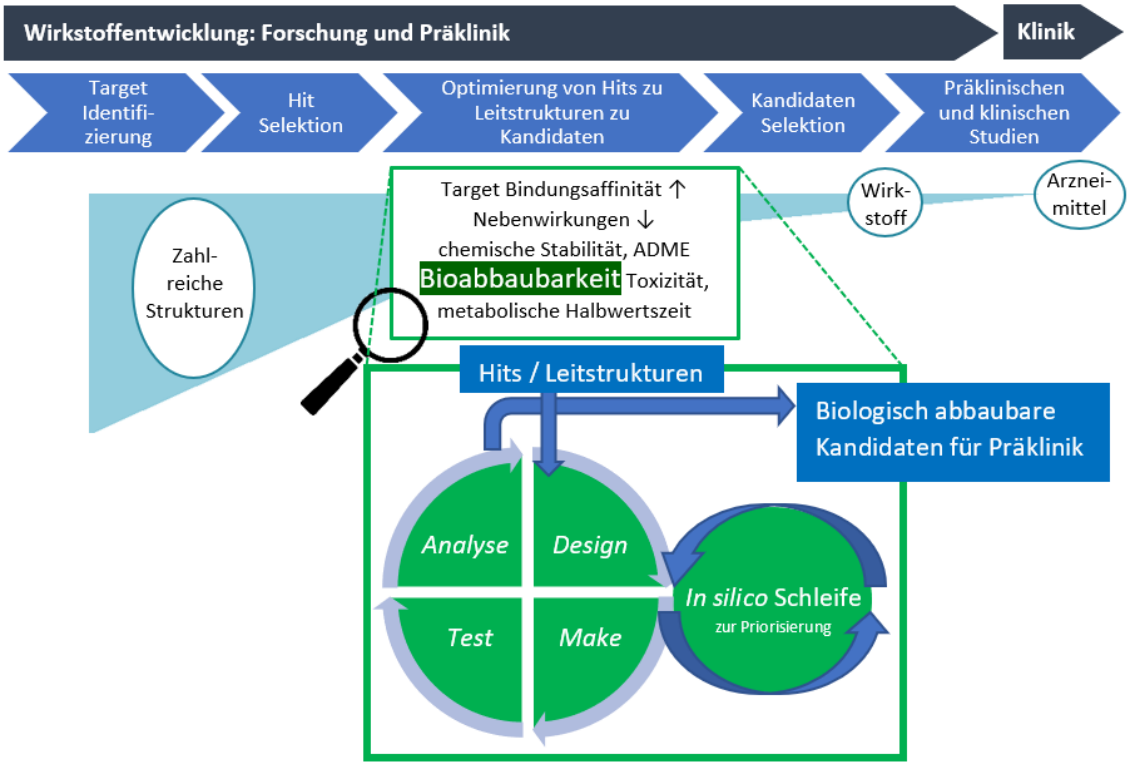

Abbildung 3: *Die Wirkstoffentwicklung von der Target Identifizierung bis hin zu den präklinischen und klinischen Studien, erweitert um das Konzept Benign by Design in der Optimierungsphase (nach Abbildung 6, P1). Die Bioabbaubarkeit in der Umwelt wird neben den typischen pharmazeutischen Parametern, wie die Target Bindungsaffinität und die metabolische Halbwertszeit, während des Design-Make-Test-Analyse (DMTA) Zyklus mitberücksichtigt. In silico Tests sind hilfreich, um Verbindungen für die Synthese (make) zu priorisieren.*

Die chemische (abiotische) und metabolische Stabilität des Wirkstoffs – benötigt für die Funktion und die Qualität entlang des Lebenszyklus bis zur Anwendung – scheinen im Widerspruch zur biologischen Abbaubarkeit in der Umwelt zu stehen. In Publikation 1 wurde allerdings aufgezeigt, dass eine ausreichende Stabilität für die Anwendung, z. B. eine für die Wirkung benötigte Halbwertszeit im Körper, bei gleichzeitiger Bioabbaubarkeit in der Umwelt erzielt werden kann, wenn das Maß an

[3] Lipinskis *Rule of Five* zur Optimierung der ADME Eigenschaft wird bei der Entwicklung eines oral zu verabreichenden Wirkstoffs berücksichtigt, da sie ein Indikator für sogenannte *Druglikeness* darstellt (Lipinski et al., 2001): ≤ 5 Donatoren von Wasserstoffbrückenbindungen, ≤ 10 Akzeptoren von Wasserstoffbrückenbindungen, Molekülmasse ≤ 500 Dalton, Oktanol-Wasser Verteilungskoeffizienten (log P) ≤ 5.

Stabilität differenzierter betrachtet wird. Es geht also darum eine entsprechende „Stabilitätslücke“ für die Umwelt zu finden (als ‚*Window of Opportunity*‘). Dabei muss berücksichtigt werden, dass Stabilitäten nicht nur von Moleküleigenschaften abhängen, sondern auch von den Umgebungsbedingungen und Kinetiken, und somit das Abbaupotenzial während der verschiedenen Phasen des Lebenszyklus des Wirkstoffs mit unterschiedlichen Umgebungsbedingungen variiert (siehe Abschnitt 1.2). Beispielsweise könnte die Verpackung in Braunglas oder braunen Blistern einen lichtempfindlichen Wirkstoff vor Licht schützen. Der Schutz vor einem photolytischen Abbau wäre nicht mehr gegeben, wenn der Wirkstoff nach Ausscheidung ins Oberflächenwasser gelangt (Kümmerer, 2007). Darüber hinaus lässt der Leitfaden ICH Q1A zur Prüfung der chemischen Stabilität von Wirkstoffen und Arzneimitteln unter dem Einfluss von Temperatur, Feuchtigkeit und Licht genügend Flexibilität. Bei wissenschaftlich vertretbaren Gründen können alternative Ansätze verwendet werden (EMA, 2003), ähnlich wie für Biologika nach dem Leitfaden ICH Q5C (EMA, 1996). Folglich bestehen Möglichkeiten, beide Kriterien, d. h. die für die Anwendung benötigte chemische und metabolische Stabilität und die (Bio)Abbaubarkeit in der Umwelt, zu erfüllen, wenn ‚Stabilitätslücken‘ gefunden werden. Dies zeigt auch das Beispiel des patentierten Antibiotikums CIP-Hemi (Leder et al., 2021). CIP-Hemi wurde so konzipiert, dass es die erforderliche Stoffwechselstabilität und antibiotische Aktivität beibehalten hat, aber unter den leicht sauren Bedingungen im Urin und nach der Freisetzung in die Umwelt zu einem inaktiven Fragment und einem abbaubaren Linker hydrolysiert.

In Publikation 1 werden Herausforderungen und Möglichkeiten für die Integration von BbD entlang des DMTA-Zyklus beschrieben. Eine große derzeitige Herausforderung für die Berücksichtigung von BbD während der Wirkstoffentwicklung stellt die limitierte Verfügbarkeit von in *silico* Modellen und *in vitro* Hochdurchsatz-Screenings dar, die für pharmazeutische Wirkstoffe geeignet sind. Daher ist das Aufzeigen bestehender, beziehungsweise das Schaffen neuer Anreize fundamental, um die Entwicklung geeigneter Methoden voranzubringen. Denkbare Anreize sind die aktuellen politischen Entwicklungen unter dem Europäischen *Green Deal*, der bestehende gesellschaftliche Auftrag mit den Zielen für eine nachhaltige Entwicklung und gesetzlich regulierte finanzielle Anreize (siehe Abschnitt 1.1), wie beispielsweise eine längere Patentlaufzeit für grünere Wirkstoffe. Wenn Unternehmen erkennen, dass die Entdeckung von biologisch abbaubaren Wirkstoffen eine Geschäftsmöglichkeit ist, wächst die Bereitschaft, Veränderungen vorzunehmen und die Herausforderungen anzugehen. Die Sichtweise von Wirkstoffentwickler:innen zu geeigneten Anreizen wurde im Rahmen des zweiten Arbeitsschwerpunkts untersucht.

3.1.3 Schlussfolgerung

Das erlangte Verständnis der Machbarkeit von BbD ist entscheidend. Es dient der Entkräftigung der von Interessenvertreter:innen geäußerten Bedenken. Das aufgezeigte Potential von BbD stimuliert Investitionen in zukünftige Forschung im Bereich von BbD. Die Erkenntnisse aus Publikation 1 zur Anwendbarkeit von BbD in der Wirkstoffentwicklung unterstützen die pharmazeutische Industrie dabei, BbD innerhalb der eigenen Firmenstrukturen zu testen, und sich dadurch auf den Weg der grünen Transformation zu machen. Dies dient der Vorbereitung auf gesetzliche Neuregelungen, die aufgrund oben genannter EU-Strategien zu erwarten sind. Eine erfolgreiche Umsetzung von BbD hängt von der Verfügbarkeit geeigneter *in silico* und *in vitro* Methoden sowie von Anreizen für die entsprechende Forschung und Entwicklung ab. Ein Wissensaustausch und eine konstruktive Zusammenarbeit zwischen pharmazeutischen Unternehmen, der Wissenschaft und Behörden ist zur weiteren Untersuchung der Anwendbarkeit von BbD und damit zur tatsächlichen industriellen Anwendung unerlässlich und wurde in P2 adressiert.

3.2 Die Sichtweise von Expert:innen aus der pharmazeutischen Wirkstoffentwicklung

3.2.1 Problemstellung und Methode

Für die Untersuchung der Machbarkeit, umweltfreundlichere Wirkstoffe zu entwickeln, bedarf es der sorgfältigen Berücksichtigung der Prozessstrukturen in der pharmazeutischen Forschung und Entwicklung (FuE) und der Sichtweise von Wirkstoffentwickler:innen auf die Implementierung umweltrelevanter Parameter in den FuE-Prozess. Allerdings wurde laut dem Deloitte-Bericht "*Options for a strategic approach to pharmaceuticals in the environment*" die Machbarkeit der Entwicklung umweltfreundlicherer Substanzen von der Industrie selbst noch nicht in nennenswertem Umfang erforscht (Deloitte et al., 2018). Die Studie von De Soete et al. (2017) zur Bewertung der ökologischen Nachhaltigkeit pharmazeutischer Produkte führte zwar Interviews und Umfragen mit Interessenvertreter:innen des Gesundheitssektors inklusive Industrievertreter:innen durch, lieferte allerdings keine Erkenntnisse zur Machbarkeit von BbD, sondern zur umweltfreundlicheren Herstellung von Pharmazeutika. Danach werden Prinzipien der Grünen Chemie für die Herstellung bereits berücksichtigt (De Soete et al., 2017). Die Machbarkeit, umweltfreundlichere Wirkstoffe zu entwickeln, wurde bis zu den Aktivitäten des zweiten Arbeitsschwerpunkts noch nicht unter der Berücksichtigung der Perspektive von Wirkstoffentwickler:innen untersucht.

Aufgrund dieser Forschungslücke war das Ziel der Publikation 2, die Machbarkeit der Entwicklung umweltfreundlicherer Wirkstoffe auf der Grundlage von Erkenntnissen aus Interviews mit Expert:innen aus der industriellen Wirkstoffentwicklung (im Folgenden FuE-Expert:innen) zu bewerten. Die Machbarkeitsbewertung beinhaltete auch die Identifizierung von Bedürfnissen der Industrie und Anreizen zur Prozessänderung. Zu diesem Zweck wurden FuE-Expert:innen aus weltweit forschenden Pharmaunternehmen anhand eines strukturierten Fragebogens (Anhang von P2) angehört. Zehn Unternehmen wurden dafür kontaktiert. Insgesamt zwanzig FuE-Expert:innen aus sieben Unternehmen waren zu einem Online-Interview bereit, darunter mindestens jeweils eine medizinische Chemiker:in pro Unternehmen. Die FuE-Expert:innen wurden von den Umweltexpert:innen ihres eigenen Unternehmens begleitet. In dem Interview wurden fünf Fragen zum FuE-Prozess gestellt, um Einblicke in den Prozess zu gewinnen, welche über Informationen aus der Literatur hinausgingen. Weitere fünf Fragen des Fragebogens richteten sich an die Berücksichtigung von Umweltparametern hinsichtlich der Exposition und Wirkung von Wirkstoffen in der Umwelt (vgl. Einleitung von P2): i) keine oder verringerte Umweltexposition, ii) (Bio)Abbaubarkeit in der Umwelt, iii) keine schädlichen oder weniger schädliche Effekte auf Umweltorganismen und iv) keine oder weniger unerwünschte funktionelle Gruppen (z. B. per- und polyfluorierte Alkylgruppen).

Die Interviewstudie folgte einem qualitativen Forschungsansatz mit dem Vorteil diversere Antworten zu ermöglichen und flexibel auf Antworten reagieren zu können. Folglich waren die in den Interviews gewonnenen Informationen überwiegend von qualitativer Natur. Für die durchgeführte qualitative Inhaltsanalyse wurden die Antworten nach Themenblöcken kategorisiert, um sie anschließend zusammenfassen und klassifizieren zu können. Die Analyse folgte einerseits bekannten theoretischen Konzepten (deduktiv; Bsp.: Theorien zu Wirkstoffentwicklungsprozessen, das BbD-Konzept) und verarbeitete andererseits erhobene empirische Daten (z. B. Meinungen hinsichtlich der Aufnahme von Umweltparametern), um weiteres theoretisches Wissen zu erlangen (induktiv). Letzteres diente der Identifizierung von Gemeinsamkeiten und Unterschieden zwischen den Befragten, beispielsweise im Zusammenhang mit der Anwendbarkeit von BbD während der Wirkstoffentwicklung. Die Analyse war somit abduktiv (Thompson, 2022). Die Angaben der Befragten wurden anonymisiert.

3.2.2 Ergebnisse

Die Befragten aller Unternehmen bestätigten, dass ihr interner FuE-Prozess dem in der Literatur beschriebenen Schema in den Grundsätzen gleicht (von Target Identifizierung bis zu klinischen Studien, vgl. **Abbildung 3**, bzw. Abbildung 1, P2). Aufgrund der Gegebenheiten, die spezifisch für das jeweilige FuE-Projekt sind, kommt es zu Anpassungen des FuE-Prozesses. Des Weiteren betonten alle Befragten, dass der FuE-Prozess in der Regel nicht linear verläuft, sondern aufgrund des erheblichen Erkenntnisgewinns sehr flexibel ist. Insbesondere die Multiparameter Optimierung mit dem DMTA-Zyklus ist sehr iterativ und dynamisch. Diese Flexibilität ermöglicht es, den Prozess zu ändern, indem weitere Entscheidungspunkte, "Stopper" oder neue Parameter hinzugefügt werden, um Probleme aus vorangegangenen Iterationen frühzeitiger zu identifizieren. In den Interviews wurden Parameter beschrieben, die standardmäßig Teil der Screening-Kaskaden im FuE-Prozess sind (z. B. Potenz, physikochemische Parameter, chemische Stabilität, Plasmastabilität, ADME und sicherheitsbezogene Parameter) und solche genannt, welche spezifisch für das jeweilige FuE-Projekt hinzukommen (Abschnitt 3.1.2, P2).

Die Befragten beantworteten die Fragen zu Umweltaspekten in der Regel sehr umfassend und zeigten sich aufgeschlossen und interessiert an dem Design umweltfreundlicherer Wirkstoffe. Einige von ihnen betonten eine Selbstmotivation des Unternehmens, zu diesem Lösungsansatz für PiE beizutragen. Andere zeigten sich auch auf persönlicher Ebene intrinsisch motiviert mitzuwirken. Diese Einstellung der Befragten führte zu lebhaften und erkenntnisreichen Diskussionen – sogar zwischen Expert:innen desselben Unternehmens, z. B. medizinische Chemiker:innen und Umweltexpert:innen, die dadurch teilweise erstmals in einen Austausch gekommen sind.

Laut den Befragten spielt das Verhalten von Wirkstoffrückständen nach ihrem Eintritt in die Umwelt gegenwärtig keine Rolle in der Entwicklung. Sie haben aber sogenannte *Win-Win*-Situationen bei der Diskussion der Standardparameter und ihrer Bedeutung für die Umwelt identifiziert. Es handelt sich um Wirkstoffeigenschaften, die für die therapeutische Anwendung benötigt werden und, wenn auch

unbeabsichtigt, zur Verringerung von Umweltrisiken beitragen können (Anhang von P2). Beispiele sind eine hohe Target Selektivität und geringe Lipophilie. Die medizinischen Chemiker:innen betonten, dass sie sich um Moleküle geringerer Lipophilie bemühen, um das Risiko für Nebenwirkungen zu minimieren. Eine schwache Lipophilie sei aufgrund des verringerten Potentials zur Bioakkumulation auch von Vorteil für die Umwelt. Bei der Diskussion des Standardparameters ‚Stabilität' verhielt es sich differenzierter als bei den *Win-Win*-Situationen. Die Befragten gaben an, dass die Ergebnisse experimenteller Stabilitätstests während der Optimierung von Leitstrukturen zum Ausschluss von Strukturen führen können, beispielsweise bei einer Instabilität unter atmosphärischen, feuchten oder oxidativen Bedingungen. Wichtig sei eine gewisse Lagerstabilität. Die Halbwertszeit im Blut müsse ebenfalls ausreichend lang sein, damit das Medikament beispielsweise nicht häufiger als ein- oder zweimal am Tag eingenommen werden müsse.

Auf die allgemeine Frage, ob Umweltaspekte neben anderen Kriterien im FuE-Prozess zukünftig berücksichtigt werden könnten, tendierten die Befragten in sechs der sieben Interviews dazu, dass eine Aufnahme potentiell möglich sei, wenn entsprechende Notwendigkeiten erfüllt würden. Umweltkriterien müssten z. B. klar definiert sein und sich auf reale Umweltbedingungen übertragen lassen. Die Berücksichtigung von Umweltaspekten würde maßgeblich von der Verfügbarkeit geeigneter *in silico* Modelle und Hochdurchsatz-Screening Methoden zur Bewertung der umweltrelevanten Kriterien abhängen. Wie bereits in P1 herausgearbeitet, müssten Tests am Anfang des FuE-Prozesses zeit- und kostensparend sein (siehe oben, Abschnitt 3.1.2). Tests in späteren Phasen könnten allerdings umfangreicher und komplexer sein. Reproduzierbarkeit, Genauigkeit, Transparenz und Robustheit sind weitere wichtige Schlüsselmerkmale für *in vitro* Methoden. Die Befragten betonten das große Potential von *in silico* Modellen, wobei es auf die Anwendungsdomäne und eine gute Vorhersagekraft ankommt. Bei stetigem Datengewinn könnten Modelle aber verbessert werden, sodass es lediglich eine Frage der Zeit sei, bis sie ausreichend gut sind. Interesse an einer zukünftigen Modellentwicklung auch im Rahmen einer Kooperation wurde mehrfach bekundet (P2, Abschnitt 3.2.4).

Die befragten Expert:innen sahen vielfältige Möglichkeiten zur Berücksichtigung und Verbesserung des Umweltverhaltens von Wirkstoffen entlang des gesamten FuE-Prozesses, von der Target Selektion bis hin zur Kandidaten Selektion (Abschnitt 3.2.2.). Der Selektion und Optimierung von Hits und Leitstrukturen wurde eine besondere Bedeutung für BbD beigemessen, da hier die Auswahl von Substanzen und weitere Modifizierungen direkt anhand von umweltrelevanten Daten beeinflusst werden können. Der Grund ist, dass in diesen Phasen die Verflechtungen der zahlreichen Moleküleigenschaften inklusive der (Bio)Abbaubarkeit und Umwelttoxizität zum Tragen kommen, welche nach ihrer Optimierung gewichtet werden müssen. Mit der Selektion des Wirkstoffkandidaten für die weitere Produktentwicklung steht die Struktur des Wirkstoffs fest, sodass keine weiteren Änderungen der intrinsischen Umwelteigenschaften möglich sind.

Anreize zur Förderung der Entwicklung umweltfreundlicherer Wirkstoffe wurden von den Befragten als potenziell hilfreich bei der Entwicklung von umweltfreundlicheren Wirkstoffen angesehen. Die

Befragten schlugen starke und weiche Anreize vor, darunter regulatorische und finanzielle Anreize, beispielsweise eine verlängerte Patentlaufzeit, sowie Bewusstseins- und Wissensbildung (Abschnitt 3.2.5 in P2).

3.2.3 Diskussion

Das Potenzial für eine Prozessänderung durch die Einführung neuartiger Kriterien kann auf andere forschende Pharmaunternehmen übertragen werden, da die FuE-Prozesse und Prinzipien der befragten Unternehmen grundsätzlich miteinander und mit verfügbarer Literatur (Blass, 2015) vergleichbar sind. Zu Generikaherstellern und auch kleinen Start-up Unternehmen können keine Aussagen getroffen werden.

Die Erkenntnis, dass der pharmazeutische FuE-Prozess sehr flexibel ist, stimmt mit Informationen aus der Literatur überein. Berggren et al. (2018) haben beschrieben, dass die Unternehmen versuchen, sehr agil zu handeln, um die Produktivität im FuE-Prozess zu verbessern, indem sie laut Gyurjyan et al. (2017) zum Beispiel einen agilen, iterativen Test-und-Lern Ansatz anwenden. Die FuE-Kultur in pharmazeutischen Unternehmen ist daher mit hochdynamischen Prozessen und ständigen Veränderungen vertraut. Die Prozessflexibilität und die flexible FuE-Kultur zeigen, dass keine prozessualen Hindernisse für die Einführung neuartiger Kriterien in den Entscheidungsprozess während pharmazeutischer FuE bestehen.

Die Befragten sahen zahlreiche Möglichkeiten für die Berücksichtigung von Umweltparametern in verschiedenen Phasen des FuE-Prozesses, beispielsweise die Einbindung solcher während der Multiparameter Optimierung. Die Begründung liegt in der hohen Flexibilität des FuE-Prozesses und der Vielzahl an Parametern zur Untersuchung und Optimierung. Ihre Meinungen gingen auseinander, wenn es darum ging, ob die Umweltkriterien früher oder später in die Phasen bis zur Kandidaten Selektion einzubeziehen sind. Es hat sich gezeigt, dass die Verfügbarkeit geeigneter *in silico* Modelle und *in vitro* Tests zu Umweltparametern ausschlaggebend für die Machbarkeit von Umweltberücksichtigungen ist. Die Testeigenschaften, z. B. hinsichtlich des Durchsatzes und der Kosten, würden entscheiden, in welcher Prozessphase sie erfolgreich eingesetzt werden könnten. Derzeitig verfügbare Methoden werden in P2, Abschnitt 4.3 diskutiert.

Nach Ansicht zahlreicher Befragter könnten einige Umwelteigenschaften mit standardmäßig erwünschten Wirkstoffeigenschaften Hand in Hand gehen, was zu potenziellen *Win-Win*-Situationen führen würde. Beispielsweise geht der aktuelle Trend bei der Entwicklung von Wirkstoffen in Richtung schwacher Lipophilie, sodass nur sehr wenige Wirkstoffe bioakkumulative Eigenschaften aufweisen dürften. Allerdings würden dann im Umkehrschluss hydrophilere Wirkstoffe entwickelt, welche in der aquatischen Umwelt mobil sein könnten. Verbunden mit einer möglichen (Pseudo-)Persistenz in der Umwelt würde dies folglich eher ein Problem für die Zukunft darstellen (Hale et al., 2020). Ein weiteres Beispiel aus den Interviews für eine *Win-Win*-Situation war die hohe Target Selektivität. Für Wirkstoffe, die im Körper hochselektiv an das Target binden und keine bekannten Nebenwirkungen hervorrufen,

werden Risiken für Effekte auf Umweltorganismen ebenfalls geringer eingeschätzt. Effekte auf Umweltorganismen, die auf dem für die Anwendung erforderlichen Wirkmechanismus beruhen, würden durch hohe Selektivität allerdings nicht vermieden werden, wenn aufgrund der evolutionsbedingten Konservierung von Targets diese auch in Umweltorganismen vorkommen. Die Datenbank Ecodrug.com ermöglicht orthologische Vorhersagen von Targets im menschlichen Körper und in Umweltorganismen (Verbruggen et al., 2018). Die Optionen zur Minderung des Risikos solcher Umwelteffekte sind weitgehend darauf beschränkt, die Umweltexposition zu verringern, z. B. durch leichte und vollständige Mineralisierung in der Umwelt nach dem BbD-Konzept.

Die weitere Diskussion zur Machbarkeit der einzelnen Umweltkriterien (3.2.3., P2) hat gezeigt, dass ihre genaue Bedeutung von den FuE-Expert:innen teilweise noch nicht verstanden ist. Es ist beispielsweise unklar, welche funktionellen Gruppen aus Umweltgründen unerwünscht sein könnten oder wie eine vollständige Mineralisierung in der Umwelt mit dem benötigten Maß an Stabilität unter der Berücksichtigung variierender Umgebungsbedingungen vereint werden könnte. Klar ist: Befragte benötigen eindeutig definierte Kriterien zu relevanten, mess- und optimierbaren Parametern für umweltfreundlichere Wirkstoffe, ob bezüglich Ökotoxizität oder zum Verbleib in der Umwelt. Dafür bedarf es einer interdisziplinären Zusammenarbeit zur Diskussion geeigneter Kriterien. Das GREENER Konzept von Moermond et al. (2022) stellt einen guten Ausgangspunkt für weitere, Akteurs übergreifende Diskussionen dar, beispielsweise zur Definition geeigneter Kriterien an die Bioabbaubarkeit in der Umwelt unter der Berücksichtigung des Eintragsvolumens und möglicher Umwelteffekte.

Basierend auf den identifizierten Bedürfnissen wurde eine wegweisende Übersicht über eine Abfolge an Aktivitäten erstellt, um die Transformation zu umweltfreundlicheren Wirkstoffen zu unterstützen (**Abbildung 4**, nach Abbildung 4, P2). Die vorgeschlagenen Aktivitäten sind: i) die Entwicklung neuer Ansätze wie z. B. zu BbD, ii) Datenerhebung, iii) Entwicklung eines Methodenpakets und iv) laufende Aufgaben. Insbesondere für Schritt i) und ii) ist die Zusammenarbeit zwischen der pharmazeutischen Industrie, verwandte Industrien (z. B. die Kosmetik- oder Pflanzenschutzmittelindustrie), der Wissenschaft, Umweltbehörden und Interessengruppen unerlässlich. Die Aktivitäten zur Transformation würden von Leitlinien und Vorschriften profitieren.

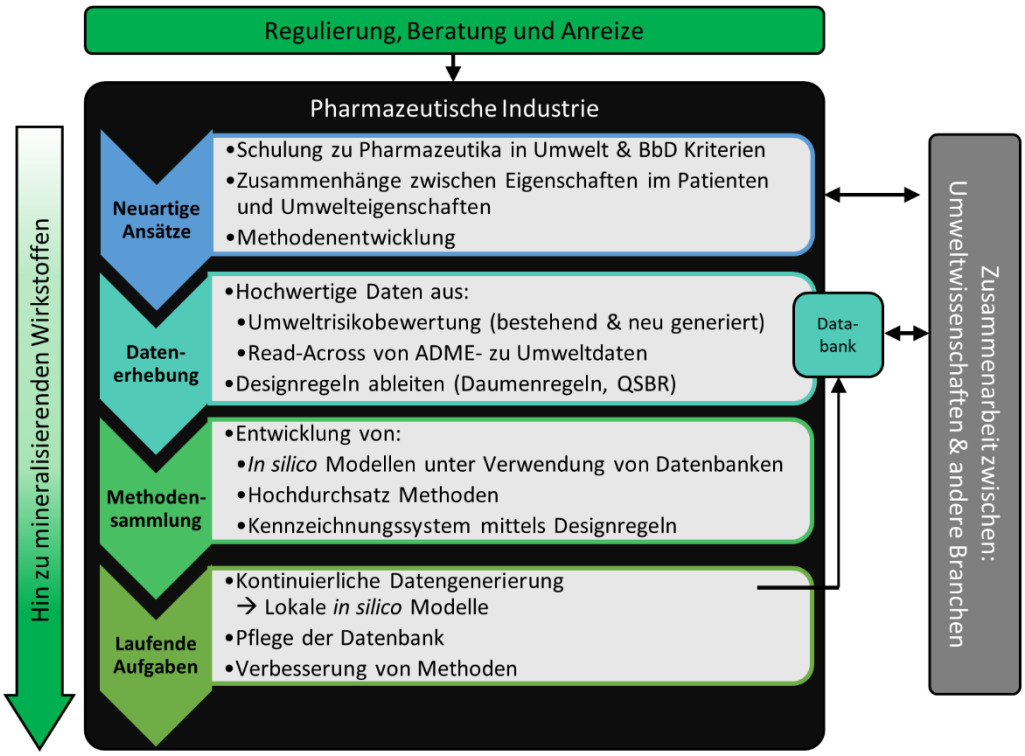

Abbildung 4: *Vorschlag für einen Weg zur Gestaltung umweltfreundlicherer Wirkstoffe (nach Abbildung 4, P2). BbD: Benign by Design, ADME: Absorption-Distribution-Metabolismus-Elimination, QSBR: Quantitative Struktur-Bioabbau-Beziehung.*

3.2.4 Schlussfolgerung

Mit dieser Interviewstudie ist es gelungen, Möglichkeiten, Bedürfnisse und Anreize für die Entwicklung umweltfreundlicherer Wirkstoffe aus Sicht von forschenden Pharmaunternehmen zu identifizieren und zu charakterisieren. Die Ergebnisse zeigen, dass die Berücksichtigung von Umweltaspekten während des pharmazeutischen FuE-Prozesses von der Hit Selektion bis zu den späten Phasen der Optimierung möglich ist. Herausforderungen für die tatsächliche Einbeziehung von Umweltaspekten bestehen allerdings. Am kritischsten sind die Herausforderungen im Zusammenhang mit dem derzeitigen Wissensstand und der Verfügbarkeit von geeigneten *in silico* Modellen und *in vitro* Tests zur Bewertung des Umweltverhaltens. Eine konstruktive Zusammenarbeit zwischen Pharma-unternehmen, Behörden und der Wissenschaft, inklusive des Austausches zwischen FuE- und Umweltexpert:innen innerhalb eines Unternehmens, könnte die industrielle Anwendung des Konzepts zum Design grünerer Wirkstoffe vorantreiben. Finanzielle, regulatorische und soziale Anreize würden die künftige Entwicklung umweltfreundlicherer Wirkstoffe unterstützen. In zukünftiger Forschung sollte der Anwendungsbereich für die Entwicklung umweltfreundlicherer Stoffe auf pharmazeutische Produkte ausgeweitet werden, beispielsweise um auch Hilfsstoffe zu beachten. Außerdem sollten weitere Prinzipien der grünen Chemie angewendet werden, um z. B. Treibhausgasemissionen bei der Herstellung zu senken.

4 Fallstudie: Das Re-Design von Sulfonamid-Antibiotika

4.1 Literaturdaten zu SUA-TP und ihr Nutzen für *Benign by Design*

4.1.1 Problemstellung und Methode

Sulfonamid-Antibiotika (SUA), ihre Metabolite und Transformationsprodukte (TP) tragen zur Umweltverschmutzung mit möglichen negativen Folgen für die planetare Gesundheit bei. Die Entstehung und Selektion von antibiotikaresistenten Keimen ist von großer Besorgnis (Baran et al., 2011; Christou et al., 2018; De Soete et al., 2017; Ezzariai et al., 2018; Felis et al., 2020). Die Berücksichtigung von SUA-TP hinsichtlich ihres Vorkommens und ihrer Umwelteigenschaften, wie die biologische Abbaubarkeit und Ökotoxizität, ist wichtig, um z. B. eine umfassende Bewertung der Umweltrisiken von SUA zu ermöglichen (Fatta-Kassinos et al., 2011; Haddad et al., 2015; Klavarioti et al., 2009; Kümmerer et al., 2019a). Dies wird beispielsweise durch die nachgewiesene Rücktransformation von *N4*-Acetyl-Sulfamethoxazol zu Sulfamethoxazol (SMX) deutlich (Radke et al., 2009). *N4*-Acetyl-SMX wird nicht nur in der Umwelt gebildet (Reis et al., 2018), sondern stellt auch ein Hauptmetabolit von SMX dar (Radke et al., 2009). *N4*-Acetyl-SMX-Konzentrationen in Abwässern von Kläranlagen können bis zu 50 % der SMX-Konzentrationen betragen (García-Galán et al., 2012b; Göbel et al., 2004). Somit könnte die Rückumwandlung von *N4*-Acetyl-SMX in Sedimenten eine kontinuierliche Quelle für SMX stromabwärts von Kläranlagenabwässern sein (Radke et al., 2009). Obwohl die Wichtigkeit des Verständnisses über TP bereits seit mehr als einem Jahrzehnt bewusst ist (Fatta-Kassinos et al., 2011), wurden Eigenschaften von TP kaum in Übersichtsarbeiten über SUA thematisiert (Charuaud et al., 2019; Ezzariai et al., 2018; Spielmeyer, 2018; Tian et al., 2020; Wang und Wang, 2018; Wohde et al., 2016). Informationen zu SUA-TP beschränkten sich in diesen Übersichtsarbeiten lediglich auf Annahmen oder generelle Aussagen, beispielsweise dass TP mit intaktem Pharmakophor stets noch antibakteriell aktiv sein könnten (Charuaud et al., 2019).

Daher war das Ziel der Publikation 3, den aktuellen Wissensstand über SUA-TP in einer Übersichtsarbeit zu erörtern, einschließlich der Forschungslücken und der Gemeinsamkeiten von SUA-TP und TP im Allgemeinen. Im Kontext der Fallstudie zum Re-Design von SUA diente P3 der Datengewinnung zur Abbaubarkeit und Bioaktivität, da TP aufgrund ihrer Strukturähnlichkeit zur Ausgangssubstanz möglicherweise eine ähnliche pharmakologische Wirkung bei verbesserter biologischer Abbaubarkeit zeigen könnten.

Die Literatur über SUA-TP wurde systematisch konsultiert, um Daten über das Vorkommen in Kläranlagen und der Umwelt, die physikochemischen Eigenschaften, Abbaubarkeit (biotisch und abiotisch) und (Öko-)Toxizität zu sammeln. 14 SUA wurden für die Literaturrecherche ausgewählt:

Sulfamethoxazol, Sulfachloropyridazin, Sulfadimethoxin, Sulfadiazin, Sulfaguanidin, Sulfisoxazol, Sulfamethoxypyridazin, Sulfamerazin, Sulfamethizol, Sulfamethazin, Sulfapyridin, Sulfasalazin und Sulfathiazol. Die Methode bestand aus einer systematischen Publikationssuche unter Verwendung der Datenbanken *Web of Science* und *SciFinder* und dessen Selektion anhand von definierten Selektionskriterien, gefolgt von der Erstellung der Datensätze zum Vorkommen und den ausgewählten Eigenschaften von SUA-TP sowie der Auswertung dieser Daten (Abschnitte 2.1 - 2.3, P3). Wichtig bei der Erstellung der Datensätze war die Festlegung von Kriterien zur Gruppierung der Daten aus unterschiedlichsten Studienkonzepten. Bei der Datenanalyse wurden umweltrelevante Aspekte herausgearbeitet, die für TP im Allgemeinen gelten, um beim künftigen Umgang mit TP als komplexe Stoffklasse zu unterstützen. Daten zu Sulfamethoxazol wurden detaillierter analysiert, um Erkenntnisse auf molekularer Ebene zu diskutieren. Darüber hinaus wurden Daten zur Abbaubarkeit und Bioaktivität von TP gesammelt, die für die BbD Fallstudie von SUA hilfreich sein könnten.

4.1.2 Ergebnisse

Die systematische Literaturrecherche resultierte in 607 SUA-TP aus 222 Publikationen. Die Charakterisierung der Publikationssammlung, ausführlich beschrieben in P3 im Abschnitt 3.1, zeigte beispielsweise, dass Sulfamethoxazol am häufigsten Gegenstand der Studie war. Unterschieden nach Studienarten (unterschiedliche Arten von Laborversuchen und Monitoring), überwog der Anteil an Studien zu erweiterten Methoden der Wasserbehandlung (abiotisch, drastische Bedingungen). Die Charakterisierung der Publikationssammlung war für eine bessere Diskussion der TP-Daten vonnöten.

Im Abschnitt 3.2 von P3 wurden die Datensätze zu den mehr als 600 Strukturen vorgestellt und diskutiert. Es zeigte sich beispielsweise, dass die meisten Strukturvorschläge auf der Tandem-Massenspektrometrie (MS/MS) basieren. Nur wenige Strukturen wurden mithilfe der Kernspin-resonanzspektroskopie aufgeklärt. Einer der Hauptgründe ist der Fokus auf Studien zur Elimination der Ausgangssubstanz mit TP als Nebenergebnis.

Daten zum Vorkommen, zu physikochemischen Eigenschaften, zum Abbau und zur (Öko-)Toxizität wurden nur für einen geringeren Teil dieser TP gefunden (4 %, 4 %, 31 % bzw. 35 %). Zudem stammten die Daten häufig aus Mischungstests mit geschwächter Aussagekraft für die Einzelsubstanzen. Beispielsweise blieb die Mineralisierungsrate der zahlreichen TP, die in Mischungstests vollständig eliminiert wurden, unbekannt, denn eine weitere Umwandlung in persistente TP konnte nicht ausgeschlossen werden. Standardisierte Tests zur biologischen Abbaubarkeit einzelner TP würden deren Mineralisierungsrate überwachen, fehlten aber fast vollständig. Mögliche Gründe sind die stark limitierte käufliche Verfügbarkeit von TP, aber auch der wissenschaftliche Fokus auf der Eliminierung der Ausgangssubstanz durch abiotische Wasseraufbereitung. Die mangelnde Vergleichbarkeit der diversen Testbedingungen erschwerte es, Aussagen hinsichtlich der Eigenschaften von TP zu treffen.

Die Daten zu den TP des Hauptvertreters Sulfamethoxazol ähnelten dem gesamten Datensatz aller SUA-TP in Bezug auf die Qualität und Quantität und wurden im Abschnitt 3.3 von P3 im Detail beschrieben.

Typische Merkmale von SUA-TP wurden identifiziert und die TP in SUA-unspezifische und SUA-spezifische TP unterteilt (Tabelle 7, P3). SUA-unspezifische TP sind solche, die für alle SUA identisch sind, z. B. Sulfanilamid. SUA-spezifische TP sind TP mit unterschiedlichen SUA-spezifischen Resten (R), aber vergleichbar transformiertem SUA-Rückgrat, z. B. desulfonierte SUA. Sulfanilamid und Sulfanilsäure wurden für zahlreiche Ausgangssubstanzen in der Literatur beschrieben. Tabelle 7 aus P3 zeigt an, welche TP für die meisten SUA in der Literatur beschrieben wurden, darunter beispielsweise Sulfanilamid, desulfonierte SUA und hydroxylierte SUA (OH-SUA). In Laborstudien kommt es häufig zur Hydroxylierung durch reaktive Hydroxyradikale, welche in wässrigen Lösungen unter verschiedensten Bedingungen entstehen können. Tabelle 7 aus P3 unterstützt die Identifizierung von zu erwartenden TP basierend auf typischen Transformationsmechanismen. Darunter ist beispielsweise das desulfonierte SMP als das Produkt der für sechsgliedrige SUA typischen Smiles-Umlagerung (Boreen et al., 2005), das bisher aber noch nicht in der Literatur beschrieben wurde. Alle beobachteten Bindungsspaltungen sind in **Abbildung 5** markiert.

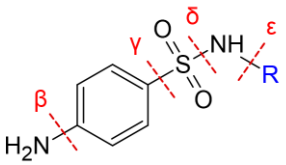

Abbildung 5: *Bindungsspaltungen bei Sulfonamid-Antibiotika (nach Boreen et al., 2004). Typische Transformationsprodukte sind Desamino-SUA (β-Spaltung), Anilin (γ-Spaltung), Sulfanilsäure und das Amin des spezifischen Rests (δ-Spaltung) sowie Sulfanilamid (ε-Spaltung).*

4.1.3 Diskussion

In P3 wurde diskutiert, wie das Problem der großen Datenlücken gelöst werden kann. Beispielsweise müssen zwingend qualitativ hochwertige Daten generiert und zusammengetragen werden, um benötigte *in silico* Modelle zu entwickeln bzw. bestehende Modelle zu erweitern. Die Begründung liegt in der limitierten Anwendung aktueller Modelle für Wirkstoffe, da die Modelle auf Daten zu klassischen Chemikalien basieren. Die große strukturelle Vielfalt von Wirkstoffen wird somit nicht ausreichend abgedeckt. Außerdem liegen Wirkstoffe bei neutralem pH im Gegensatz zu Bulkchemikalien meist als Ionen vor (Lorenz et al., 2021). Der Effekt gesteuerte Ansatz ist eine weitere Möglichkeit, um Strukturen für eine genauere Datenerhebung nach geeigneten, standardisierten Methoden zu priorisieren (Escher und Fenner, 2011; Hensen et al., 2020; Umweltbundesamt, 2019). Die limitierte käufliche Verfügbarkeit bzw. kostspielige Synthese von TP bleibt dabei allerdings eine entscheidende Hürde, und unterstreicht nochmals die Bedeutung von einer computerbasierten Vorhersage.

Eine Möglichkeit zur Überwindung dieser Hürden, d. h. der nicht käuflichen TP und der Grenzen von *in silico* Modellen, wäre die schnelle und einfache Erzeugung von TP beispielsweise mittels Photolyse mit anschließender Untersuchung der TP-Mischungen nach einer standardisierten Methode, z. B. von der Organisation für wirtschaftliche Zusammenarbeit und Entwicklung (OECD). Testergebnisse aus standardisierten Methoden haben u. a. den Vorteil der besseren Vergleichbarkeit, selbst wenn sie aus unterschiedlichen Studien stammen, sodass studienübergreifend und dadurch ressourcensparend mit den Daten gearbeitet werden kann. Dieser Ansatz zur Generierung (mittels Photolyse) und Untersuchung von TP-Mischungen wurde bereits von Rastogi et al. (2014, 2015a, 2015b) am Beispiel der Betablocker verfolgt. Untersucht wurden die leichte Bioabbaubarkeit im Wasser (nach OECD 301D und 301F) und weitere pharmazeutisch relevante Parameter. Kurz darauf wurde der Ansatz von Su et al. (2016) am Beispiel von Sulfamethoxazol genutzt. Untersucht wurde die Bioabbaubarkeit von Photo-TP in einem Wasser-Sediment System (nach OECD 308).

Die Auswahl der Methoden aufgrund geeigneter Endpunkte bleibt eine weitere Herausforderung, da beispielsweise die Ökotoxizität durch viele Endpunkte beschrieben werden kann. Ökotoxizitätsstudien zur Ausgangssubstanz oder möglichen TP werden v. a. dann von Bedeutung für BbD, wenn eine vollständige Abbaubarkeit nicht erzielt werden kann. Der Ökotoxizitätsstandard im Rahmen der Umweltrisikobewertung von Wirkstoffen ist die Prüfung der chronischen Auswirkungen auf Algen, Daphnien und Fische (EMA, 2024; Europäische Kommission, 2004). Als Ergebnis des Taxa-Sensitivitätsvergleichs zur Reduzierung von Tests an Wirbeltieren sollten sich die Tests von SUA als Antibiotikagruppe auf Algen und Cyanobakterien konzentrieren (Schwarz et al., 2021). Dies gilt gleichermaßen für solche SUA-TP, welche stets antibiotisch wirken. Insbesondere bei größeren strukturellen Unterschieden zur Ausgangssubstanz könnte sich die Taxa-Sensitivität aber auch verändert haben, sodass andere Ökotoxizitätstests relevant würden.

In Publikation 3 wurde eine Übersicht der Vielfalt an SUA-TP gegeben, und damit einhergehend große Wissenslücken identifiziert. Diese ergaben sich u. a. aufgrund genannter Herausforderungen bei der Erhebung von umweltrelevanten Daten. Die Ergebnisse verdeutlichen die Dringlichkeit, BbD als präventiven Ansatz zu etablieren. Die großen Wissenslücken spiegeln die sogenannten bekannten Unbekannten wieder (Bsp.: unbekannte Persistenz). Hinzukommen die sogenannten unbekannten Unbekannten (Bsp: unbekannter Effekt eines noch unbekannten Endpunkts). Erst seit dem Jahr 2002 gibt es eine international anerkannte, wissenschaftliche Definition von endokrinen Disruptoren (WHO, 2002) und seitdem ein wachsendes Bewusstsein für einen weiteren (öko)toxikologisch relevanten Endpunkt. Was kommt in den nächsten zwanzig Jahren?

4.1.4 Schlussfolgerung

SUA-TP müssten für eine umfassendere Bewertung möglicher Auswirkungen des Umwelteintrags von SUA und ihren Metaboliten berücksichtigt werden. Gründe sind mögliche Rücktransformation zur Ausgangssubstanz oder die Bildung von persistenten und ökotoxischen TP. Die Übersichtsarbeit identifizierte allerdings große Datenlücken. Es fehlen insbesondere hochwertige Daten nach anerkannten, standardisierten Methoden zum Verbleib und zu Effekten von SUA-TP in der Umwelt. Dies liegt u. a. an der geringen käuflichen Verfügbarkeit von TP bei gleichzeitig großer Vielzahl an möglicherweise umweltrelevanten TP. Letzteres erhöht die gesamte Vielzahl an möglichen Stoffen in der Umwelt und damit die Schwierigkeit, die Datenlage zu Stoffen in der Umwelt zu verbessern. Diese Problematik gilt für TP im Allgemeinen. Publikation 3 verdeutlicht die Bedeutung von Maßnahmen zur Verhinderung bzw. Reduzierung solcher komplexen Stoffgemische in der Umwelt, wie beispielsweise die Anwendung des BbD-Konzepts. In der Übersichtsarbeit gewonnene Erkenntnisse zu SUA-TP dienten dem Einstieg in eine weitere Datengenerierung im Rahmen dieser Arbeit für das Re-Design von SUA nach dem BbD-Konzept.

4.2 Experimentelle Analysen zur antibiotischen Aktivität und Bioabbaubarkeit generierter SUA Photo-TP – Implikationen für *Benign by Design*

4.2.1 Problemstellung und Methoden

SUA und ihre Metabolite können in die Umwelt gelangen und sich negativ auf die Umwelt und menschliche Gesundheit auswirken, beispielsweise aufgrund der Bildung und Selektion von SUA-resistenten Keimen (siehe Abschnitt 1.3). Um die Bildung von Antibiotikaresistenzen im Menschen oder der Umwelt zu verlangsamen, werden seit über 10 Jahren zahlreiche Strategien und Aktionspläne verfolgt, die u. a. auf einen umsichtigen Umgang mit Antibiotika abzielen (ECDC). Im Fall von SUA werden zur Vermeidung von Resistenzen Kombinationspräparate mit dem antibakteriellen Wirkstoff Trimethoprim verabreicht (siehe Abschnitt 1.3). Eine weitere wirksame Lösungsstrategie insbesondere für den unvermeidbaren Anteil an Antibiotika stellt *Benign by Design* bzw. *by Re-Design* dar (Kümmerer, 2007, 2010c; Lorenz et al., 2021): Ein neues umweltfreundlicheres SUA-Derivat würde die gewünschten pharmazeutischen Eigenschaften wie beispielsweise die Bindungsaffinität zur Dihydropteroat-Synthase aufweisen. Gleichzeitig würde dieses SUA-Derivat beim Eintrag in die Umwelt (bzw. Kläranlagen) vollständig mineralisieren und somit keine negativen Effekte auf Umweltorganismen, inklusive der SUA-Resistenzbildung v. a. in Kläranlagen als sogenannte *Hotspots* (Rizzo et al., 2013), verursachen. *Benign by Re-Design* von SUA wurde bisher allerdings noch nicht wissenschaftlich erforscht, geschweige denn von der pharmazeutischen Industrie untersucht (P2; Deloitte et al., 2018).

Literaturdaten zur (Bio)Abbaubarkeit und antibiotischen Aktivität von Transformationsprodukten von SUA (SUA-TP) mit intaktem Pharmakophor, aber leichter struktureller Veränderung könnten für BbD nützlich sein (Lorenz et al., 2021). In der Literatur wurden über mehr als 600 SUA-TP beschrieben (Publikation 3). Es konnte jedoch keine Verbindung identifiziert werden, die für BbD von Interesse wäre, hauptsächlich wegen fehlender Daten zur antibiotischen Aktivität und (Bio)Abbaubarkeit. Somit bestand Bedarf an weiteren Experimenten zur Gewinnung dieser Daten. Einige Hinweise aus der Literatur dienten dennoch als gute Ausgangspunkte für das Re-Design von SUA (siehe Einleitung, P4).

Das Hauptziel der Publikation 4 war es daher, die Anwendbarkeit von BbD auf SUA zu untersuchen. Dafür wurden TP von SUA mittels Photolyse generiert (Photo-TP) und hinsichtlich der BbD relevanten Eigenschaften (die antibiotische Aktivität und Bioabbaubarkeit) analysiert. Sechs SUA wurden mittels Ultraviolettstrahlung (UV-Strahlung) photolysiert: Sulfaquinoxalin-Na (SQX), Sulfisoxazol (SIX), Sulfamethoxypyridazin (SMP), Sulfamethoxazol (SMX), Sulfathiazol (STZ) und Sulfamethizol (SMT). Die erzeugten UV-Mischungen wurden mithilfe der Hochleistungsflüssigkeitschromatographie (HPLC)

gekoppelt mit einem UV-MS/MS Detektor analysiert. Mithilfe des Leuchtbakterientests (LBT, nach Menz et al., 2013) wurden sie auf Bioaktivität (akute und chronische Leucht- und Wachstumshemmung nach 30 min, 24 h bzw. 14 h) und mithilfe des manometrischen Respirationstests (MRT, OECD 301F) auf leichte Bioabbaubarkeit in der Umwelt untersucht. Bei dem 24-stündigen LBT gilt die Wachstumshemmung als Indikator für eine antibiotische Aktivität. Die Leuchthemmung ist lediglich ein erster Anhaltspunkt dafür, könnte aber auch auf andere, ökotoxische Effekte zurückzuführen sein. Neben den Untersuchungen der UV-Mischungen wurde die leichte biologische Abbaubarkeit von drei im Handel erhältlichen hydroxylierten Sulfanilamidderivaten getestet (MRT). Die verwendeten Testmethoden wurden ausführlich in Publikation 4 beschrieben.

4.2.2 Ergebnisse und Diskussion

Die Photolyse der sechs SUA führte zu UV-Mischungen mit je 8 - 15 detektierten TP pro Ausgangssubstanz, in Summe 66 SUA-TP (Tabelle 1, P4). Darunter waren einige TP, die aufgrund der Literaturdaten zu erwarten waren (Vgl. Tabelle 7, P3): die Arylamine als spezifischer Rest aller SUA (NH_2-R; δ-Bindungsspaltung siehe **Abbildung 5**), hydroxylierte SUA (OH-SQX, -SIX, -SMP, -SMX, -STZ, -SMT), desulfonierte SUA (Desulfo-SQX, -SMP, -SMX, -SMT), *p*-Nitroso-SUA (NO-SQX, NO-SMP), SUA-Isomere (drei SIX-Isomere und je ein SMP-, SMX- und STZ-Isomer). Die SIX-Isomere und das SMP-Isomer wurden bisher noch nicht in der Literatur beschrieben.

Ergebnisse und Diskussion des Leuchtbakterientests

Die LBT-Ergebnisse zeigten, dass die Lumineszenz des gramnegativen Bakteriums *Allivibrio fischeri* signifikant durch die UV-Mischungen gehemmt wurde. Die UV-Mischungen sind charakterisiert durch eine abnehmende SUA-Konzentration bei steigendem Anteil an SUA-TP entlang der andauernden Bestrahlungszeit (**Abbildung 6**). Der Vergleich mit der Dosis-Wirkung-Beziehung der reinen SUA-Substanz zeigte, dass in einigen Fällen der TP-Anteil, also nicht alleine der SUA-Rückstand, zur Hemmung beitrug (**Abbildung 6**, rot umrandete Balken). Der weitere Vergleich mit den TP-Kinetiken, d. h. den Konzentration-(bzw. Peakfläche-)Bestrahlungszeit Kurven, und Literaturdaten diente der Identifizierung solcher TP, die möglicherweise zur Hemmung beigetragen haben könnten (**Tabelle 2**). Darunter sind das SIX-Isomer und das STZ-Isomer. Letzteres könnte das in der Literatur beschriebene Haupt-Photo-TP Promizol sein, ein Antituberkulotikum (Spielmeyer et al., 2015), welches aber nach der Isomerisierung keine Sulfonamid-Gruppe mehr aufweist und daher als BbD-Kandidat in P4 ausgeschlossen wurde.

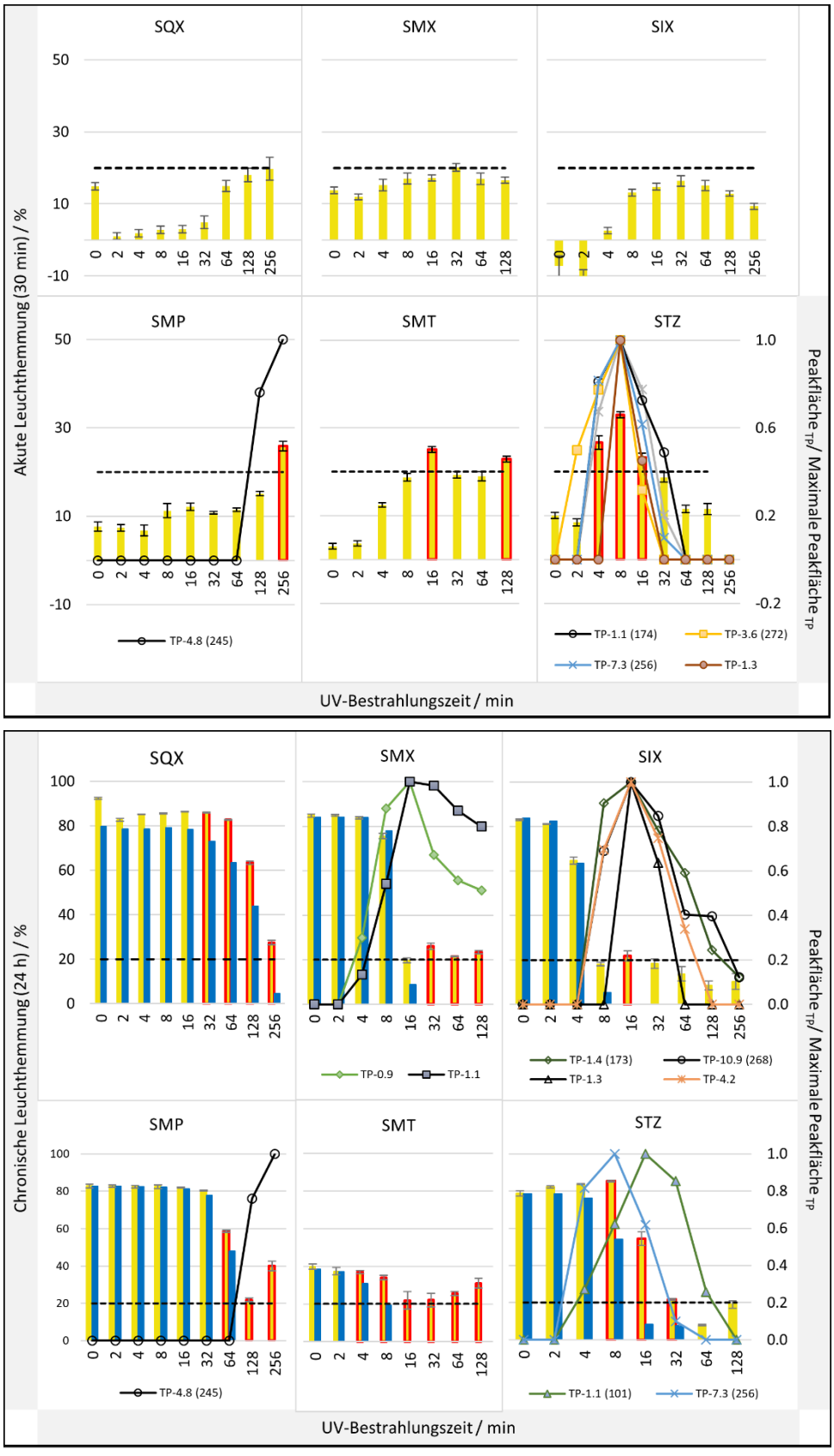

Abbildung 6: *Akute (oben) und chronische Leuchthemmung (unten) durch SUA-TP-Mischungen nach 0 bis 256 min UV-Bestrahlung (gelbe Balken) und Konzentration-Zeit Kurven von möglicherweise bioaktiven SUA-TP (aus P4).*
Rot umrandet: UV-Mischungen, bei denen die Leuchthemmung auch auf den TP-Anteil zurückgeführt werden kann.
Blaue Balken unten: Die theoretische Hemmung, die ausschließlich durch den SUA-Rückstand hervorgerufen würde, und dem Vergleich zur Entscheidung über bioaktive TP diente.
Gestrichelte Linie: Schwellenwert für signifikante Hemmung von 20 %.

Die signifikante Leuchthemmung durch die UV-Mischungen von SQX (akut) und SMT (akut und chronisch) konnte durch den Vergleich mit TP-Kinetiken keinen spezifischen TP zugeordnet werden. Dies unterstreicht die Herausforderungen der Interpretation von Testergebnissen komplexer Mischungen, u. a. aufgrund von möglichen Mischungseffekten (z. B. additiv oder synergistisch) und fehlender Informationen über die Zusammensetzung (z. B. hinsichtlich Konzentrationen und nicht detektierte TP).

Tabelle 2: *Transformationsprodukte (TP) nach Bestrahlung von Sulfonamid-Antibiotika (SUA), welche zur akuten und/oder chronischen Leuchthemmung beigetragen haben könnten.*

Mischung SUA-X mit X = Bestrahlungszeit / min	**Leucht-hemmung**	**Möglicherweise aktive TP** Bezeichnet nach Retentionszeit (und wenn vorhanden Masse-Ladungsverhältnis)	**Strukturvorschlag**
SIX-16	chronisch	1,4 (173), 10,9 (268), 1,3 und 4,2	Sulfanilamid SIX-Isomer keine Information
SMP-64-256	akut, chronisch	4,8 (245)	ohne Pharmakophor
SMX-32-128	chronisch	0,9 und 1,1 Aus der Literatur bekannte aktive SMX-TP wurden nicht detektiert	keine Information
STZ-8-128	akut, chronisch	1,1 (101), 1,1 (140), 1,1 (174), 3,6 (272), 7,3 (256), 1,3	ohne Pharmakophor, ohne Pharmakophor Sulfanilsäure OH-STZ STZ-Isomer keine Information

Im Gegensatz zur Leuchthemmung war die Wachstumshemmung der UV-Mischungen nicht signifikant. Eine Wachstumshemmung kann aber für die in den Mischungen enthaltenen TP nicht ausgeschlossen werden. Der Grund dafür ist, dass die TP in den UV-Mischungen in geringeren Konzentrationen als die Ausgangssubstanz vorkamen, da zahlreiche TP aus einer Substanz entstanden, und die höchste Konzentration der meisten Ausgangssubstanzen (25 $mg \cdot L^{-1}$) ebenfalls zu keiner signifikanten Wachstumshemmung führte.

Ergebnisse und Diskussion des manometrischen Respirationstests

Die Mineralisierungsraten der getesteten SUA lagen unter dem Schwellenwert für den Sauerstoffverbrauch von 60 % des theoretischen Sauerstoffbedarfs, weswegen sie als nicht leicht bioabbaubar eingestuft wurden (Rohdaten im Anhang von P4). Auch wenn die HPLC-Analyse der Ausgangssubstanzen (unbestrahlte SUA Proben) die Tendenz von SUA zur vollständigen Primärelimination nach 28 Tagen im MRT anzeigte, bestätigten die geringen Mineralisierungsraten die Widerstandsfähigkeit von SUA gegen den vollständigen biologischen Abbau (Mahmoud et al., 2013; Nationales Zentrum für Biotechnologieinformationen, 2023a, 2023b).

Die Mineralisierungsraten der UV-Mischungen lagen in ähnlicher Größenordnung wie die Mineralisierungsraten der SUA. Die HPLC-Analyse ergab allerdings, dass ein Drittel der SUA-TP während der Inkubation um mindestens 25 % primär eliminiert wurde. Dies waren erste Hinweise auf

bioabbaubare TP. Unter ihnen waren z. B. einige hydroxylierte SUA. An dieser Stelle ist allerdings zu betonen, dass eine hohe Eliminierungsrate eines TPs lediglich ein Hinweis ist, aber keine Auskunft über dessen Mineralisierungsrate gibt (nötige Information für BbD). Die Eliminierung kann auch auf die Bildung von stabilen, organischen TP und/oder Adsorption an den Klärschlamm zurückzuführen sein. Dies war der Fall bei den Ausgangssubstanzen selbst.

Ob sich die Hydroxylierung tatsächlich positiv auf die biologische Abbaubarkeit ausgewirkt hatte, musste daher näher untersucht werden. Drei hydroxylierte Sulfanilamidderivate wurden als Einzelsubstanzen im MRT mit Sulfanilamid als Referenz getestet. Sie wurden jedoch nicht leicht biologisch abgebaut (<< 60 %, siehe **Abbildung 7**) und zeigten auch nur geringe Eliminierungsraten (< 50 %, siehe P4). Somit lag nahe, dass die festgestellte Eliminierung der hydroxylierten SUA nicht auf verbesserte Mineralisierung, sondern auf die Bildung von stabilen TP und/oder Adsorption zurückzuführen ist.

Grund für einen primären Abbau von SUA-ähnlichen TP (z. B. OH-SUA und SUA-Isomere), wie auch von SUA selbst, könnten SUA-abbauende Bakterien sein, die sich durch die Exposition gegenüber SUA in der Kläranlage vermehren konnten (García Galán et al., 2012a; Ingerslev und Halling-Sørensen, 2000). Das Auftreten von SUA-spezifisch abbauenden Bakterien könnte auch das Verschwinden strukturell ähnlicher TP (OH-SMX, OH-STZ, SMX- und STZ-Isomer) unter biotischen Bedingungen erklären (Tabelle 4, P4), da festgestellt wurde, dass die am SUA-Abbau beteiligten Enzyme eher klassenspezifisch als verbindungsspezifisch sind (Ingerslev und Halling-Sørensen, 2000).

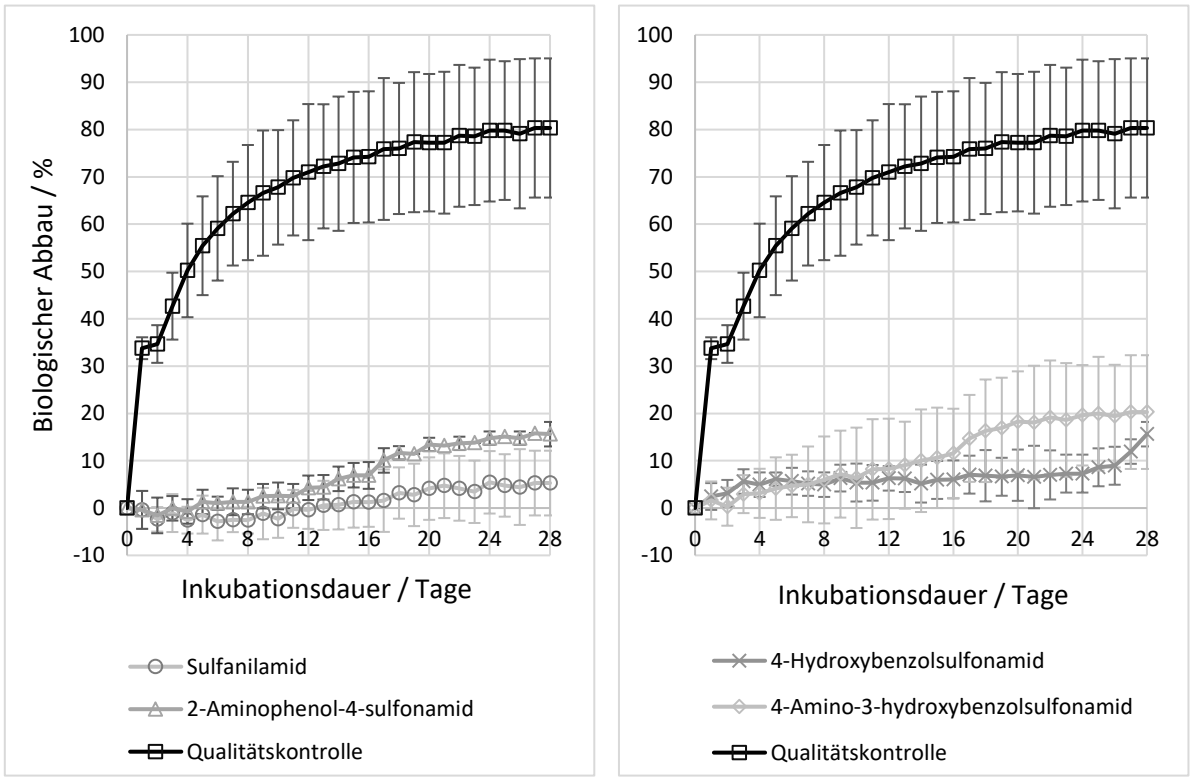

Abbildung 7: *Biologischer Abbau von Sulfanilamid und drei hydroxylierten Sulfanilamidderivaten während des manometrischen Respirationstests.*

Der Vergleich der HPLC-Ergebnisse von UV-Mischungen verschiedener SUA führte zu einer weiteren Gemeinsamkeit für ähnliche TP (Tabelle 3, P4). Die Arylamine NH_2-R (R: SUA-spezifische Arylgruppe), die für fünf SUA in den MRT-Proben detektiert wurden, nahmen im Allgemeinen während der Inkubation in ihrer Peakfläche zu. Dies stimmt mit der Literatur überein, da die Arylamine NH_2-R als *Dead-End* TP des SUA-Bioabbaus beschrieben werden (Ricken et al., 2013).

Interessante Transformationsprodukte und Schlussfolgerungen für *Benign by Design*

Aufgrund der HPLC-, LBT- und MRT-Ergebnisse waren lediglich fünf TP für BbD von Interesse (Tabelle 4, P4, blau markiert), denn zahlreiche Photo-TP wurden bereits aus strukturellen Gründen, beispielsweise wegen fehlendem Pharmakophor, ausgeschlossen (siehe auch Methodenteil, P4).

Bei drei der interessanten TP handelte es sich vermutlich um OH-SIX, OH-SMX und OH-STZ (Position der Hydroxygruppe blieb unbekannt), welche noch ein intaktes Pharmakophor aufwiesen und im MRT eliminiert wurden. Aufgrund des MRT-Ergebnisses zu den hydroxylierten Sulfanilamid-derivaten (siehe vorheriger Absatz) wurden diese Strukturen allerdings als BbD-Kandidat ausgeschlossen. Die Faustregel, dass Hydroxylierung die aerobe biologische Abbaubarkeit begünstigt (Boethling et al., 2007; Kümmerer, 2010c), trifft hier nicht zu. Die übrigen TP, die für BbD von Interesse waren, sind SIX-TP-1.2 (286) und SIX-TP-1.4 (173) (Tabelle 4, P4). Bei letzterem handelt es sich aufgrund des Massespektrums vermutlich um Sulfanilamid. Eigene Testdaten (siehe **Abbildung 7**) und Literaturdaten nach OECD 301D (Mahmoud et al., 2013) zeigen, dass Sulfanilamid in der aquatischen Umwelt nicht leicht biologisch abbaubar ist. Damit scheidet TP-1.4 (173) als BbD-Kandidat aus. Eine genauere Betrachtung des Massenspektrums von TP-1.2 (286) führte ebenfalls zum Ausschluss aufgrund unzureichender Modifizierung. Lediglich der spezifische Rest wurde leicht modifiziert, nicht aber der SUA-Grundkörper. Daher wurde kein BbD-Kandidat identifiziert. Dennoch wurden nützliche Daten und Erkenntnisse für die zukünftige Forschung zu BbD gesammelt.

Die Verbesserung der Bioabbaubarkeit in der Umwelt wurde bei dem auf SUA angewendeten, nicht zielgerichteten BbD-Ansatz als größere Herausforderung identifiziert als die Beibehaltung der Bioaktivität. Die MRT-Bewertung von UV-Mischungen führte zu keiner Identifizierung eines Kandidaten mit einer verbesserten biologischen Abbaubarkeit, wohingegen der LBT auf bioaktive TP hinwies und auch andere Studien bakteriostatische SUA-TP identifizierten (Bsp. Majewsky et al., 2014; Mirzaei et al., 2018; Osorio et al., 2016). Daher sollten sich künftige Arbeiten auf die Verbesserung der (Bio)Abbaubarkeit fokussieren. Ein abiotischer Abbauschritt zur Inaktivierung des SUAs könnte der biotischen Mineralisierung vorausgehen (z. B. Hydrolyse; vgl. Leder et al., 2021), um einen als Resistenzmechanismus fungierenden, enzymatischen Abbaumechanismus sicher auszuschließen (Ricken et al., 2017; Vila-Costa et al., 2017).

Eine wichtige Erkenntnis aus dieser Fallstudie für BbD im Allgemeinen ist, dass, obwohl bioaktive TP-Mischungen und Eliminierungsraten einzelner TP mittels LBT bzw. MRT bestimmt werden können, es schwierig ist, Hinweise zu BbD-interessanten TP zu finden. Hier könnten zukünftige Arbeiten

ansetzen. Als Variante des in dieser Publikation verfolgten nicht zielgerichteten Re-Designs könnten interessante UV-Mischungen beispielsweise mithilfe der Festphasenextraktion fraktioniert werden, sodass ausgewählte Fraktionen mit einer deutlich reduzierten TP-Diversität hinsichtlich ihrer Eigenschaften untersucht werden könnten. Dadurch sollten Schlussfolgerungen zu einzelnen TP leichter zu ziehen sein. Zukünftige Arbeiten könnten auch den Ansatz des zielgerichteten Re-Designs verfolgen, indem die SUA-Struktur auf Grundlage von Expert:innenwissen gezielt verändert wird. Dabei könnte auch das Pharmakophor modifiziert werden (vgl. Majewsky et al., 2014), solange die pharmazeutisch relevanten Strukturmerkmale erhalten blieben. Eine Zusammenarbeit zwischen Umweltwissenschaftler:innen und Expert:innen der Pharmazie oder Wirkstoffentwicklung wäre hierbei sehr hilfreich.

4.2.3 Schlussfolgerung

Diese Studie füllt Datenlücken zu UV-Mischungen von SUA und verbessert das Verständnis über das BbD-Konzept und dessen Anwendbarkeit auf SUA. Die Photo-TP der untersuchten UV-Mischungen können die Lumineszenz von *Allivibrio Fischeri* hemmen. Zusammen mit Literaturdaten zu antibakteriellen SUA-TP gibt es somit Hinweise auf bakteriostatische SUA-Derivate, die für BbD von Interesse sind. Dennoch erweist sich keins der Photo-TP als potentiell umweltfreundlichere Alternative zu den herkömmlichen SUA, da es keine ausreichenden Hinweise auf ein biologisch abbaubares SUA-Derivat gibt. Selbst hydroxylierte Sulfanilamidderivate sind nicht leicht biologisch abbaubar. Dies zeigt, dass bekannte Faustregeln zu Strukturmerkmalen, welche die Bioabbaubarkeit begünstigen bzw. behindern, nur eine allgemeine Orientierung bieten können und von Fall zu Fall überprüft werden müssen.

Die Erkenntnisse untermauern folglich die hohe Stabilität des SUA-Grundkörpers gegenüber dem biologischen Abbau in der Umwelt. Sie können zu neuen Design-Ansätzen anregen, wie etwa die nicht zielgerichtete Generierung von TP-Mischungen mit anschließender Fraktionierung oder das zielgerichtete Design. Dabei können auch die Möglichkeiten zur Änderung des Grundkörpers untersucht werden. Künftige Arbeiten sollten sich auf die Verbesserung der biologischen Mineralisierung konzentrieren, welche auch durch einen abiotischen Schritt eingeleitet werden könnte.

5 Diskussion der Anwendbarkeit von BbD auf Wirkstoffe

Teilziel 1: Bereitstellung eines Konzepts zur Anwendung von BbD in der Wirkstoffentwicklung

Im Rahmen der ersten beiden Publikationen wurde untersucht, ob und wie das Konzept BbD in der Wirkstoffentwicklung angewendet werden kann – ein bedeutsamer Schritt auf dem Weg zur industriellen Umsetzung (Deloitte et al., 2018; Europäische Kommission, 2020b, 2021). Die wichtigsten Schlüsselerkenntnisse der Literaturarbeit (P1), welche durch die Interviewstudie (P2) bestätigt, genauer differenziert oder erweitert wurden, sind in **Tabelle 3** beschrieben. Beispielsweise wurde durch P2 genauer differenziert, welche Anforderungen an einen pharmazeutischen Wirkstoff Hand in Hand mit der Umweltfreundlichkeit gehen, und welche eine Herausforderung für BbD darstellen (**Abbildung 8**). Letzteres ist dennoch nicht zwangsläufig ein Ausschluss für die Mineralisierung in der Umwelt, wenn ausgenutzt wird, dass Umgebungsbedingungen und damit die Abbaubarkeit entlang des Lebenszyklus des Wirkstoffs variieren (**Tabelle 3,** Schlüsselerkenntnis Nr. 1). Das Konzept aus P1 (s. o. **Abbildung 3**) wurde um den Vorschlag für einen Weg zu dessen Umsetzung (s. o. **Abbildung 4**) erweitert. Wichtige Meilensteine auf diesem Weg sind die Verfügbarkeit von geeigneten Designregeln, QSBR- und Hochdurchsatz-Methoden zur (Bio)Abbaubarkeit in der Umwelt (Schlüsselerkenntnisse Nr. 2 - 4), wofür finanzielle, regulatorische und soziale Anreize hilfreich sind (Schlüsselerkenntnis Nr. 5).

P1 und P2 liefern Argumente für die prozessuale Machbarkeit von BbD. Dazu zählen die Vereinbarkeit der pharmazeutisch- und umweltrelevanten Eigenschaften (**Tabelle 3,** Nr. 1) und die zahlreichen Beispiele von eingesetzten Wirkstoffen, die in der Umwelt mineralisieren. Darüber hinaus haben die Einblicke in die industrielle Wirkstoffentwicklung (P2) gezeigt, dass die vorherrschenden Strukturen die Anwendung von BbD nicht behindern. Im Gegenteil, die bestehenden flexiblen Strukturen ermöglichen stetige Prozessanpassungen. Des Weiteren sind Wirkstoffentwickler:innen bereits geübt, mehrere Parameter gleichzeitig zu berücksichtigen und zu optimieren (eine Industrievertreter:in: „*Why not one more?*“). Das Aufzeigen der prozessualen Machbarkeit und die Diskussion neuer Geschäftsmöglichkeiten (P1 und P2) bei zunehmender Relevanz des Themas PiE auf politischer Ebene (siehe Einleitung) sowie die Gespräche mit Industrievertreter:innen an sich (P2) appellieren an die Verantwortung auf höherer Entscheidungsebene und regen zu künftigen Umweltberücksichtigungen bei FuE-Projekten in Pharmaunternehmen an. Dies ist von großer Bedeutung, da der Fokus bisher vorwiegend auf einer umweltfreundlicheren Produktion lag (Ang et al., 2021; Becker et al., 2022; De Soete et al., 2017), während BbD noch keine Anwendung in der Industrie gefunden hat und Bedenken hinsichtlich hoher Kosten und technischer Hürden bestehen (Deloitte et al., 2018).

***Tabelle 3**: Anwendung von BbD in der Wirkstoffentwicklung – Fünf Schlüsselerkenntnisse der Literaturarbeit (P1), durch die Interviewstudie (P2) bestätigt (blau), genauer differenziert bzw. erweitert (grün), durch weitere Literatur eingeordnet (grau).*

Nr.	Schlüsselerkenntnis und Einordnung mittels weiterer Literatur
1	Pharmazeutisch relevante Eigenschaften, inklusive einer genügenden Stabilität, können mit der Mineralisierung in der Umwelt in Einklang gebracht werden, wenn ausgenutzt wird, dass Umgebungsbedingungen entlang des Lebenszyklus des Wirkstoffs variieren. Von Bedeutung ist das Verständnis von einem ausreichenden Maß an Stabilität, gekennzeichnet durch chemische (abiotische) Stabilität während der Herstellung, dem Transport und der Lagerung sowie metabolische Stabilität während der Anwendung im Körper, bei vollständiger Mineralisierung in der Kläranlage oder Umwelt (P1, P2).
	Die Zusammenhänge zwischen pharmazeutisch relevanten Stoffeigenschaften und den Umwelteigenschaften aus P1 wurden durch P2 erweitert (**Abbildung 8**).
	Vidaurre et al. (2024), eine interdisziplinäre Autorenschaft inklusive medizinischer Chemiker:innen, bauten darauf auf und richteten sich mit ihrem Artikel gezielt an medizinische Chemiker:innen.
2	Designregeln können bei der Identifizierung einer ‚Stabilitätslücke' helfen, sofern sie unterschiedliche Umgebungsbedingungen (Licht, pH, T, Enzyme) berücksichtigen (P1, P2).
	Wirkstoffentwickler:innen benötigen Designregeln und Struktur-(Bio)Abbau-Beziehungen, um BbD während der Optimierungsphase anzuwenden, denn es wird im DMTA-Zyklus bereits mit Struktur-Eigenschaftsbeziehungen gearbeitet (P2).
	Die Designregeln aus P1 müssten künftig angepasst werden, um die metabolische Stabilität gezielt zu berücksichtigen. Denn während Hydroxylgruppen und Carbonsäuren Merkmale sind, die in Wirkstoffen toleriert werden könnten, wird die Einführung der meisten funktionellen Gruppen, die den biologischen Abbau in der Umwelt begünstigen (z. B. durch enzymatische Hydrolyse), als kritisch für das pharmazeutische Profil angesehen (Vidaurre et al., 2024). Folglich müssten die Designregeln aus P1 erweitert werden, um variierende Umgebungsbedingungen, inklusive abiotischer Faktoren, zu berücksichtigen. Dies könnte auch dem Re-Design von SUA nutzen, da die Sulfonamidgruppe die Bioabbaubarkeit behindert (Längin et al., 2009) und eine Hydroxylierung, welche in der Regel die aerobe biologische Abbaubarkeit begünstigt (Boethling et al., 2007; Kümmerer, 2010c), nicht ausreicht, um dem entgegenzuwirken (P4).
3	Die Optimierungsphase mit dem DMTA-Zyklus ist eine geeignete Phase, um BbD in die Wirkstoffentwicklung zu integrieren (P1, P2).
	Weitere Möglichkeiten zur Anwendung und Unterstützung von BbD bestehen sowohl vor als auch nach der Optimierungsphase. Beispielsweise könnten Daten aus standardisierten Bioabbaubarkeitsstudien, die erst nach der Kandidaten Selektion erhoben werden und somit keinen direkten Einfluss auf das aktuelle FuE-Projekt haben, in eine Datenbank einfließen (P2).
	Im Rahmen des Projekts PREMIER und im Sinne der Reform der EU-Gesetzgebung zu Humanarzneimitteln (Europäische Kommission, 2023) wird ein Datenbank- und ein Bewertungssystem entwickelt. Daten könnten genutzt werden, um bestehende QSBR Modelle hinsichtlich ihrer limitierten Anwendungsdomaine zu verbessern, beziehungsweise neue Modelle zu entwickeln (Lorenz et al., 2021).
4	Um BbD frühzeitig in die Wirkstoffentwicklung zu integrieren, fehlt es an geeigneten *in silico* Modellen und *in vitro* Hochdurchsatz-Screenings zur Abbaubarkeit in der Umwelt (P1, P2).

4	Darüber hinaus wünschen sich Wirkstoffentwickler:innen eine Methodensammlung zur Anwendung in den Screening-Kaskaden des FuE-Prozesses (P2). Die Merkmale der Methode entscheiden darüber, in welcher FuE-Phase sie zum Einsatz kommen kann.
	Bramke et al. (2023) haben *in vitro* und *in silico* Testmethoden für Umweltparameter zur Anwendung in frühen und späteren FuE-Phasen zusammengetragen. Aufgeführte, erste Ansätze zu Bioabbau-Hochdurchsatz-Screenings liefern allerdings z. B. noch keine Auskunft über die Mineralisierungsrate (François et al., 2016; Martin et al., 2017). In den Projekten PREMIER und TransPharm werden daher *in silico* Methoden sowie ein miniaturisierter Bioabbautest basierend auf der Methode von Friedrich et al. (2013) entwickelt.
5	Finanzielle, regulatorische und soziale Anreize sind hilfreich, damit Anstrengungen in der Erprobung und Anwendung von BbD während der Wirkstoffentwicklung unternommen werden (P1, P2).
	Einige Befragte gaben an, dass der öffentliche Druck, die Bereitschaft der Unternehmen und persönliches Interesse der Wirkstoffentwickler:innen bereits greifen würden. Offenheit gegenüber BbD, persönliches Interesse und intrinsische Motivation waren in der Tat in allen Interviews ersichtlich (P2).
	Der öffentliche Druck und die Bereitschaft begünstigen künftige Anstrengungen im Bereich von BbD. Ordnungspolitische Instrumente sind dennoch von großer Bedeutung, um Aktivitäten zu BbD zu lenken und standardisieren, zu fördern und honorieren (Europäische Kommission, 2020a).

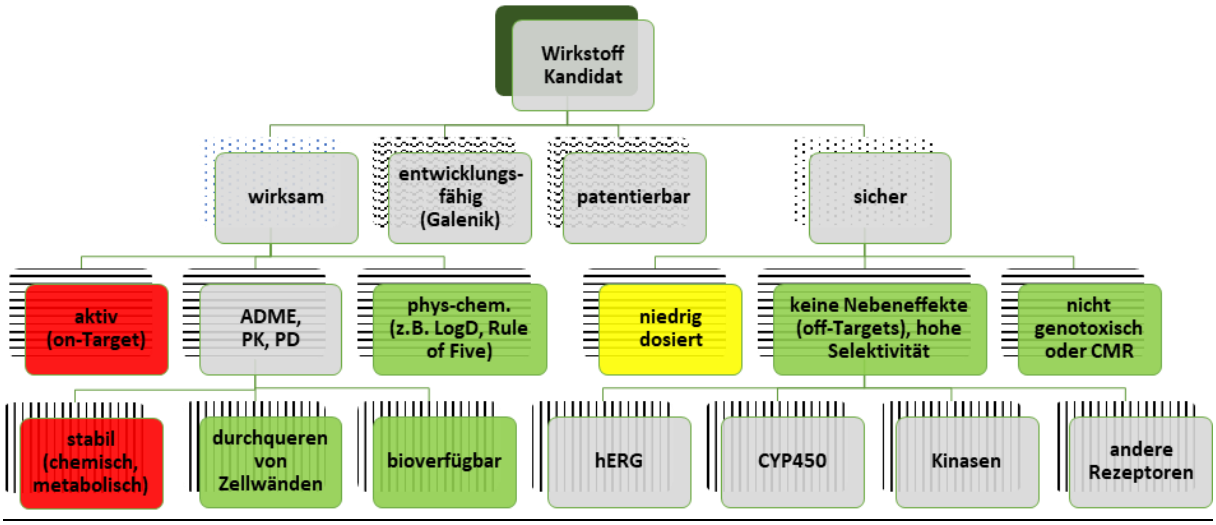

Abbildung 8: *Kriterien für einen Wirkstoff-Kandidaten und ihre Kompatibilität mit der Abbaubarkeit in der Umwelt oder der reduzierten Ökotoxizität (angelehnt an Abbildung 2, Puhlmann et al., 2022).*
Kompatibilität: Einige Kriterien gehen mit Umweltfreundlichkeit Hand in Hand (grün markiert), andere stellen eine Herausforderung für BbD dar (rot markiert). Bei „niedrig dosiert" kommt es auf den Ansatz zur Dosis-Reduzierung an (gelb markiert).
Erklärung der Kriterien: Wirkstoff-Kandidaten sollen wirksam und sicher sein (gepunktet), sodass sie zu einem pharmazeutischen Produkt weiterentwickelt und patentiert werden können (Wellen). Wirkstoff-Kandidaten müssen viele Kriterien erfüllen (Beispiele in horizontal-gestreift), um wirksam und sicher zu sein. Für die Kriterien „ADME, Pharmakokinetik (PK), Pharmakodynamik (PD)" und "keine Nebeneffekte" wird eine weitere Ebene mit detaillierten Aspekten angezeigt (Beispiele in vertikal-gestreift), um mehr Details zu liefern. Beachte: einige Kriterien hängen voneinander ab (Bsp.: Dosis hängt ab von Aktivität, PK, etc.).

Teilziel 2: Ein Beitrag zur Entwicklung umweltfreundlicherer Sulfonamid-Antibiotika

Die Literaturrecherche (P3) zum Einstieg in die experimentelle SUA-Fallstudie (P4) steht im Einklang mit dem vorgeschlagenen Arbeitsablauf zu BbD und *in silico* Methoden von Lorenz et al. (2021). Der frühzeitig gewonnene Überblick über bereits erhobene Daten diente der Vermeidung überflüssiger Tests (Vgl. Konzept *one substance – one assessment; Europäische Kommission, 2020a*) und der gesamten weiteren Planung der Fallstudie.

In P3 und P4 wurden Erkenntnisse über SUA-TP gewonnen, welche signifikant zum BbD von SUA beitragen. Dazu zählen ein besseres Verständnis von Transformationsprozessen der SUA und ihren Produkten (Tabelle 7, P3) sowie die Hinweise zur Eignung von TP für BbD, auf welche aufgebaut werden konnte (P3 und P4). Bezüglich des Letzteren haben sich insbesondere die Studien von Radke et al. (2009), Majewsky et al. (2014), Su et al. (2016) und Achermann et al. (2018) als hilfreich erwiesen. In P4 wurden bioaktive TP vorgeschlagen. Darunter waren auch solche vertreten, die zuvor noch nicht in der Literatur beschrieben wurden. Durch die Literaturarbeit (P3) und die eigenen experimentellen Studien (P4) konnten keine TP identifiziert werden, welche möglicherweise besser in der Umwelt abgebaut werden, denn Bioabbaudaten zu Einzelsubstanzen aus der Literatur (P3) und erste Hinweise auf leicht und vollständig abbaubare SUA-Derivate fehlten (P4). Letzteres ist auf die herausfordernde Interpretation von Daten aus Mischungstests zurückzuführen.

Hier könnten zukünftige Arbeiten ansetzen, um die erhobenen Daten von Mischungstests besser aufklären und im Detail interpretieren zu können. Beispielsweise könnten UV-Mischungen flüssig chromatographisch (analytische präparative HPLC; Fabel, 2023) oder mittels Festphasenextraktion fraktioniert (Balakrishnan et al., 2006; Schemeth et al., 2019) werden. Ausgewählte Fraktionen mit deutlich reduzierter TP-Vielfalt könnten im Anschluss mithilfe von Aktivitätstests (für SUA z. B. nach Balouiri et al., 2016; Wiegand et al., 2008) und einer Kombination aus abiotischen und biotischen Abbaustudien untersucht werden. Darüber hinaus könnte auch der zielgerichtete Design-Ansatz angewendet werden, indem die SUA-Struktur basierend auf Fachexpertise modifiziert wird. Anschließend könnte durch computerbasierte Methoden eine vielversprechende Struktur zur Synthese priorisiert werden. Beim zielgerichteten Ansatz könnte auch das Pharmakophor eine Änderung erfahren, beispielsweise an der *para*-Position (Majewsky et al., 2014), solange relevante Merkmale für die Dihydropteroat-Synthase Hemmung erhalten bleiben. Die Zusammenarbeit z. B. mit Expert:innen aus der Wirkstoffentwicklung würde sich anbieten, um diese und andere alternative Ansätze anzuwenden und damit die Chance auf ein neues umweltfreundlicheres SUA zu erhöhen.

Teilziel 3: Die Förderung der Anwendung von BbD im Allgemeinen durch die SUA-Fallstudie

Die Vielzahl an vorgeschlagenen TP-Strukturen und die damit einhergehende Herausforderung für die Umweltrisikobewertung (P3) unterstreichen die Bedeutung von BbD als effektive Präventivmaßnahme (P4). Die bekannte TP-Problematik begründet neben den Strategien zur Priorisierung der umweltrelevanten TP (Escher und Fenner, 2011; Hensen et al., 2020; Umweltbundesamt, 2019) und Ansätzen zur Berücksichtigung von Cocktail-Effekten (Europäische Kommission, 2020a) auch die bisherige und zukünftige Forschung zu BbD. BbD als Präventivmaßnahme folgt dem Vorsorgeprinzip, ein etabliertes Grundprinzip, welches in der gesamten europäischen Gesundheits- und Umweltpolitik verankert ist (Europäisches Parlament, 2015).

Allerdings geht die Implementierung des Design-Ansatzes sowohl mit Möglichkeiten als auch mit Hürden einher, die in P4 aufgezeigt wurden. Möglichkeiten der Anwendung von BbD bestehen in dem Effekt gesteuerten Ansatz (beschrieben z. B. von Escher und Fenner, 2011) mit vorangehender Photolyse. Dies ermöglichte die Identifizierung aktiver TP-Mischungen. Gleichzeitig erlaubt dieses Vorgehen erste Rückschlüsse auf möglicherweise antibakteriell wirksame TP trotz ihrer limitiert käuflichen Verfügbarkeit. Gleichzeitig ist die Interpretation der Daten von komplexen Photolyse-Mischungen eine wesentliche Hürde des BbD Ansatzes. Diese Herausforderung war aufgrund der Arbeiten von Lorenz (2023) zum nicht zielgerichteten Re-Design von Fluorchinolonen bereits zu erwarten. Varianten des nicht zielgerichteten Re-Designs und das zielgerichtete Re-Design stellen mögliche Alternativen dar (s. o. letzter Paragraph zu Teilziel 2).

Die aufgezeigten Herausforderungen der Fallstudie zeigen, wie wichtig die Zusammenarbeit von Expert:innen unterschiedlicher Fachrichtungen für die Anwendung von BbD ist, denn sie erfordert transdisziplinäre Expertise zu diversen Kriterien für umweltfreundlichere Wirkstoffe. Außerdem ist die Anwendung von BbD aus unterschiedlichen Perspektiven zu beleuchten. Insbesondere die Interviewstudie mit FuE-Expert:innen (P2), publiziert in der pharmazeutischen Zeitschrift *European Journal of Pharmaceutical Sciences*, unterstützt den transdisziplinären Austausch zwischen und innerhalb der FuE- und Umweltexpert:innen. Eine zweite Interviewreihe mit Interessenvertreter:innen des Gesundheitssektors wird zum Zeitpunkt des Verfassens dieser Dissertationsschrift im PREMIER Projekt durchgeführt, um die Machbarkeit des Designs von umweltfreundlicheren Wirkstoffen und dessen Anwendung in einem breiteren Kontext zu untersuchen und damit die Anwendung von BbD weiter zu fördern.

Übergeordnetes Ziel: Ein Beitrag zur Anwendung von BbD im Wirkstoffentwicklungsprozess

Diese Arbeit liefert aufgrund oben genannter Aspekte einen direkten Beitrag zu BbD – ein wirksamer Lösungsansatz und daher Teil der EU-Chemikalienstrategie für Nachhaltigkeit (Europäische Kommission, 2020a) und des strategischen Ansatzes der EU zu Arzneimitteln in der Umwelt (Europäische Kommission, 2019) unter dem Europäischen *Green Deal*. Die konzeptionellen Studien (P1, P2) zeigen, dass BbD auf Wirkstoffe angewendet werden kann, wenn geeignete Hochdurchsatz- und *in silico* Modelle zur Untersuchung der (Bio)Abbaubarkeit in der Umwelt bereitstehen und Bewertungskriterien definiert sind. Die SUA-Fallstudie (P3, P4) liefert Analysen und Daten zur Entwicklung mineralisierender SUA und demonstriert die Möglichkeiten und Herausforderungen des nicht zielgerichteten Designansatzes. Dieses verbesserte Verständnis von BbD fördert die industrielle Anwendung von BbD und damit die Erfüllung von BbD als zentrale Rolle in den derzeitigen Aktionsplänen unter dem *Green Deal*.

Indirekt schärft diese Arbeit den Blick auf den gesamten Lebenszyklus eines Wirkstoffs und damit die Berücksichtigung aller Prinzipien der Grünen Chemie und Pharmazie (Anastas und Warner, 1998), z. B. hinsichtlich der Rohstoffgewinnung, Synthese, Produktion und des Verbleibs des Wirkstoffs in der Umwelt. Die Betrachtung des gesamten Lebenszyklus ist nicht nur zentral für BbD, sondern für Grüne Chemie im Allgemeinen. Um fundierte und begründete umweltbegünstigende Entscheidungen bei der Entwicklung treffen zu können, müssen verschiedene Aspekte hinsichtlich der Umweltfreundlichkeit von pharmazeutischen Wirkstoffen (z. B. Treibhausgasemissionen, Bioabbaubarkeit in der Umwelt) beleuchtet und abgewogen werden (Moermond et al., 2024). Somit regt diese Dissertation an, weitere Aspekte der Grünen Chemie und Pharmazie entlang des gesamten Lebenszyklus einer Chemikalie, eines Wirkstoffs oder Produkts zu berücksichtigen. Solche Arbeiten zur Grünen Chemie sind nötig für einen ganzheitlichen Klima- und Umweltschutz. Sie dienen als Wegbereiter, um den Weg der grünen Transformation zu gehen, ob auf freiwilliger Ebene oder aufgrund zukünftiger gesetzlicher Vorschriften (siehe Einleitung).

6 Fazit

Die Erkenntnisse aus dieser Arbeit liefern einen wertvollen Beitrag zur Anwendung von BbD im Wirkstoffentwicklungsprozess. Erstmals wurde ein Konzept zur Anwendung von BbD auf Wirkstoffe entwickelt und eine Vorgehensweise zu dessen Umsetzung vorgeschlagen. Dabei wurde gezeigt, dass die vorherrschenden Strukturen und Abläufe der Wirkstoffentwicklung zahlreiche Anwendungsmöglichkeiten für BbD erlauben. Darunter ist die Multiparameter Optimierung am entscheidendsten und gleichzeitig am besten geeignet. Mit dem Identifizieren der diversen Möglichkeiten, aber auch Hürden für BbD im Allgemeinen und für den nicht zielgerichteten Design-Ansatz im Speziellen wurde das Verständnis von BbD verbessert. Darüber hinaus wurden Datenlücken zu SUA-TP gefüllt, welche nicht nur aus umwelttoxikologischer Sicht von Bedeutung sind, sondern auch dem Re-Design von SUA nutzen. Die weitere Erprobung von BbD-Ansätzen wird durch das gewonnene Verständnis und durch die aufgezeigten, bestehenden und zu erwartenden Anreize stimuliert.

Die empfohlene Vorgehensweise zur Umsetzung (P2) des eigens entwickelten Konzepts (P1) in der pharmazeutischen Industrie ist richtungsweisend für künftige Aktivitäten zu BbD in und außerhalb der Wissenschaft, um die identifizierten Hürden zu überwinden. Ein Beispiel ist die Entwicklung und Bereitstellung einer geeigneten Sammlung an Testmethoden zu klar definierten Umweltparametern. Es besteht ein dringender Bedarf insbesondere an geeigneten *in silico*- und Hochdurchsatz-Methoden zur Untersuchung der Bioabbaubarkeit in der Umwelt. Dies wurde auch im Rahmen der SUA-Fallstudie deutlich. Damit *in silico* Modelle entwickelt werden können, sind vermehrt hochwertige Bioabbaudaten aus standardisierten Tests (z. B. nach OECD 301D) zu generieren. Des Weiteren stellen die zusammengetragenen Designregeln hinsichtlich der Bioabbaubarkeit in der Umwelt (P1) einen geeigneten Ausgangspunkt für künftige Diskussionen mit Wirkstoffentwickler:innen dar, um die Designregeln durch die Berücksichtigung der physiologischen Umgebungsbedingungen zu verfeinern. Die erweiterten Designregeln könnten für das zielgerichtete *de novo* Design oder Re-Design, beispielsweise von SUA, herangezogen werden. Zukünftige Re-Design Ansätze von SUA könnten auch auf die gezielte Modifizierung des Pharmakophors abzielen, wobei pharmazeutisch relevante Eigenschaften zu wahren sind.

Darüber hinaus sollte zukünftig näher untersucht werden, unter welchen Bedingungen umweltfreundlichere Arzneimittel auf höherer Entscheidungsebene in Unternehmen und von den zahlreichen Akteuren des Gesundheitssektors berücksichtigt bzw. bevorzugt werden können oder sollen. Die Konzept- und Fallstudien zu BbD geben Anlass für diese Untersuchungen insbesondere wegen der aufgezeigten prozessualen Machbarkeit. Eine transdisziplinäre Zusammenarbeit zwischen Expert:innen

aus der Pharmazie und den Umweltwissenschaften ist bei anstehender Forschung zu BbD von großem Vorteil. Diese Zusammenarbeit könnte beispielsweise auf der Interaktion während der Interviewstudie aufbauen, in welcher sich erstmals die große Bereitschaft von medizinischen Chemiker:innen gezeigt hat (P2). Transdisziplinarität könnte ebenfalls das gezielte Re-Design von SUA unterstützen (P4).

Zusammen mit zukünftiger Forschung im Bereich der Grünen Chemie dient diese Arbeit als wichtiger Wegbereiter, um Instrumente wie BbD zu implementieren und so den Weg der grünen Transformation der pharmazeutischen Industrie zu ebnen und zu gehen. Gewonnene Erkenntnisse lassen sich auch auf andere Industrien, wie beispielsweise die Kosmetikindustrie, übertragen.

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Towards the design of active pharmaceutical ingredients mineralizing readily in the environment

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Active pharmaceutical ingredients (APIs), their metabolites, and transformation products (TPs) occur globally in the environment. They pose a risk to both environmental and human health. These alarming circumstances highlight the strong need for efficient measures, which is also reflected in EU strategies on sustainable chemicals and on pharmaceuticals in the environment. The design of APIs for mineralization in the environment according to the concept Benign by Design (BbD) is a promising approach to tackle this challenge. However, its implementation into the industrial API discovery process has not been discussed yet. To stimulate such a discussion and to better understand the applicability and limitations of this approach, the generic API discovery process is reviewed, including procedure, principles, and paradigms based on publicly available information. In addition to the concept of BbD itself, workflow scenarios such as *de novo* design and re-design are presented to provide a better understanding of its feasibility. Bringing these aspects together, we conclude that the optimization phase within drug discovery seems to be the most appropriate place to implement environmental considerations. At this early stage, costs are low and the potential impact of design and structural variation on the outcome is high. We found that pharmacological parameters required for application are sometimes even in line with biodegradability in the environment, since the conditions in the human body and the environment differ. However, the effects of optimizing pharmacological parameters such as toxicity and stability on environmental biodegradability of APIs must be considered together with design rules for biodegradability. Understanding the feasibility of BbD can mitigate the concerns pointed out by stakeholders and encourage them to invest in research and development, as well as support pharmaceutical companies to be prepared for upcoming regulations, since the aforementioned EU strategies announce further political regulations. We found also that successful implementation of BbD depends on the availability of suitable tools and methods as well as on incentives for research and development within constructive collaboration of industry, academia, and authorities.

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Introduction

Active pharmaceutical ingredients (APIs) and their metabolites occur ubiquitously in the environment. They enter the environment mainly *via* wastewater as a result of patient use since, on average, an estimated 60–70% of APIs and their metabolites are excreted *via* urine, with varying excretion rates for the individual substances.[1] Most APIs do not degrade completely in wastewater treatment plants (WWTPs), despite much research and effort having already been invested in the field of wastewater treatment.[2] Furthermore, 80% of wastewater worldwide is not treated at all because of a lack of treatment facilities.[3] Another entry path of APIs into the environment is *via* contaminated manure spread on fields. In the environment, some APIs are fully mineralized, *i.e.* degraded to water, carbon dioxide, and inorganic salts, for example acetylsalicylic acid.[4] Some are persistent, *e.g.* carbamazepine,[5] whilst others are only partly mineralized, like fluoroquinolones.[6] Incomplete mineralization results in a highly diverse cocktail of transformation products (TPs) since several or many TPs are often formed based on one parent API.[7] Additionally, several thousand APIs are marketed worldwide and the diversity and volume will only increase due to increasing living standards, population, and age. Thus, there is a ubiquitous occurrence of APIs, their metabolites, and TPs in the environment. Although their environmental fate and effects are often unknown, some

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effects of APIs in environmental concentrations on environmental organisms, but also at a population level, have been demonstrated, *e.g.* cytological effects on fish.[8,9] Nevertheless, the chemical formulas, fate, and effects of TPs are mostly unknown. In some cases, it has been found that TPs are even more toxic than the parent API, as is the case for methadone and its TP *N*-nitrosodimethylamine.[10,11] Adverse effects of compound mixtures, including APIs, metabolites and TPs, are difficult to predict.[12]

These alarming circumstances highlight the strong need for efficient measures against the presence of APIs, their metabolites, and TPs in the environment, which is also reflected in the EU Chemicals Strategy for Sustainability Towards a Toxic-Free Environment[13] based on the Zero Pollution Action Plan,[14] as well as in the Pharmaceutical Strategy for Europe.[15] In addition to measures like adapted emission management or discouraging unnecessary use and inproper disposal of unused drugs, the design of greener APIs according to the concept of Benign by Design (BbD) is a promising method of input prevention: it is an effective measure at the source ("beginning of the pipe")[16] and a sustainable long-term solution alongside the across-the-board approach and the precautionary principle.[17] Thus, the implementation of BbD is urgently needed. However, short-term solutions are favored by the industry, likely due to concerns about the high costs and technical challenges associated with long-term solutions such as BbD. Accordingly, the pharmaceutical industry has not yet taken significant steps to implement the concept BbD into the generic API discovery process,[16] albeit it would offer economic advantages as new compounds will result in new business opportunities.[2] The reasons for this reluctance have to be better understood.

Even through an environmental risk assessment (ERA) has been required within the marketing authorisation application in the EU since 2006,[18] authorization cannot be denied for human pharmaceuticals because of the ERA result. Accordingly, it is typically conducted late during drug development.[19] Thus, environmental considerations are not integrated effectively, *i.e.* early within the API discovery process, as would be desirable for a greener design to gain the full functionality needed within the product life cycle (*eco*-pharmacovigilance).[20] The implementation of BbD into regular API discovery could be stimulated by providing an appropriate strategy. Workflows showing how environmental biodegradability can be considered beside the indispensable pharmaceutical activity[21] and demonstrating the feasibility of BbD[22,23] are available in existing literature. On a laboratory scale, the combination of *in silico* prediction and *in vitro* confirmation has been used as a very powerful strategy to achieve BbD.[24,25] This combination is already implemented during the industrial process for endpoints such as toxicity: *In silico* prediction is well integrated due to cost and time savings. However, tools predicting ready biodegradability are scarce, even though rules of thumb for structure–biodegradability relationships are well-described.[26–30]

To the best of our knowledge, there is no article discussing the implementation of the concept BbD within the industrial API discovery process. Therefore, the overarching goal of this study is to foster a better understanding of the pros and cons and motivate academia and industry to investigate the feasibility and opportunities of the concept of BbD for the discovery of APIs mineralizing readily in the environment. Herein, we provide an overview on the feasibility of the concept of BbD from a scientific point of view to mitigate the concerns pointed out by stakeholders and encourage them to invest in research and development. Furthermore, this overview is intended to support pharmaceutical companies to be prepared for upcoming regulations since the aforementioned EU strategies announce further political regulations.

Neele Puhlmann

Neele Puhlmann was born in Neumünster (Germany) in 1991 and received her diploma and first state examination in food chemistry from Martin-Luther-University Halle-Wittenberg in 2016, and the second state examination from the Institute for Hygiene and the Environment in Hamburg in 2017. After two years of experience as an expert for market compliance of consumer products, she started her PhD at the Institute of Sustainable Chemistry at the public Leuphana University of Lüneburg. Neele is working for the IMI-project PREMIER (Prioritisation and Risk Evaluation of Medicines in the EnviRonment), funded by the European Commission and EFPIA members.

Renate Mols

Renate Mols was born in Weert, the Netherlands in 1994 and moved to the United States at the age of 9. In 2014, she moved back to the Netherlands to obtain her bachelor's degrees in Chemistry and Environmental Science at Utrecht University. She then completed her joint Chemistry master's degree at the University of Amsterdam and the Vrije Universiteit Amsterdam as well as a minor in Fundamentals of Business and Economics at Utrecht University. During her thesis internships, she worked on cultured meat innovation and environmental impact assessment in collaboration with Maastricht University and Mosa Meat.

To achieve these objectives, the generic API discovery process, including the generic procedure, principles, and paradigms, was studied based on publicly available information. Next, the concept of BbD is presented including suggested test methods and tools. Thereby, the role of abiotic degradation, *e.g.*, by direct and indirect photodegradation, is not discussed in this article although such processes may play a role under environmental conditions (*e.g.*, access to light in contrast to the human body) and could in some cases initiate or at least support and accelerate biodegradation. However, photolytic and hydrolytic degradation are not expected to lead to complete mineralization. For example, photolysis is mediated by radicals (directly formed or by hydroxy radicals), which are of low selectivity due to their reactivity. The absorption spectrum has changed after the reaction. Often, no further absorption of light with sufficiently high energy takes place and/or no hydrogen atoms can be abstracted anymore resulting in stable TPs. In general, more polar TPs can be formed *via* abiotic degradation that are not necessarily better at mineralizing environmentally, or being of lower ecotoxicity, but which are more mobile in the aquatic environment and also under discussion as persistent, mobile, and toxic (PMT) substances with growing concerns.[31] Biotic degradation, on the other hand, is mediated by enzymes, leading to a lower TP diversity as the reaction pathways are more specific. Therefore, we focus in this review on biodegradability of APIs due to the potential for complete mineralization.†

Furthermore, we present different BbD workflow scenarios for varying starting points, such as *de novo* design ("from scratch") and re-design ("re-engineering", *i.e.* improvement of already existing molecules by structure variation for the same or another purpose) to present a variety of perspectives. In the final discussion, we highlight where the BbD concept can be included in the generic, well-established API discovery process.

The generic workflow for the discovery of APIs: procedures, principles, and paradigms

The generic discovery of APIs is well established[32–35] and therefore only briefly described, including its principles and paradigms, before we discuss the needed improvements and promising points of possible intervention.

Traditional target-based drug discovery in the pharmaceutical industry (Fig. 1) is subdivided into (i) target identification, (ii) hit selection, (iii) from hit to lead optimization, (iv) from lead to candidate optimization, and (v) preclinical trials for candidates as a stepping stone to further drug development.[36]

Target identification and hit selection

First, the target for the disease needs to be identified, which is typically assumed to be a protein. For this selected target, large molecule libraries are used to find compounds with potential activity on the selected target, resulting in up to tens of thousands of compounds. By an appropriate screening method, *e.g.* high throughput screening, structures with confirmed activity on the chosen target are selected. These so-called hits can then be further optimized.[33]

†According to common understanding, "complete mineralization" refers to the share of an organic compound that is not used by degraders as building blocks for growth. Thus, in terms of full mineralization, this remaining share is completely metabolised to water, carbon dioxide and inorganic salts (*i.e.*, catabolism excluded).

Oliver Olsson

Oliver Olsson was born in Hanover (Germany) in 1975 and received his diploma and doctoral degree in civil-engineering from Leibniz University of Hanover in 2003 and 2009. He is lecturer and research scientist at the Institute of Sustainable Chemistry at the public Leuphana University of Lüneburg since 2011. He has several years of experience in academic research of water quality management and has training in international transdisciplinary project management. Specific interest lies on multi-scale methods assessing the emission, transport and fate of chemicals and pharmaceuticals in the aquatic environment.

J. Chris Slootweg

Chris Slootweg started his independent scientific career in 2006 at Vrije Universiteit Amsterdam. He was promoted to Associate Professor in 2014, and moved to the University of Amsterdam in 2016. The mission of his laboratory is to educate students at the intersection of fundamental physical organic chemistry, main-group chemistry, and circular chemistry. Chris is co-founder and scientific advisor of SusPhos BV, a pioneering company focused on upcycling of phosphate rich waste streams to generate high-quality alternatives to replace current fossil-sourced products.

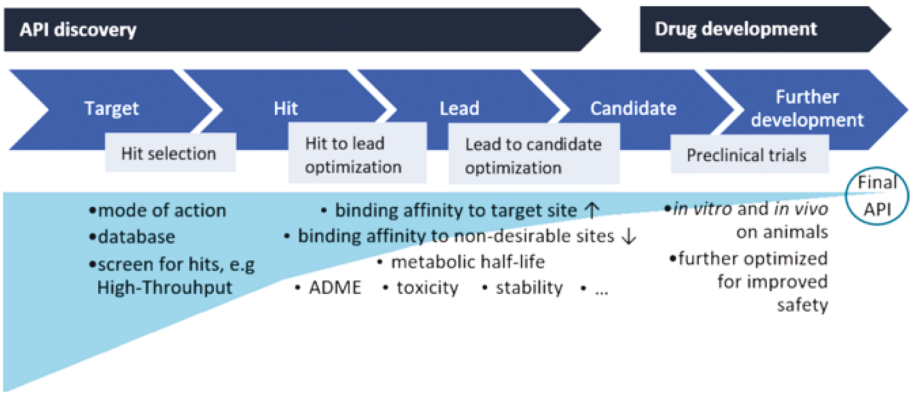

Fig. 1 Scheme of a traditional research process in drug discovery, inspired by Messinger *et al.*[36]

From hit to lead to candidate optimization

The optimization steps 'from hit to lead' and 'from lead to candidate' follow. Leads are the most promising optimized hits. They are safe, patentable, have desired *in vitro* pharmacokinetic properties (absorption, distribution, metabolism, and excretion (ADME)) and show activity in preliminary *in vivo* models. Next, selected candidates are suitable for preclinical development.

This optimization strategy is challenging for medicinal chemists due to the many parameters that need to be investigated.[32] At the same time, it is most crucial to include all the required properties in this multiparameter space, such as activity, selectivity, ADME, toxicology, target specificity, druggability and patentability.[36] The synthetic route of the "optimal" candidate compound needs to be highly flexible to allow for the implementation of bioactive groups. Therefore, the optimization process is conceived as a repetitive cycle, the so-called design-make-test-analyse (DMTA) cycle,[37,38] that is executed many times. Since drug discovery is a very competitive field, the factor *time* affects the process significantly. To reduce the cycle time for optimization as much as possible and to enable many runs during the optimization phase (1–2 years), automated, combinatorial, or parallel concepts are employed.[36] Selected assays of the *in vitro* test phase that run in parallel can provide 80% of all data within just ten working days. In order to cope with the challenge of multi-parameter design and to improve the quality and the effectiveness of the DMTA cycle, effective collaboration within a dedicated multidisciplinary design team, consisting of medicinal, synthetic, and computational chemists as well as drug metabolism and pharmacokinetics experts, is essential.

It must be emphasized that the quality of a candidate compound which dictates the success of development is decided already at the point of design where cost of idea finding is low and the chances of success are variable.[37] Therefore, most companies perform *in vitro* pharmacology profiling already at this early stage, even though only one *in vitro* pharmacology assay is required by regulatory authorities: the assay for effects on the human ether-à-go-go-related gene (hERG) cardiac potassium channel (ICH Guideline S7B).[39] Importantly, an early screening of compounds against a broad range of targets (receptors mainly of the G protein coupled receptor superfamily, ion channels, enzymes, transporters) that are different from the intended therapeutic target(s) can reduce safety-related attrition (*i.e.* due to adverse drug reactions) in the later stages of drug discovery and development. In order to perform an effective *in vitro* pharmacological profiling early during API design, Bowes *et al.*[40] recommend 44 targets as a minimal panel and the use of data for the development of *in silico* tools.

Preclinical trials for candidates

After optimization, candidates that fulfil all criteria are transferred to preclinical development to ensure safe application of the drug using *in vitro* tests and *in vivo* tests on animals. At this point, the required quantities of compounds increase from grams to kilograms and up to tons, for which process chemists are developing the optimal synthetic route. The final

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clinical development of the promising candidate(s) follows *via* tests on humans which, if successful, will ultimately lead to an effective and safe drug.[32,33,35,36,41]

Overarching principles and paradigms

In all of the steps in the drug discovery process, quality assurance is an overarching principle. For this purpose, organic and genotoxic impurities are analysed and physical properties and stability, *e.g.* regarding temperature and humidity (stress testing), are controlled according to defined standards (ICH Guideline Q1A(R2)).[42] However, these experimental tests take time and are usually only conducted once the optimal synthetic route is decided. Therefore, the Quality by Design approach, recommended by the European Medicines Agency (EMA), would be more effective, as it applies statistical, analytical, and risk-management methodology in each stage of the process from discovery to manufacturing.[43] For example, a control strategy within the development of a robust aniline-containing API could limit the amount of potential genotoxic aryl nitroso and hydroxylamine impurities that arise from the reduction of an aromatic nitro-compound.[44]

Another overarching principle is to further improve the efficacy of the process under competitive conditions. For this purpose, computational tools are indispensable today throughout the entire process from target selection to drug development. *In silico* tools are diverse and have various pros and cons.[45,46] In the broad field of computational screenings for hits, structure-based screenings are predominant compared to ligand-based screenings, which in turn tend to provide more potent hits.[46] Starting out with fragments, hot spot analysis can be applied. Continuing with further optimization, druggability prediction, (quantitative) structure–property/activity relationship ((Q)SAR) methods, molecular docking, machine learning, and deep learning models play a role for endpoints such as bioactivity, solubility, synthetic feasibility, ADME properties, and toxicity (ADMET).[45]

Computational calculations routinely predict characteristics defined by Lipinski's rule of five, *e.g.*, $\log P < 5$ using (Q)SAR, for prospective structures within API discovery.[32] Through molecular docking, on-target as well as off-target effects can be calculated. The limitations of the accurate simulation of relative binding free energy[46] can be overcome using the guidelines provided by Cournia *et al.* based on examples encouraging free energy methods.[47] A prominent example of successful application is the prediction of effects on the hERG cardiac potassium channel early during the design phase of the DMTA cycle, which has led to reduced safety-related attrition rates. Based on the predicted effects on the hERG channel, a prioritization for subsequent synthesis and *in vitro* testing is possible.[48] Driven by business needs, *i.e.* minimizing overall attrition and costs, machine learning is also recently being implemented at all stages of the drug discovery and development process. For example, machine learning was successfully applied to lead optimization by designing compounds with desirable properties, but this required an enormous amount of training data.[49] In general, API discovery could benefit more from artificial intelligence (AI), as it is currently only partially implemented; AI has the potential to become a more integral part of API discovery.[50]

These principles, *i.e.* quality assurance and process efficacy, are both well-established in contrast to the concept of Benign by Design, here aimed specifically at the principle *Design for Environmental Degradation.*[51] This is further explained in the following section.

The concept benign by design

Benign by Design (BbD) is the targeted design of a compound with optimized characteristics for its application and fast and complete mineralization after it has entered the environment. It means that ready degradability after use or application is considered even before an API's synthesis.[52] BbD is based on the tenth principle of green chemistry described by Anastas and Warner: *Design for Degradation*.[51] The design of degradable APIs will contribute to United Nations Sustainable Development Goals 3 (health), 6 (clean water), 9 (sustainable industries), 11 (sustainable cities), and 12 (responsible production and consumption).[53] Beyond ready environmental degradability as part of full functionality in terms of BbD, an ideal and modern API is expected to be effective and efficient, receptor specific, with reduced or without unwanted effects, and metabolized to harmless metabolites.[27]

BbD takes advantage of the fact that changes in the chemical structure of a molecule alter its chemical and physical properties including its biodegradability. If changes are made at parts of the molecule that are not mandatory for its activity, environmental biodegradability can be improved without compromising its activity.[25] However, changes at the core structure delivering activity are possible as well, and may go hand in hand with improved properties needed for application and at the same time increased environmental biodegradability.[23,54,55] Thus, this BbD concept can be used to design APIs to be fully biodegradable in the environment without decreased pharmacological activity or increased human toxicity during use.[21]

Ideally, the entire life cycle of a product should be made circular, meaning that there are no resource inputs and outputs and only limited energy inputs during its entire life cycle.[56,57] Achieving resource circularity is particularly difficult in the case of APIs, as they are emitted at non-point sources into sewage systems, soil, and surface water. After emission, collection is impossible as it is too energetically, financially, and environmentally taxing to derive them for recycling purposes.[52] However, if cleverly designed APIs mineralize at the end of their life cycles through complete environmental degradation into carbon dioxide, water, and inorganic salts there will be no end of life issues.[58] These resulting inorganic degradation products can then re-enter the biological cycles by incorporation into plant and microbial biomass, which can then in theory be used as a renewable resource for pharmaceutical production, thereby closing the cycle to the best currently feasible extent.[52,58] Furthermore, such an

approach protects water resources without upgrading sewage treatments. This saves (financial) resources, chemicals, and the need for advanced treatment as well as the resulting associated issues related to products of incomplete mineralization.[57,59] In fact, this concept can be applied everywhere, even if there is no wastewater treatment available, which is the case for 80% of effluent on the planet.[3] Furthermore, because of climate change there will be an increased need for water reuse, *e.g.* in agriculture which requires non-polluted water.[53,59,60]

Tools and test methods needed for BbD

In silico. *In silico* models based on (Q)SAR, for example, are very efficient in predicting biodegradability because of lower costs and time savings when compared to *in vitro* tests. Therefore, *in silico* methods are an appropriate tool when dealing with a huge number of compounds, which is often the case during API discovery. However, their application is not trivial and requires expertise, as only (Q)SAR models with defined and validated applicability domains should be used. Additionally, the application of more than one model makes the predictions more reliable. Ideally, statistical models should be used in combination with expert rule-based models as recommended by the ICH M7 guidelines for toxicity.[61] The user needs to review and evaluate the predicted results, and be aware of which experimental tests provide the data for the *in silico* model. Rücker and Kümmerer[62] have provided practical information *e.g.* on the availability and limitations of reviewed prediction models like the Biowin1-7 of the US EPA, CASE Ultra from Multicase, Model Applier from Leadscope, VEGA, OECD QSAR Toolbox and many others, which could be of interest for an API designer. Two essential challenges for the prediction of environmental biodegradation are the sometimes poor reproducibility of data caused by the variability of bacterial populations and the lack of qualitative experimental data.[62] However, high quality experimental data is a prerequisite for good predictions and needs to be generated by further research. Some available (Q)SAR software packages (*e.g.* CASE Ultra from Multicase and ModelApplier from Leadscope) allow the user to integrate new data into models. Thus, within experimental research, results can be used to amend existing databases and establish models for prediction of biodegradability in order to improve the quality of predictions.

To increase the reliability of results, predictions *via* (Q)SAR based models can be expanded, *i.e.* as *in silico* test batteries, using the Read-Across approach, which is accepted for the assessment of chemicals according to the REACH regulation (Annex XI, 1.5.). Read-Across is a technique used for the prediction of a property for one substance (target), by using data on the same property for (an)other reference substance(s). Structural similarity, *e.g.* regarding common functional groups, is a prerequisite for the grouping of substances and subsequent application of the Read-Across approach.[63,64] The ECHA developed the Read-Across Assessment Framework as an internal tool for examining predictions about environmental fate based on Read-Across and according to the REACH regulation. It is emphasized that expert judgement is required to use this approach.[63] To use Read-Across to determine fate during biodegradation, environmental conditions must be defined using proper judgement during the testing of the reference substances. However, recently Fenner *et al.*[65] have demonstrated broader application of Read-Across: half-lives from biodegradation tests with mixtures of chemicals in activated sludge can be used to predict half-lives in soils. Thus, extensive work and resource-intense regulatory simulation studies might no longer be needed. In theory, bacterial populations in soil and activated sludge could be functionally similar regarding the enzymatic degradation of diverse molecules of environmentally relevant concentrations.[65] A practical quantitative Read-Across procedure is proposed for the prediction of Ames mutagenicity by Benigni.[66] Although environmental biodegradability has not been addressed, this specific approach can help in using Read-Across in a simplified, standardized, and reliable manner, as is the case for (Q)SAR. This approach can be especially useful for parameters for which (Q)SAR has limitations.[66]

In vitro. A plethora of *in vitro* biodegradation tests are available.[67–69] However, standards, *e.g.* ISO 7827, ISO 9408, ISO 14593,[70] and OECD tests reflecting the latest state of science and techniques are accepted internationally as standard methods. OECD tests (series 301 for ready biodegradability, test 308 for aquatic sediments) are recommended by the EMA for the environmental risk assessment of pharmaceuticals.[71]

Since the biodegradation tests vary in conditions (type of inoculum, concentration of substance(s), light and oxygen availability, and detection and evaluation method), different types of biodegradability can be determined (Table 1).

Table 1 Definitions regarding biodegradability[72]

Ultimate biodegradation	Complete metabolism and thus mineralization
Primary biodegradation	Any chemical change caused by microorganism involvement
Readily biodegradable	Generally used to indicate a chemical having passed OECD tests for biodegradability, typically agreed upon to indicate that a chemical will biodegrade completely in aquatic environments with aerobic conditions
Inherently biodegradable	Undeniable evidence of biodegradation in a test has been demonstrated
Half-life ($t_{0.5}$)	Time it takes for 50% of a substance's chemical composition to change in a biodegradability test when first-order kinetics can be used to describe this change
Disappearance time 50 (DT50)	The time it takes for the initial concentration of a compound to halve during a biodegradability test

Table 2 Biodegradability test choice[75]

Test	Analytical method	Suitability for compounds which are:		
		Poorly soluble	Volatile	Adsorbing
DOC Die-Away (301 A)	Dissolved organic carbon	−	−	+/−
CO_2 Evolution (301 B)	Respirometry: CO_2 evolution	+	−	+
MITI (I) (301 C)	Respirometry: oxygen consumption	+	+/−	+
Closed Bottle (301 D)	Respirometry: dissolved oxygen	+/−	+	+
Modified OECD Screening (301 E)	Dissolved organic carbon	−	−	+/−
Manometric Respirometry (301F)	Oxygen consumption	+	+/−	+

According to the concept of BbD, ready and ultimate biodegradation is the goal, *i.e.* complete degradation resulting in full mineralization that rapidly initiates once a pharmaceutical has exited the body, in order to enable complete degradation even in surface water.[72–74] This is needed since 80% of wastewater worldwide is not treated due to a shortage of treatment facilities.[3] Thus, the tests for ready biodegradability (OECD test series 301)[75] are highly recommended during drug discovery. The test choice within series 301 is primarily dependent on solubility, volatility, and absorbance of the compound (Table 2). For example, the Closed Bottle Test (CBT) and the Manometric Respirometry Test (MRT) are suitable for a broad range of properties, *i.e.* solubility, volatility, and absorbance.[75]

When putting BbD into practice, the CBT and MRT were found to function as suitable elements of BbD.[67,68,76,77–79] During the CBT (OECD 301 D), the compounds to be tested are treated with an inoculum of low concentration (10^4–10^6 cells per L) *e.g.* received from secondary effluent from a domestic sewage treatment plant or from surface water. The inoculum for the MRT (OECD 301 F), on the other hand, is received, *e.g.*, from activated sludge or sludge effluent and has a higher concentration (10^7–10^8 cells per L). Thus, ready biodegradability during the CBT is the gold standard for BbD, because a compound that biodegrades readily during the CBT will degrade readily in the environment. Furthermore, during the MRT, solubility problems may occur for poorly soluble compounds, since 10 times as much test substance as in the CBT (50–100 mg ThOD L^{-1} *vs.* 5–10 mg ThOD L^{-1}) is used.[75]

However, the 301 OECD tests take 28 days and may be time intensive from an industry perspective. As mentioned previously, the test phase of the DMTA cycle is attempted to be shortened by the industry.[37] Approaches to OECD test simplification already exist. During the OECD test 301 D, the monitoring of oxygen consumption using the optode method instead of the laborious electrode method saves time and effort as well as having analytical advantages.[80] Furthermore, efforts have been made to simplify the elaborate water-sediment test 308 of the OECD. Thereby, the use of an artificial and standardized medium and the measurement of pressure difference in closed vessels (OxiTop®) turned out to be an easier screening method.[81] These examples indicate that there are opportunities to simplify the standard tests.

Furthermore, high throughput screenings are demanded for competitive drug design where time is a crucial factor. AstraZeneca developed a high throughput biodegradation screening test (HT-BST) based on a colorimetric assay for aromatic compounds. It was thereby evaluated for use as a high volume test method for the screening and prioritization of compounds based on their biodegradability equal to OECD tests for ready biodegradability. 20 000 tests could run in parallel and for the same cost as an analogue OECD standard test.[82] This HT-BST reveals to be a good indicator of relative biodegradability and a tool to prioritize APIs and to investigate the structural rules of biodegradation toward establishing reliable *in silico* models.[62,83] Structure–property relationships have been derived from this HT-BST. One example of this is the influence of the electrophilicity of the substituent group at the *meta* position of phenols on their biodegradability, as indicated by the statistically significant correlation with the substituent's Hammett constant on this *meta* position. A general trend was found for increased biodegradability through decreased electrophilicity of the substituent at the benzene ring. These findings could serve as future design rules. However, the HT-BST has limitations as mineralization is unexplored and TPs that may interact with the color reagent are not considered, which could lead to a false-negative result. Thus, the combination with high throughput screenings developed for ultimate degradation[84] is recommended, so as to take advantage of both methods.[82]

Workflow scenarios of BbD and case studies of greener APIs

We can differentiate between two procedures of BbD: *de novo* design and re-design. The *de novo* approach is defined as design from scratch, whereas re-design aims to improve already existing molecules by structure variation.[2] In the case of re-design, the complex optimization process discussed above might be less challenging, as already existing knowledge about the starting molecule is available.

Furthermore, we can differentiate between two types of molecule generation as the crucial starting point for the workflow of BbD: targeted and non-targeted synthesis.[21] In targeted synthesis, structural features known to improve properties are intentionally inserted into the molecule, *e.g.* computer aided. In doing this, expert knowledge on design rules regarding desired properties can help lead to successful development of compounds satisfying the concept BbD. However, the optimization process *via* stepwise structural variation can be time-intensive. Every step includes an *in silico* screening ((Q)SAR,

Docking) for pharmaceutically important properties (*e.g.* ADMET) and further *in vitro* testing of promising compounds (selected ADMET endpoints, stability, and environmental biodegradability). Finally, the most promising candidate(s) can be investigated during subsequent preclinical development. Non-targeted synthesis, on the other hand, is based on an undirected, arbitrary generation of a large pool of compounds, for example *via* photolysis, which can be screened simultaneously. Therefore, less expertise is necessary for this "quick and dirty" approach, which is also cheaper than targeted synthesis.[21] Even an arbitrarily generated hit without any grounded influence can be found, as a matter of luck, and there are examples of success presented as a case study for non-targeted re-design at the end of this section.

The combination of procedures and molecule generation leads to four approaches to designing new compounds, namely non-targeted *de novo* design, targeted *de novo* design, non-targeted re-design, and targeted re-design (Table 3).

Non-targeted *de novo* design. Typical API discovery presents an appropriate example for non-targeted *de novo* design. Starting with thousands of molecules from large databases, this process can be modified for the purpose of BbD if environmental considerations are implemented in the screening and optimization process. The typical API discovery process and potential environmental considerations are explained in detail in the next section.

Targeted *de novo* design. Fragment-based methods for API discovery are a good example of targeted *de novo* design. Small structural fragments are combined to make new more complex molecules through the use of expert knowledge and information on the structure of fragments and their activity.[85] Considering design rules for biodegradability, API discovery using this method can be modified according to BbD. For example, 3-β-D-galactopyranosyloxymethyl-4-sulfatomethylfuran was selected as the starting compound for a new mode of action. By systematic structural variation, a pool of compounds was designed and tested regarding efficacy. At the same time, biodegradability was considered by environmental chemists involved in the design and selection of compounds with improved properties.[86]

Non-targeted re-design. Studies by Rastogi *et al.*[24,25,79] present examples of non-targeted re-design for environmental biodegradability, using non-biodegradable β-blockers such as propranolol (Fig. 2). In this approach, mixtures of derivatives of the starting compounds were generated arbitrarily, *e.g. via* photolysis (alternative to the strategy of Rastogi *et al.*: *in silico* prediction of TPs). As a rule, oxidation such as hydroxylation took place, so that degradation-favored oxygen could be introduced into the molecule. Subsequently, the mixtures were screened using an *in vitro* assay for environmental biodegradability, *e.g.* the CBT (OECD 301D[75]). After structure elucidation of potential biodegradable derivatives with an intact drug moiety, these were investigated regarding druglikeness, absence of mutagenicity and genotoxicity, as well as other ADMET parameters using *in silico* tools (QSAR, Docking). Then, promising candidates were synthesized and experimentally tested for environmental biodegradability, pharmaceutical activity, selected ADMET endpoints, and behavior of main metabolites.[24,25,79] In the case of propranolol, the derivative 4-hydroxypropranolol was identified to be an appropriate candidate, having an experimentally determined EC_{50} in a receptor assay for the desired activity in the same range as propranolol.[25] Most promising candidates could be investigated during subsequent preclinical development.[21]

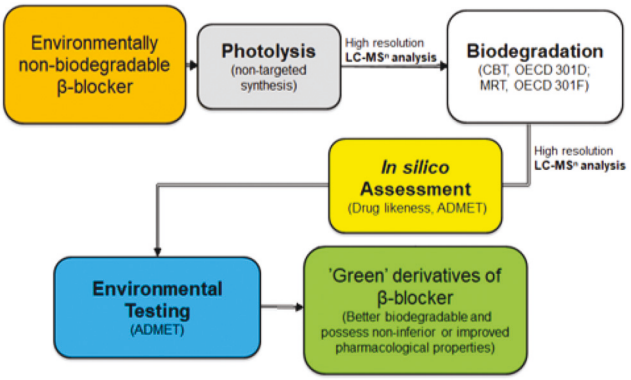


Fig. 2 The re-design of β-blockers for environmental biodegradability through non-targeted synthesis of derivatives and subsequent screening, taken from Kümmerer[2] and marginally modified.

Targeted re-design. The targeted re-design approach was applied to improve the antibiotic ciprofloxacin, since it is poorly biodegradable in the environment, mainly because of the fluorine atom on the aromatic ring.[54,55] As a rule of thumb, electron-withdrawing substituents make a ring less favorable for an attack by oxygenase enzymes using electrophilic oxygen.[26] Occurrence in the environment, given the highly antibiotic effect of this fluoroquinolone, is quite concerning with regards to potential increased microbial resistance. Ciprofloxacin was re-designed *via* systematic variation of functional groups at the R^1 and R^2 positions on the core structure (Fig. 3) using multiple types of side chains.

Table 3 Four approaches according to the concept of BbD by the combination of procedures and molecule generation

Approach	Description
Non-targeted *de novo* design	Computational screening of large number of *in silico*-compounds for required properties
Targeted *de novo* design	Combination of small structural fragments to new more complex molecules by expert knowledge
Non-targeted re-design	Non-targeted structural variation of existing molecules and testing to improve properties
Targeted re-design	Targeted modification of existing molecules by expert knowledge to improve properties

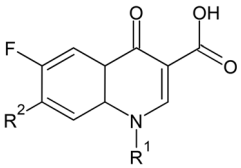


Fig. 3 Core structure of antibiotic ciprofloxacin. R^1 and R^2: positions for systematic substitution.

In silico prediction of the properties of these designed derivatives led to promising candidates. The structures Cipro-Prolin and Cipro-P2C were patented as active but at the same time environmentally biodegradable quinolone antibiotics.[22] Another patent applies for environmentally degradable quinolone antibiotics with hemiaminal structural feature, *i.e.* the hemiaminal-linker of Cip-Hemi and CG008-Hemi as well as further substituents at C7 of fluoroquinolone.[23] Cipro-Prolin and Cip-Hemi are the most successful candidates (Fig. 4) with increased degradability in the environment and only slightly decreased antibiotic activity compared to ciprofloxacin, which is one of the most active fluoroquinolones. Their degradation *via* hydrolytic cleavage of the amide or the hemiaminal, respectively, leads to a core structure (still including R^1 or R^2, respectively) with highly reduced antibacterial activity and consequently to a possibly reduced selection pressure in the environment. The hemiaminal is unstable at low pH values, but showed sufficient stability in humans (liver, blood serum).[54,55] The success of this project is demonstrated by the acquisition of patents for these greener active compounds. However, further effort is needed to design an environmentally degradable core structure to achieve the gold standard, complete mineralization. Research is currently being conducted on this.

Further examples for (unintendedly) biodegradable APIs

There are examples of biodegradable APIs from the anti-cancer drug group that typically are quite stable and have concerning effects on the environment. The chemical structures of anti-cancer drugs, such as ifosfamide and 5-fluorouracil, were changed by glycosylation to improve drug efficacy or the treatment spectrum, incidentally leading to increased environmental biodegradability.[77,78,87] For example, the anti-cancer drug 5–fluorouracil, acting as a uracil anti-metabolite, was

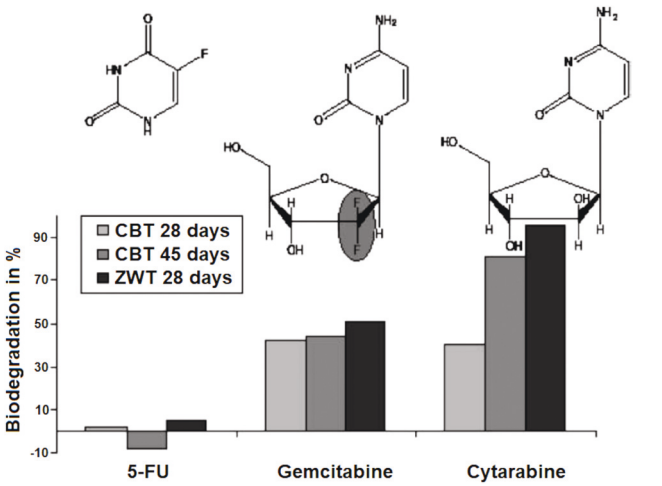


Fig. 5 Results of the CBT and ZWT reported by Kümmerer,[73] which illustrate the increased biodegradability after the re-design of 5-fluorouracile *via* addition of the arabinose or doubly fluorinated arabinose moiety to the structure of cytosine.

modified to gemcitabine (2′,2′-difluorodeoxycytidine) and cytarabine (cytosine arabinoside) using doubly fluorinated arabinose and arabinose, respectively. Gemcitabine was biodegraded to 42% and 50% in the CBT and Zahn–Wellens Test (ZWT, OECD 302 B), respectively. Cytarabine showed between 40% to almost 80% biodegradation in the CBT, depending on the treatment period, and more than 95% in the ZWT.[77] The molecular structures and results of these tests are illustrated in Fig. 5.[73] Even if these results are mainly below the 60% threshold required to deem a substance readily biodegradable according to OECD guidelines, they are better biodegradable than 5-fluorouracil, which is due to the added arabinose and doubly fluorinated arabinose moieties. Cytarabine is better biodegradable than gemcitabine, because the sugar moiety without fluorine atoms allows for environmental biodegradation of the cytosine moiety.[77] There are exceptions to this. Gemcitabine and cytarabine were found to have the same mode of action and similar or improved pharmacological properties.[73] Thus, cytarabine and gemcitabine present greener alternatives to 5-fluorouracil. However, since they are less stable than their parent compound and degrade under acidic conditions in the human stomach, they must be administered intravenously instead of orally.[88] Improving the delivery during

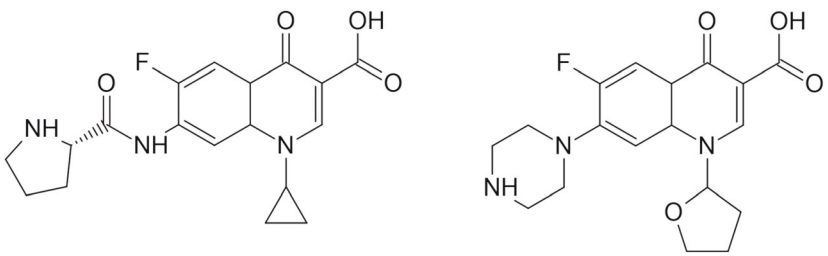


Fig. 4 Structures of the most successful antibiotic candidates. Cipro-Prolin[22] (left) and Cip-Hemi[23] (right).

Table 4 Biodegradability of selected pharmaceuticals from various classes in different OECD tests, taken from Kümmerer and Hempel.[27] Criterion for ready and inherent biodegradability (301 and 302 tests, respectively) is >60%

Active compound	OECD 301 D	OECD 301 F	OECD 302 B
Isosobiddinitrate	>90		
Mesalazine	>90	>90	
Acetylsalicylic acid		81	
Penicillin V (Phenoxymethylpenicillin)	27		>90
Glufosfamide	53		72
Piracetam		>90	>90
Hydroxamic acid	50	90	
Valproic acid	72	78	
Cytarabine	40		>90

drug development, however, can help researchers to overcome the challenge of pH dependent stability within a sustainable approach.[89]

Another example of the fact that glucosidation can increase biodegradability is that of glufosfamide (β-D-glucosylisophosphoramidmustard), which resulted from improving structurally related ifosfamide (not biodegradable) for increased resorption and reduced side effects. Although the core structure of ifosfamide is not present anymore, glufosfamide (readily biodegradable) also belongs to the class of nitrogen mustard-derived alkylating agents[78] and made it into late clinical development.[87]

Furthermore, there are existing marketed high-volume APIs that are biodegradable in the environment.[27] These active compounds (Table 4) demonstrate that pharmacologically relevant properties are not generally speaking in contrast to environmental biodegradability.

Some pharmaceutical companies published the results of environmental risk assessments, including environmental degradation tests, in order to show their engagement in tackling the issue of pharmaceuticals in the environment.[90,91] Among the listed pharmaceuticals, there are several examples of APIs showing inherent biodegradation, *e.g.* Rosiglitazone,[90,91] APIs even showing ready biodegradation, *e.g.* Interferon alfa-2a and Enfuvirtide, as well as the complete group of monoclonal antibodies, such as Emicizumab and Ocrelizumab.[91] ß-Lactams are known to be sensitive against hydrolysis at certain pH, even though, they are used in high amounts. The prodcuts of hydrolysis are inactive and often readily biodegradable (*e.g.* Penicillin V, see Table 4, Amoxicillin). This again shows that pharmacological properties are not necessarily in conflict with biodegradability in the environment.

Discussion: implementation of BbD into the generic design process

The main goal of the common drug discovery process is to find new APIs that result in a new medicine that is safe, efficacious, and of assured quality according to legal requirements. The BbD approach simply adds environmental aspects to be included into the understanding of safe, efficacious, and of assured quality. Accordingly, the understanding of pharmacovigilance is extended to *eco*-pharmacovigilance, thereby including unwanted side effects of APIs in the environment. To reduce or even better to avoid unwanted effects by the presence of APIs in the environment, targeted design for environmental degradation or complete mineralization, respectively, is indispensable. The shown examples of marketed APIs and APIs designed by academia that are environmentally biodegradable, whether intended or not, indicate that it is feasible to design compounds that are both more environmentally biodegradable and (still) active.

Tailored to the industrial process, environmental considerations according to BbD should be included early during the drug discovery process, *i.e.* where they are most effective and the chances of introducing biodegradability are the greatest. It is proposed to add a filter for environmental biodegradability to the multi parameter space of the optimization process, since at this stage many compounds are designed and tested in cycles of many sets with high flexibility to find candidates that combine all required properties into one structure. It has been shown through many examples, such as the hypothesis-driven drug design by Plowright *et al.*, that the optimization phase is appropriate for lowering overall attrition and costs.[37]

Within this early phase, the use of *in silico* tools can help to prioritize compounds for synthesis within the DMTA cycles. The presented workflows by Leder *et al.*[21] and Rastogi *et al.*[25] show this combinational approach for BbD, even if not tailored to the industrial API discovery process. The implemented hERG test strategy, for example, demonstrates on an industrial level that the combination of *in silico* and *in vitro* methods is a powerful strategy and very strongly demanded. In the same effective way, environmental biodegradability should be tested as one of many filters during the optimization cycles.

Another fundamental reason for early implementation is that environmental biodegradability of an API is already influenced (unintentionally) and can even be increased within the optimization phase which is discussed in the following section.

Hit and lead optimization – impact on environmental biodegradability of APIs

For the improvement of ADME properties, it is suitable to apply Lipinski's rule of five as a rule of thumb for druglikeness, since most drugs are administered orally. For example, the rules on the presence of no more than 5 H-bond donors and 10 H-bond acceptors balance hydrophilicity for solubility against absorption as well as lipophilicity for membrane permeability. This balancing is also reflected in the rule stating log *P* should be below 5. These rules are generally important for API's pharmacokinetic (ADME) properties and beneficial for cellular uptake, be it for human cells or for microbial cells, including those in the environment. The same holds true for

the rule on molecular weights smaller than 500 Dalton,[92] which also has a positive effect on biodegradability.

Reducing human toxicity can affect biodegradability in the environment in various ways, depending on how the potential candidate is modified to alleviate a given mechanism of toxicity. For example, hypersensitivity and immune response, caused by greater compound reactivity, as well as off-target effects, caused by low drug specificity, can be alleviated through increasing size or branching with the aim of reduced reactivity and/or improved API specificity.[93] Both approaches (reduced reactivity, improved specificity) may decrease biodegradability due to a more complex structure. However, there are many exceptions since this depends on the added functional groups and the entire molecule. For example, monomethyl, in contrast to trimethyl, branching does not hinder biodegradation.[26] Further strategies, also later within drug development, like improving on-target specificity *via* controlled release formulations, can also reduce toxicity due to improved delivery, targeting, or specificity without the decrease in environmental biodegradability.

Increased stability is aimed at ensuring the function and quality of the API from shelf to target site. Stability is influenced by temperature, humidity, and light, among other factors, and can be tested according to ICH Q1A guidelines. The latter defines the exemplary stability data package for a new drug substance or drug product but leaves sufficient flexibility to consider varying practical situations due to specific scientific reasons.[94] High stability can strongly reduce biodegradability in the environment. We want to emphasise that extreme stability is superfluous due to the following reasons.[73]

Stability, like reactivity, is not an intrinsic property; in addition to the stereochemistry and electronic properties of the molecule, it also depends strongly on the environmental conditions present. Thus, at varying stages along the life-cycle of the substance (from shelf, human body, wastewater, to the environment, *e.g.* surface water) with varying conditions, the potential for degradation varies as well. For example, microbes present in the human gut (mostly anaerobic) and in sewage treatment plants or surface water (mostly aerobic) as well as their enzymatic portfolio and activity are different due to differences in pH, the availability of nutrients (type and concentration), and access to light. In case compounds are sensitive to light, packing in brown glass or brown blisters protects the compound against light. This protection is not present after the API has been excreted or is present in surface water, allowing for its photolytic degradation.[73] Therefore, biodegradability in the environment is not synonymous with biodegradation in the human body, *i.e.* metabolization. Design for environmental degradation and mineralization of APIs is not prohibitive for stability during application, efficacy, and other properties necessary for an API to be applied as a pharmaceutical, as demonstrated with the example of the patented antibiotic Cip-Hemi.[54,55] Thus, at this point rethinking must take place, as stability from shelf to target site in the body does not necessarily contradict ready biodegradability in the environment. Small changes in the chemical structure of a pharmaceutical can drastically alter its environmental biodegradability.[73] This fact needs to be considered during the variation of lead structures.

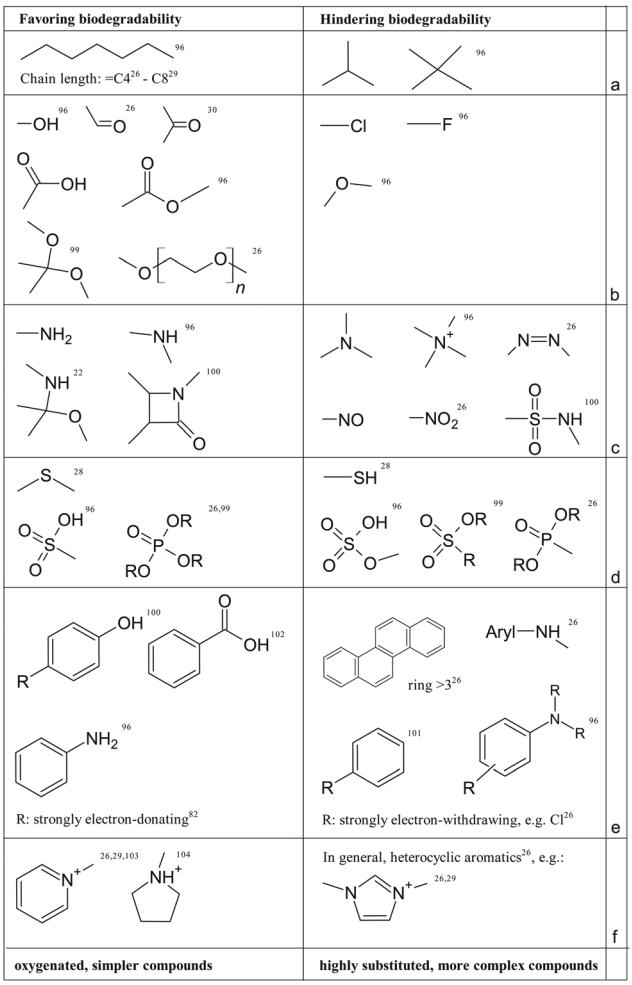
Consequently, we have to be aware of the impact of optimization (*i.e.* reducing toxicity, increasing stability) on environmental biodegradability. Drug designers could balance the multi parameters for new derivatives in such a way that introduced structural features also benefit, or at the very least do not hinder, environmental biodegradability. The application of design rules could support both stability during application and environmental degradability to ensure full functionality, meaning good performance along the entire life cycle of the drug in the understanding of complete pharmacovigilance, *i.e.* *eco*-pharmacovigilance.[20,95]

Design rules for biodegradability

Biodegradation depends on microbial bioavailability, which is influenced by molecule size, because larger molecules (>1000 Da) are less readily taken up by bacterial cells, and by hydrophobicity; the substance should not be too hydrophilic or hydrophobic. These criteria fit nicely with the requirements for the oral administration of drugs.[92] After uptake of the substance, its metabolism inside of the bacterial cells is affected by structural features of the molecule and impacts biodegradability. Thereby, the availability of enzymes plays a crucial role. Widespread esterases show broad specificity and are therefore ubiquitously involved in biodegradation processes. Oxygenases catalyze the insertion of oxygen when molecular oxygen is present. This initiating degradation step is beneficial for small molecules that already contain oxygen.[26] There are many well-known rules of thumb (Table 5) for structural features that favor or hinder biodegradability. For example, unbranched alkenes are better biodegradable than branched alkanes.[98] The main pathway of alkane degradation is terminal oxidation, which is widespread among bacteria. The respective alcohol is dehydrogenated by dehydrogenases *via* the corresponding aldehyde to form the fatty acid, which is then degraded gradually *via* β-oxidation to C2 units as acetyl-CoA.[96] In addition to the shown structural features, natural structural elements such as amino acids,[22] peptides,[97] or sugar moieties[77] can promote biodegradation as well. Thereby, the stereochemistry must be considered. Naturally occurring L-amino acids and D-saccharides are favored for BbD since they fit well with enzymes in the environment.

A comprehensive view of the presented examples for biodegradable APIs (Table 6) reflects the rules of thumb, *e.g.*, that oxygenated molecules of medium polarity favor biodegradation since hydroxy groups are often a good starting point for further biodegradation. In many cases, hydrolysis of bonds like glycosidic linkages, amides, and esters is expected to initiate biodegradation processes. However, in contrast to the β-lactam antibiotics Amoxicillin and Penicillin V, structurally related Piperacillin does not represent a fully biodegradable API in spite of the hydrolysis of β-lactam. The benzene ring in contrast to the phenol (Amoxicillin) and phenoxy (Penicillin V) moiety prevents further degradation, as

Table 5 Rules of thumb for structural features regarding biodegradability



Categorized into a) alkanes, b) oxygen and halogen compounds, c) nitrogen compounds, d) sulfur and phosphorus compounds, e) aromatics, f) heterocycles.

well as the tertiary amino groups.[100] As for Amoxicillin, but also 4-hydroxypropranolol, phenol rings can be oxidized at the *ortho* position by monooxygenases.[96]

The design rules can be considered as guidelines for the design of environmentally biodegradable APIs.[26–28,30,98] However, the entire molecule and its biodegradation mechanisms must be taken into account. For example, degradability depends also on the position(s) of the substituents (*e.g.*, halogens) and on the degree of substitution (*e.g.* by methyl groups).[26] Furthermore, there are always exceptions. For example, the quaternary ammonium group of choline is expected to hinder biodegradability, as demonstrated for cations of ionic liquids (ILs) with cholinium covalently bound to amino acids.[29,105] However, ILs with choline (cation) and several amino acids (anion) showed ready biodegradability.[26,106] Thus, after first indications of biodegradability have been gathered using rules of thumb, the entire molecule must be evaluated considering the reaction conditions to avoid generalization. And even with the application of expert knowledge in biochemistry, testing must be carried out to determine actual biodegradability because of the complex occurring interplay outside and inside of the bacterial cell (*i.e.*, between structural properties needed for uptake and metabolization with selective enzymes and changing reaction conditions). For example, Zumstein and Fenner[97] recently investigated the deviating stability of peptide-based antibiotics in blood plasma and in solutions with extracellular wastewater peptidases as a potential BbD approach. However, rules of thumb as well as *in silico* tools are important to give guidance and to speed up the total process of API discovery.

Enhanced process flow for the design of APIs mineralizing readily in the environment

Based on the reviewing process and the discussion regarding the implementation of the concept of BbD into generic API discovery, an enhanced process flow for the design of environmentally biodegradable APIs has been developed (Fig. 6). The modified DMTA cycle as key element of the enhanced optimization phase is zoomed out and explained in the following.

Design of new compounds (design). At the start of the DMTA cycle, the lead structures are designed by considering the structural features, for which effective collaboration between experts is required. The designed leads are screened by an available and appropriate *in silico* model in order to prioritize leads for synthesis. If there is no suitable model available, *e.g.*, because the structures do not belong to the applicability domain of the model, the proposed Read-Across approach can remedy this. Note that Read-Across should also be used to evaluate results of (Q)SAR models. In the long term, machine learning and other pattern-recognition strategies can be used in conjunction with expert-based Read-Across. Data generated according to standard methods can be of great use for later purposes, mainly as a basis for new models. Therefore, it is important that the data is made available to the community through an open database.

Synthesis of designed compounds (make). The results of the computational analyses will be confirmed through *in vitro* tests. Therefore, promising candidates will be synthesized, *i.e.* compounds predicted to be biodegradable besides optimized regarding all pharmaco-relevant properties like activity.

Testing in various assays (test). For a first screening of many compounds, high throughput tests such as the outlined HT-BST by Martin *et al.*[82] might be an appropriate *in vitro* method to indicate biodegradability and prioritize APIs for intensive testing. For this testing, the presented standardized *in vitro* methods that are provided by OECD are suitable options, especially the CBT due to reasons discussed above. However, there is a need to develop corresponding methods further to enable high throughput screenings.

Analysis of test results (analyze). All generated data is analyzed and considered for the design of further compounds to run the next cycle. Candidates that fulfil all criteria, also environmental biodegradability, are transferred to preclinical

Table 6 Comprehensive consideration of discussed biodegradable APIs: assignment of the APIs to the expected starting reaction of the degradation process based on structural features leading to biodegradable TPs

Expected starting reaction	Active compound	Structural feature
Hydrolysis of glycosidic linkage	3-β-D-Galactopyranosyloxymethyl-4-sulfatomethylfuran	β-D-Galactopyranose, furan, sulfate
	Cytarabine	Arabinose, amino-pyrimidin-2-on
	Gemcitabine	Fluorinated arabinose, amino-pyrimidin-2-on
	Glufosfamide	D-Glucose, phosphoramide
Hydrolysis of amide/peptide, lactam, hemiaminal, and/or ester	Amoxicillin	β-Lactam, amide, amine, phenol, carboxylic acid
	Penicillin V	β-Lactam, amide, phenol, thioether, carboxylic acid
	Piracetam	γ-Lactam, amide
	Rosiglitazone	Amide, thioester, phenol, 2-pyridinylamin
	Cipro-Prolin	L-Proline
	Enfuvirtide	Peptide
	monoclonal antibodies, *e.g.*, Emicizumab and Ocrelizumab	Peptide
	Cip-Hemi	Hemiaminal ether
Possible further oxidation due to electron donating substituent; and ring cleavage	4-Hydroxypropranolol	1.4-Dihydroxynaphthaline
	Acetylsalicylic acid	2-Acetoxybenzoic acid
	Mesalazine	*o*-Hydroxy-benzoic acid, amine
β-Oxidation (*cf.* fatty acid degradation) *via* acetyl-CoA pathway	Valproic acid	Carboxylic acid

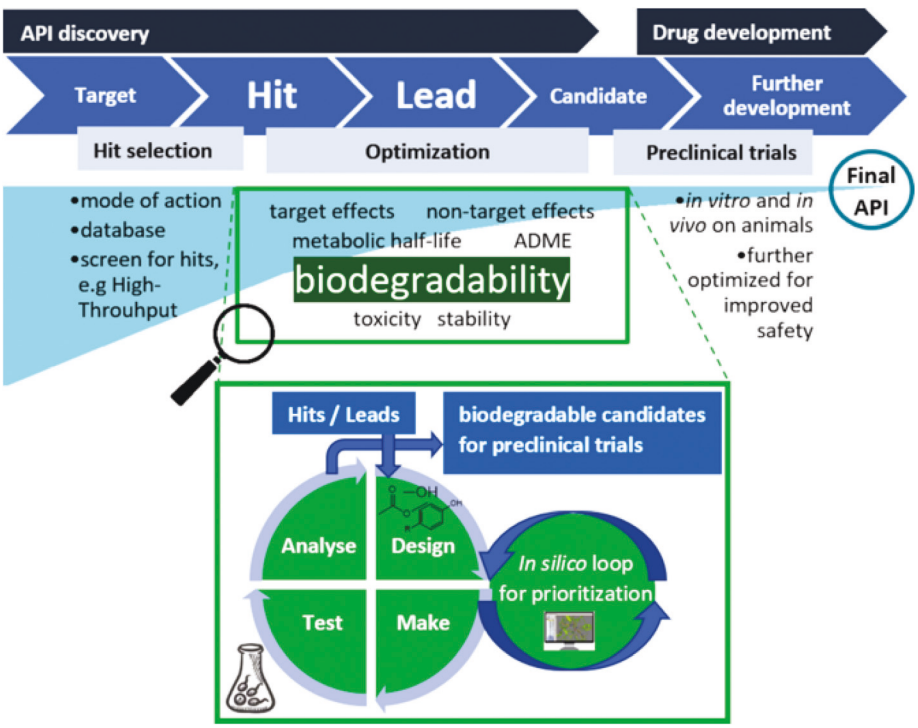


Fig. 6 Common API discovery enhanced by the concept of BbD during the optimization process.

development. All data, independent of results, should be made available to the community, preferably in open databases, and quality criteria need to be highlighted with the long-term objective of developing *in silico* models.

If minor structural changes cannot lead to success, there are other options. Namely, the improvement of drug delivery systems, *e.g.* by nanoencapsulation using appropriate excipients, can lead to increased bioavailability and improved delivery and

efficacy.[107] This leads to smaller doses and finally to reduced input into the environment. Another possible solution is the design of biodegradable non-covalent derivatives (NCD) as cocrystals to improve physicochemical properties.[104] For example, the stability of gemcitabine (presented above) was improved reasonably *via* co-crystallization using *p*–toluenesulfonic acid to develop an orally available anti-cancer prodrug.[108]

Challenges and perspectives

We go along the DMTA cycle to discuss challenges and perspectives of enhancement through the concept of BbD. Starting with the design phase, the dedicated design team must consider many parameters, always giving the highest priority to safety, followed by efficacy and quality. It could be discouraging to consider yet another parameter. However, examples such as the patented CIP-Hemi[23] demonstrate business opportunities for new benign APIs.[2] Additionally, there are some industrial pharmaceuticals without targeted design which indicate the feasibility of BbD, such as valproic acid,[109] acetylsalicylic acid,[4] and ß-lactams (see also Table 4 and mentioned industrial databases).[100] When compared to chemicals, the targeted design of environmentally mineralizing pharmaceuticals poses greater challenges because of further requirements such as pharmaceutical activity. Yet, if the application of BbD is possible for this demanding substance class, it should also be possible for many other chemicals.[13] Indeed, many chemicals, such as linear alkylbenzenesulfonates, EDDS, and organic phosphorous esters, have been designed already for mineralization in the environment.[73]

Furthermore, it is essential to understand to which extent environmental biodegradability is affected by improvement of other parameters, *e.g.* toxicity and stability. Thereby, stability does not contradict environmental biodegradability *per se* because of their strong dependence on system conditions. In fact, sometimes these factors change parallel to one another (see above *e.g.* Lipinski's rule of five), assuming the absence of extreme stability. Thus, an important paradigm shift, especially among conservative stakeholders, is needed to avoid superfluous extreme stability and make BbD feasible.

To push forward this paradigm shift, it is recommended that legal requirements for API stability are adjusted to more realistically fit needs during storage and use without preventing degradation after use. Thus, a more nuanced approach to designing drugs for "stability" is advocated, similar to biologics (*e.g.* antibodies, proteins, nucleic acids or even viruses and cells). As these biopharmaceuticals are sensitive to environmental factors (*e.g.*, temperature changes and light), specific requirements regarding stability have been developed (ICH guideline Q5C)[110] to overcome limitations regarding storage, for example. The application of biologics demonstrates that requirements for stability can be adjusted to a reasonable amount. However, if natural compounds are modified such structural modifications may lead to reduced environmental biodegradability. More data and knowledge have to be gained to provide a better understanding of the need and feasibility of BbD for biologics.

Within the design phase, *in silico* tools are an integral part of the conventional DMTA cycle. They can facilitate and accelerate the consideration of ready environmental biodegradability, too. However, sufficient qualitative experimental data is missing for the development of high quality (Q)SAR models for biodegradability prediction, because not enough testing is done and because of sometimes weak reproducibility of biodegradation due to test-specific parameters. Data gaps need to be filled by intensive experimental testing of ready environmental biodegradability according to standardized methods such as OECD methods. OECD methods are highly recommendable as they are also used in a regulatory context for chemicals, resulting in additional data, which can be used to build better models. In terms of efficiency, pharmaceutical companies, academia, and authorities should cooperate and share knowledge in order to combine the various strengths and prevent the waste of resources by duplication. This is in line with the philosophy of the EU strategy for sustainability in the broader field of chemicals.[13] Furthermore, machine learning, which should also be used to collect and publish qualitative data, as well as the Read-Across approach are highly desirable to help overcome limitations of existing (Q)SARs. Therefore, open-mindedness is essential[50] which can be promoted by highlighting existing incentives.

After the design phase, *in silico* predictions need to be confirmed for prioritized and synthesized compounds by an appropriate OECD method. However, the time-intensive biodegradation tests of 28 days according to OECD could be unattractive for industrial application, as currently attempts are made to shorten the DMTA cycle, *e.g.* to 10 days for 80% of experimental data within this phase.[37] The development of the aforementioned HT-BST offers promising solutions. These also take 28 days, which is absolutely needed to investigate complete environmental fate up to mineralization, but allow for the study of numerous compounds. Further research in this direction is needed as this approach is practice-oriented, and therefore of great interest for the proposed advanced DMTA cycle. Ideally, HT-BST should investigate ready biodegradation, including environmental mineralization of a broad range of designed lead structures.

Existing incentives include current political developments, clearly recognizable in EU strategies for a toxic-free environment.[13–15] These can be seen as upcoming unwanted limitations, but even better as new business opportunities for biodegradable APIs given increased competitiveness of sustainable EU industries. Furthermore, the effective contribution to greener pharmacy through the design of environmentally degradable APIs (compare Sustainable Development Goals 3, 6, 9, 11 and 12)[53] can be seen as a social mandate. Additionally, to be one of the frontrunners of greener pharmacy can be adapted as part of a marketing strategy. To add another incentive, the high costs and time effort required for an extensive environmental risk assessment could be saved

since this is not or only to a small extent necessary for environmentally biodegradable APIs. Longer patent lifetime, fast track approval, and public reputation can all be considered as new incentives. Thus, existing and coming incentives should encourage stakeholders to try out the design phase enhanced by BbD and to fill identified data gaps by further research.

When companies recognize that the discovery of environmentally biodegradable APIs is a business opportunity,[2] the willingness to make changes grows and the identified challenges can be tackled. Examples for APIs satisfying the concept of BbD push forward the discussion on its feasibility and demonstrate business opportunities. Among them are ß-blockers with retained activity[24,25,79] and recently approved patents for new APIs,[22,23] both designed for better biodegradability.

Conclusion

The design of APIs readily mineralizing in the environment (Benign by Design, BbD) is an effective measure toward avoiding the presence of pharmaceuticals in the environment and is therefore urgently needed. Reviewing literature regarding the common API discovery process as well as case studies of BbD, including test methods and tools for biodegradation, has enabled us to demonstrate the feasibility of BbD and to propose its implementation into commonplace API discovery procedures. These results should encourage pharmaceutical companies to invest in research and development, as well as support them to be prepared for upcoming legal changes, since the above-mentioned EU strategies announce further political regulations.

The optimization phase seems to be the most appropriate step to implement environmental considerations. Thereby, pharmacological parameters are not necessarily in conflict with biodegradability in the environment. However, the success of BbD within the DMTA cycle depends on the availability of suitable *in silico* tools and test methods. Since high quality data needed for the development of *in silico* test models is rare, further research and data publishing are urgently needed. Furthermore, new *in vitro* test strategies, such as HT-BST, need to be developed and integrated into everyday API discovery. Constructive collaboration of industry, academia, and authorities could accelerate the data gathering and processing. Existing incentives need to be highlighted and new ones created, so that the willingness to make changes to commonplace API discovery processes grows and the identified challenges are tackled.

Conflicts of interest

There are no conflicts of interest to declare.

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Designing greener active pharmaceutical ingredients: Insights from pharmaceutical industry into drug discovery and development

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ABSTRACT

Active pharmaceutical ingredients (APIs), their metabolites and transformation products (TPs) are found as pollutants in the environment. They can impact human and environmental health. To address this issue, an efficient, long-term prevention strategy could be the design of APIs that have less impact on the natural environment, i.e. *the design of greener APIs,* by the implementation of environmental parameters into the drug discovery and development process (also abbreviated R&D for 'research and development'). Our study aimed to evaluate the feasibility of *the design of greener APIs* based on insights from drug design experts working in large, research-based pharmaceutical companies. The feasibility evaluation also identified needs and incentives for process modification. For this purpose, 30 R&D and environmental experts from seven globally active pharmaceutical companies were interviewed along a structured questionnaire.

Main findings are that the interviewed experts saw manifold opportunities to include properties rendering APIs greener in different stages along the R&D process. This implementation would be favoured by the fact that the pharmaceutical R&D process is very flexible and relies on balancing multiple parameters. Furthermore, some API properties that reduce environmental risks were considered compatible with common desirable properties for application. Environmental properties should be considered early during R&D, i.e. when molecules are screened and optimized. It has been found that availability of suitable *in silico* models and *in vitro* assays is crucial for this environmental consideration. Their attributes, e.g. throughput and costs, determine at which process stage they can be successfully applied.

An intensified exchange between R&D and environmental experts within and outside companies would push the industrial application of the benign by design approach for APIs forward. Collaboration across pharmaceutical companies, authorities, and academia is seen as highly promising in this respect. Financial, social, and regulatory incentives would support future design of greener APIs.

1. Introduction

Active pharmaceutical ingredients (APIs) are essential for human 'Good Health and Well-Being', one of the UN's Sustainable Development Goals (United Nations, 2015). At the same time, residues of APIs, their metabolites and transformation products (TPs) are frequently found as pollutants in the environment (aus der Beek et al., 2016; European Commission, 2020b, 2020a, 2020d; Hester and Harrison, 2015; Wilkinson et al., 2022). This is a long standing and well-known issue (Daughton and Ternes, 1999; Jones et al., 2001; Kümmerer and Hempel, 2010). Their presence potentially impacts ecosystems, drinking water resources, crops, and eventually humans (WHO, 2017). For example, some progestins and estrogens (hormones), oxazepam (anxiolytic), diclofenac (nonsteroidal anti-inflammatory drug), carbamazepine (anticonvulsant) and metoprolol (beta blocker) have been reported to chronically affect individual fitness and population health of aquatic organisms (Blazer et al., 2021; Brodin et al., 2013; Fent, 2015; Kidd et al., 2007; Triebskorn et al., 2007).

One of the concepts, which both the scientific and the policy community see as having potential to reduce pollution of the environment by pharmaceuticals, is the design of APIs that have less impact on the natural environment, i.e. *the design of greener APIs* (Kümmerer, 2007,

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2016). This concept is included amongst the proposed actions of policy initiatives addressing pharmaceutical pollution, including the European Union's 'Strategic Approach to Pharmaceuticals in the Environment' which highlights 'green pharmaceutical design' as a means to develop pharmaceuticals intrinsically less harmful to the environment (European Commission, 2019). Similarly, the OECD's work on pharmaceutical residues in freshwater includes amongst its proposed preventive measures the 'rational design' or 'benign by design' approach when developing pharmaceutical compounds (OECD, 2019).

The design of greener APIs is an approach directed at the source of pollution with the ultimate aim of designing APIs that have less impact on the environment (Kümmerer et al., 2020). The comparative of *green*, i.e. *greener*, is used to underline that it is about a comparison between common design and the design of more environmentally friendly APIs. Thus, the aimed-for environmental properties are not rigidly defined, but rather significant improvement is sought, analogous to the best-in-class approach (LaMattina, 2013). Moreover, it is not sufficient to consider only the impact by environmental pollution for a comprehensive assessment of the greenness of an API. For example, criteria for green production (Anastas and Warner, 1998) would also play a role, but are out of the scope of this study and the term *greener*. Recently the GREENER concept was published which proposes approaches for designing APIs with reduced environmental impacts after excretion based on well-known aspects (Moermond et al., 2022). These aspects can be divided into (i) those to reduce environmental exposure and (ii) those to reduce adverse environmental effects. The first aspect encompasses the second one and reduces environmental pollution by pharmaceuticals independently of known (in present) or to be discovered (in future) unwanted effects. In this respect the first approach is closer to the precautionary principle, which is a basic principle underlying all European environmental policy (European Parliament, 2015).

On this basis, we understand *greener APIs* as APIs that have less environmental impact after use, i.e. after excretion by patients, due to either reduced environmental exposure or at least reduced adverse environmental effects.

Reduced environmental exposure

A central approach to reduce environmental exposure significantly from the very beginning is the design of compounds that mineralize completely in the natural environment, i.e. degrade to innocuous inorganic compounds, such as carbon dioxide, water and inorganic salts (in the case of hetero atoms), by biotic and abiotic processes. Fast and full environmental mineralization leads to reduced environmental concentrations of the compound and its transformation products (TPs, i.e. molecules formed in the environment, including wastewater treatment plants, by incomplete biotic and abiotic transformation of the parent compound, e.g. an API) (Kümmerer, 2007; Puhlmann et al., 2021). In the best case, such a complete mineralization in the environment also occurs in the absence of sewage treatment, i.e. in surface waters. This presents a true sustainable solution of the problem as it is estimated that worldwide over 80% of wastewater (from municipalities, industry and agriculture) is released to the environment without adequate treatment (UN Water, 2017). Another design approach for achieving reduced environmental impact is having a lower therapeutic dose, which intrinsically leads to reduced API excretion by the patient. A lower dose may be achieved for example by improved bioavailability in patients, improved drug delivery or higher specificity (Baron, 2012; Daughton, 2003).

Reduced excretion can also be approached at a level other than drug design, e.g. through optimized drug prescribing (Daughton, 2003, 2014; Fortunak et al., 2015; Hamburg and Collins, 2010; Taylor, 2015). Environmental loads may also be reduced *via* wider adaptation of precision (personalized) medicine approaches that aim to replace suboptimal (e.g. ineffective) drug treatments with such that better account for individual genetic, environmental and lifestyle variabilities (European Commission, 2020c).

Reduced adverse environmental effects

Reduced adverse environmental effects could be achieved considering i) adverse environmental effects studied within regulatory environmental risk assessment (ERA; (EMA, 2006, 2018; European Commission, 2004a, 2004b)) and ii) adverse environmental effects that are linked to the therapeutic mode of action. For the latter, the presence/absence of drug-target orthologues in environmental organisms is a key factor to predict species susceptibility for drug exposure at low concentrations (Gunnarsson et al., 2019). Orthology predictions of drug targets can be obtained from the database Ecodrug.com (Verbruggen et al., 2018).

Ågerstrand et al. (2015) have proposed that potential ecotoxicity could be assessed earlier in the drug discovery and development process (also abbreviated R&D for 'research and development') to identify problematic APIs correspondingly earlier. They also recommend including environmental risks in the benefit-risk assessment when a medicinal product is considered for marketing authorization.

According to the Deloitte Report 'Options for a strategic approach to pharmaceuticals in the environment', the concept of designing greener substances has not yet been significantly explored by industry possibly because of concerns that it might be costly and technically challenging (Deloitte et al., 2018). Moreover, in contrast to the scientific and policy community's explicit backing, there is to the best of our knowledge no data so far in the public domain on how the pharmaceutical industry evaluates the feasibility of this concept, and which preconditions would have to be met for its implementation.

Addressing this gap, we conducted interviews with company experts, such as medicinal chemists, process chemists, and environmental risk assessors, from globally active, research-based pharmaceutical companies. A structured questionnaire was used to evaluate the feasibility of including environmental criteria for APIs into the drug discovery and development process (also abbreviated R&D for 'research and development') based on experts' insights. The feasibility evaluation also included identifying needs and incentives for *the design of greener APIs*.

2. Method

2.1. The questionnaire

A questionnaire (see supplementary) was developed consisting of two sections of five questions each:

Section I dealt with topics on the pharmaceutical R&D process, such as different phases and decision points within the process (Fig. 1), as well as criteria that play a role in decision-making. This section aimed to validate publicly available information on the R&D process provided, for example, by Blass (2015) and Summerton et al. (2015).

Moreover, the first section aimed to complement literature data with more detailed information on actual R&D practice. Understanding the main features of the R&D process was a prerequisite to evaluating the feasibility of *the design of greener APIs* based on interviewees' opinions.

Section II covered where environmental considerations are already implemented in a company's R&D process or can be integrated in future to enable *the design of greener APIs*. The aim of this section was to elicit information of interviewees on the current role of environmental criteria and their opinions regarding the feasibility of including environmental criteria into the pharmaceutical R&D process in future. The discussion on future integration of *the design of greener APIs* included key attributes for any *in silico* model or *in vitro* assay, related to their use at specific points in the R&D process.

In scope of environmental criteria were aspects related to APIs that are greener due to less impact on the environment after patient excretion (cf. introduction for a more detailed discussion of our understanding of

Fig. 1. Generic workflow of a common R&D process inspired by Messinger et al. (2016). The first four phases – from target selection to candidate selection, which aim to discover an API candidate – are placed under the label drug discovery (Research, i.e. the R in R&D). The last two phases, i.e. preclinical and clinical studies, deal with the development of the formulated drug (drug Development, i.e. the D in R&D). Explanation of the terminologies 'target', 'hit', 'lead' and 'candidate' can be found elsewhere (Messinger et al., 2016).

the concept). Out of scope were aspects of green chemistry addressing greener production methods such as synthesis routes that use less inputs and the use of renewable materials within R&D.

The following four environmental criteria were used in the questionnaire:

a) *No/reduced environmental exposure* to the compound reduces risks in the environment. Exposure should not exceed a limit value for ecotoxicological effects, like the predicted no effect concentration (PNEC).
b) *Environmental (bio)degradability* or environmental persistence of an API has major influence on the actual environmental concentration and thereby exposure. Complete and fast environmental (bio) degradation (i.e. mineralization) reduces or even prevents environmental risks (no risk in case of no exposure).
c) *No/reduced adverse environmental effects* reduces risks in the environment. Adverse environmental effects include adverse effects studied within regulatory environmental risk assessment and those that are linked to the therapeutic mode of action.
d) The concept behind *no/reduced undesirable moieties* (e.g. PFAS moieties CF_2 and CF_3 including C-CF_3 as precursor of trifluoroacetic acid (Cahill, 2022; Joudan et al., 2021; Rüdel et al., 2020; Scheurer and Nödler, 2021)) is to avoid, if possible, the use of molecular groups that are known to have a negative impact on the environment (e.g. due to persistence or ecotoxicity). Thus, this concept is linked to all previous criteria.

Before conducting the interviews, the questionnaire was tested on two R&D experts from medicine agencies who worked in pharmaceutical companies' R&D earlier in their careers. Question scope, comprehensibility and scheduling feasibility were tested. The questionnaire was revised in favour of comprehensibility by using more common terms. Time slots for blocks of questions were adjusted.

2.2. Interview request and interviewees

An interview request was sent to representatives of 10 globally leading pharmaceutical companies, which do research, development and manufacturing of pharmaceutical products. The request was accompanied by a two-page background summary of the challenge of pharmaceuticals in the environment, and the questionnaire to show the interview scope and allow for preparation (both in supplementary). The contacted company representatives forwarded the request to the addressed R&D and environmental experts within their company; particularly medicinal chemists, but also computational chemists, process chemists responsible for the development of APIs and environmental risk assessors (in the following "company experts").

In total, 30 company experts working for seven out of the ten contacted companies accepted the request. All companies are large-sized pharmaceutical companies and members of the European Federation of Pharmaceutical Industries and Associations (EFPIA), with head offices in Europe (6 companies) or in the USA (1 company). Interviewed experts were mostly medicinal chemists (at least one per company), others were staff with expertise in other disciplines, e.g. environmental risk assessment (ERA) (Table 1).

2.3. Conducting and evaluating the interviews

Interviews lasted 2 h and were conducted online between April and July 2021 using the video conferencing platform *GoToMeeting*. After a brief round of introductions, the interview was conducted along the two sections of the questionnaire:

I description of the R&D process within the particular company (00h:10m - 01h:10m),
II current role of environmental considerations for APIs and potential in future (01h:11m - 02h:00m).

Before the interview started, interviewees agreed to a recording for the purposes of completing the transcript by the recorder. It was ensured that the group answers followed the questionnaire's structure, to obtain the specific data required for answering this study's research questions and to ensure comparability between the interviews. Interview transcripts were used as basis for a qualitative and systematic evaluation of all answers and additional comments. Statements that were not directly asked for were considered individually, but only if they were within the scope of this study. As confidentiality was agreed, results are presented anonymized and generalized, so that statements cannot be traced back to an individual company or employee.

2.4. Data analysis

Data generated in the interviews was predominantly qualitative. The qualitative data analysis conducted was abductive in nature (straddling a middle ground between deductive and inductive approaches; cf. Thompson, 2022). On the one hand, the analysis follows theoretical concepts related to the topic, such as the phases and decision points in the pharmaceutical R&D process and the environmental criteria presented in 2.1). On the other hand, the analysis engaged inductively with the empirical data generated, identifying relevant patterns and themes in the different responses to one and the same question and summarizing the data available for these patterns/themes. In particular, emphasis was placed on themes and patterns related to potential for uptake and implementation, as well as barriers hereto.

Analysis was conducted per question. For each question, data was condensed and restructured into a synthesis of the meaningful information provided in the responses.

Table 1
Number of company experts from different disciplines in the 7 interviews.

Discipline	Number per interview I-VII							Σ
	I	II	III	IV	V	VI	VII	
Medicinal chemistry	1	3	1	2	2	2	3	**14**
In silico chemistry						1		**1**
Process chemistry		2				1	1	**4**
Product Stewardship						1		**1**
ERA	1		1	2	1	2	3	**10**
∑ experts	**2**	**5**	**2**	**4**	**3**	**7**	**7**	**30**

3. Results

The interviewees usually answered the questions very comprehensively and were open-minded and interested towards the approach *the design of greener APIs*. Some of them expressed intrinsic motivation to contribute to this solution approach for pharmaceuticals in the environment. Interviewee's attitude led to lively, data-providing discussions – also between experts from the same company.

3.1. The drug discovery and development process, R&D (Q1–Q5)

3.1.1. Variability in pharmaceutical companies' R&D process (Q1–Q3)

All interviewees confirmed that in general terms their company's R&D workflow follows the generic workflow described in the literature (Fig. 1). Differences in how phases are named were seen as insignificant. Additional phases to those presented in Fig. 1 were mentioned by interviewees. However, they were not considered relevant to the design of a greener API. An example is synthesis route selection (deciding on the best process to synthesize the API for larger-scale production), which usually occurs after candidate selection and has therefore no impact on the drug design. Additional important decision points apart from those covered by Fig. 1 were not identified in the interviews.

In general, modification of the workflow of R&D occurs due to project- or programme-specific circumstances. For example, if a project is targeting a new or less well-known biological target it will require more intensive validation, leading to significantly more testing and thus to some R&D phases taking longer, than if the biological target is well known. However, whereas projects can have very significant differences in individual phase length, approach applied, resources and so on, such modifications do not change the overall picture and logic of the process. The overall picture is also still valid when taking into account that the R&D process evolves rapidly e.g. due to new testing methods and approaches, especially in the field of computational chemistry.

All interviewees highlighted that the R&D process is typically not linear, but rather highly cyclical and flexible due to significant learning steps within the process. Particularly, the phases of hit optimization and lead optimization, where multiple parameters are optimized (using Multi-Parameter Optimization, MPO), are highly iterative and flexible. This flexibility allows for the process to be changed by adding decision points or "stoppers" (red flags), or new parameters to be considered with further assays to filter out problems encountered in previous iterations. However, decision points are not significantly affected when returning to a previous stage in an iteration, as the same level of characterization of every potential API is needed. If information may be drawn from the failures that lead to the iteration, decision points can be merely refined by more extensive data (leading e.g. to specific follow-up assays). Interviews revealed that there is a trend towards more continuity of team members along the R&D chain, more exchange within and between teams, and more collaboration between different experts from different departments within companies. This indicates not only the possibility of flexibility but also that companies rate this as important. Accordingly, interviewees stated that these changes are seen as helping to lower compound failure rates during the process (attrition rates).

3.1.2. Decision criteria (Q4)

According to the interviewees, the parameters listed in the questionnaire (supplementary, Q4) are by default part of screening cascades in the R&D process as they are crucial for the development of an approvable pharmaceutical product. Examples are potency, physicochemical properties, adsorption, distribution, metabolism, excretion (ADME) parameters, stability (chemical, plasma, shelf life), pharmacokinetic, efficacy and safety related parameters such as off-target effects.

The list of parameters can be expanded to include project-specific parameters as needed. Some decision point criteria can vary with disease area (nervous system, oncology, etc.). For example, possible off-target effects for headache medication are less acceptable than for oncology drugs.

Several parameters being optimized for in R&D have been discussed during the interviews also in terms of their environmental significance (listed in the supplementary with literature-based information). Examples are lipophilicity and stability. It was highlighted by medicinal chemists that they try to strive for less lipophilic molecules. Regarding stability, interviewees said that results of experimental stability tests during lead optimization phase can lead to red flags. For example, if a lead compound is unstable under atmospheric, humid, or oxidative conditions this leads to a red flag for the decision criteria, as it would shorten the specified shelf life considered necessary to deliver the drug to the patient. Moreover, API stability in the human body is desired for treatment regimens conducive to patient compliance, e.g. no fast metabolism of compound, or half-lives in blood allowing for e.g. oral dosing no more than once or twice daily.

3.1.3. Company's use of a target product profile (Q5)

A so-called *target product profile* (TPP) builds the basis for the development of the pharmaceutical product. The TPP outlines the desired pharmaceutical profile of a new product. For example, topics such as dosage and administration are part of the TPP. Consistently, interviewees summarized their TPPs as living guidance documents which are systematically developed and refined along the entire R&D process.

3.2. Environmental considerations (Q6–Q10)

3.2.1. Current role of environmental considerations in any phase of the R&D process (Q6)

According to interviewees, current consideration of environmental aspects within their R&D process is limited to aspects related to manufacturing, for example, the use of fewer and safer raw materials, and lower carbon footprint. Since manufacturing-related aspects are out of the scope of this study they are not discussed here in more detail. Currently, then, the interviewed companies do not consider the environmental behaviour or profile of potential APIs when designing APIs.

Whereas there is currently no explicit consideration of environmental criteria of an API during the different phases of drug discovery, several interviewees identified aspects related to properties that medicinal chemists aim for in an API, and which may also result in better environmental properties. The consideration of these criteria by medicinal chemists may thus result in beneficial outcomes for the environment (albeit unintentionally) too, which can be seen as a win-win situation. These identified aspects are:

- low dose drugs (if enabled for reasons other than increased potency; see section Q8b)
- high target specificity
- aqueous solubility balanced against lipophilicity
- high bioavailability
- no toxicity (e.g. carcinogenic, mutagenic, reprotoxic)
- no/reduced adverse effects.

A low administered dose can be expected to translate into low amounts of API being excreted by the patient and thereby to result in lower environmental concentrations of an API or its metabolites. High specificity leads to less off-target effects in the human body, which may translate to less adverse effects in environmental organisms.

There is currently a trend in drug design to aim for lower lipophilicity (which enables better plasma solubility and minimizes non-specific, off-target binding, amongst other things). A consequence of the design towards lower lipophilicity is that newly developed APIs have lower potential for bioaccumulation. On the other hand, the development towards more molecular complexity appears not to be positive from an environmental perspective. According to an interviewee, complexity is generally on the rise because of the exploration of a wider

chemical space aiming for drugs that are superior to current drugs. API complexity (e.g. due to more extensive branching and number of functional groups) might hinder complete mineralization in the environment.

3.2.2. Potential for uptake of environmental considerations in the pharmaceutical R&D process (Q7–Q8)

On the general question if environmental considerations can be included amongst other criteria in the target product profile, interviewees in six of the seven interviews tended towards an uptake being potentially possible, associated with conditionalities, however. The implementation would depend on suitable models or assays being available to evaluate the environmentally relevant criteria. Environmental criteria would need to be clearly defined, be translatable to parameters that play a role in environmental evaluation, and have an importance attached to them, e.g. if there were legal requirements to meet these criteria.

3.2.2.1. Potential uptake of environmental considerations in the different phases of the R&D process. According to interviewees, already at **target selection** a first environmental risk evaluation for an API could be possible based on intended volume of use and population. For example, contraceptives would be a high-risk area, which contrasts strongly in volume of use and population with e.g. some antineoplastics for small patient populations and specific indications. An evaluation at this stage could inform the degree and extent of uptake of environmental criteria in the R&D process.

Hit identification is the phase where the highest number of compounds is screened and therefore the phase with the highest chances of finding promising structures meeting environmental criteria. The minimum requirement for a potential assay would be that it has "at least a medium throughput, is not too costly, and does not require a too large amount of substance." However, adding further criteria to the early stages could hamper drug discovery, in the opinion of some interviewees. Medicinal chemists generally want to avoid potentially filtering out promising scaffolds. Moreover, it would be difficult to evaluate compound's environmental properties in early phases as there would be a very large question mark regarding a compound's characteristics, including metabolites. One interviewee highlighted that hit structures selected will not necessarily resemble the evolved lead structures.

At **hit selection and optimization**, a standard toolbox would be needed that is suitable for routine screening of environmental parameters. For example, problematic structural elements could be identified at an early stage using a standard filter. Models could be based on parameters that are measured anyway in the process. Several interviewees mentioned the possibility that environmental criteria could be part of the parameters evaluated in the design-make-test-analyse (DMTA) cycle. DMTA consists of the **d**esign of new analogues, their synthesis (**m**ake) and **t**esting, and **a**nalysis of results. The cycle is performed iteratively in hit optimization and lead optimization to optimize multiple parameters using MPO. Analogues result from various modifications in the molecular structure. Whether structural changes are kept, developed further, or discarded is decided on the basis of test results. For example, structural changes are kept if improved potency (and/or other properties) results from the change. According to interviewees, it would be ambitious to implement molecular changes to the minimum environmental impact within MPO, but feasible.

Lead optimization was seen as the phase where environmental considerations should play an important role, because in this phase *in vitro* assays to characterize a compound's environmental properties can have much lower throughput and greater expense. The more precise evaluation of environmental parameters possible in this phase could ensure that any decisions taken are based on adequate and relevant data. Longer assay turnarounds, while not desirable, would not necessarily slow down processes in this phase, as the significantly smaller number of molecules that reach this phase will already undergo a detailed characterization. Interviewees also highlighted the potential to use the high-quality data that could be generated in this phase for improving the corresponding *in silico* models.

Moreover, at this phase a selection of compounds is prioritized, and choices can still be made when optimising parameters as part of MPO. Some parameters, such as dose-related ones, may go hand in hand with environmental risk. Opportunities were identified to consider environmental biodegradability in this phase by using *in silico* testing in combination with the ADME profile, as an extension of it. An interviewee showed interest in the approach of benign by design and mentioned the literature example of the β-blocker propranolol optimized regarding biodegradability (Rastogi et al., 2015).

When considering environmental criteria in MPO, it could be difficult to weigh different parameters (e.g. different ecotoxicity parameters against each other, or ecotoxicity parameters and those related to other issues) since there would already be a balance between the standard pharmaceutical parameters. The question arose of how to decide which is the most important environmental parameter. Interviewees highlighted that in medicinal chemistry, tests are almost never a yes-no decision, but rather balancing several criteria on different sliding scales of acceptability. Interviewees assumed that an evaluation of environmental criteria and prioritising compounds based on an environmental score is possible. In case of a selection of compounds that are similar in terms of pharmaceutical properties, prioritization due to environmental properties is considered feasible. It may become challenging if the outcome of an assay evaluation greatly reduces the variety of structures of different scaffolds, resulting in only one series of a compound with a solid scaffold, but with poor environmental characteristics.

The latest process point identified with potential for incorporating environmental criteria in decision-making is right before **candidate selection** (effectively the "spout of the funnel"). The reason is that the molecular structure is set with candidate selection. Once a development candidate builds momentum it is considered very difficult to go back to design alternatives that may have better properties. However, results of environmental tests at these later stages would be valuable for data and knowledge-generation purposes.

In **preclinical and clinical studies** metabolites could potentially be investigated, e.g. regarding potency, to figure out how they should be assessed from an environmental perspective. In clinical phases I and II, the therapeutic range of dosing is determined. There could be some potential for choices that can be made to the dosing regimen (e.g. choosing a lower individual dose with several daily doses, while considering patient compliance) which could reduce the amount of unchanged compound excreted by the patient.

3.2.3. Suitability of environmental criteria for uptake (Q8b)

Asked for concrete environmental criteria that could be suitable for uptake in pharmaceutical R&D, interviewees discussed the following greener aspects (Section 2.1, a - d) proposed in the questionnaire.

3.2.3.1. No/reduced environmental exposure. Exposure data is needed (together with effect data) to estimate a potential environmental risk. Early on in the R&D process, environmental exposure of the API could be estimated very roughly based on assumed dosage and market penetration which impacts environmental concentration. Compound dosage becomes more clearly specified later, typically during preclinical and clinical studies.

According to some interviewees, there are possibilities for adjusting drug dosage in ways that could lead to reduced excretion from the human body, and thus emissions into the environment. Interviewees, however, stressed that the efficacious dose in humans needed for administration would be one of the most important of multiple criteria, i.e. the dose would never be lower than needed for efficacy.

For the interviewees of six of the seven companies, no/reduced low dose toxicity is compatible with parameters currently optimized in the R&D process, as meeting toxicity requirements is necessary from a pharmaceutical point of view. APIs after all need to be safe for the patient. All the efforts carried out during the R&D process to reach a low dose can in general be seen as reducing risks of toxic effects on patients, and potentially also on the environment.

The feasibility of a lower dose is favoured by higher compound potency. However, highly potent APIs may already exert toxicity to environmental organisms at low doses. Therefore, high potency needs a more detailed consideration than the use of a simple rule of thumb. An interviewee explained that, in general, the more potent one would make the drug, the more one would have to build in some additional properties to avoid side effects, for example, a very specific way of drug delivery. After *in vivo* efficacy it would be desirable that the API is metabolized to less potent metabolites.

In addition to the aspect of low dose, an interviewee argued that for new substances designed for specific diseases of a small patient population, no release in huge quantities is expected. The low quantities of these substances would never provoke any risks in the environment.

3.2.3.2. Environmental (bio)degradability. All interviewees agreed in principle on the desirability of APIs with a higher environmental (bio) degradability, but there was no clear agreement amongst them on the potential to implement this criterion in practice. An interviewed medicinal chemist feared that the consequences of designing APIs that are degradable in the environment have not yet been sufficiently researched, and was therefore sceptical of the aim of environmentally biodegradable drugs. Multiple transformation products could be formed in the water that are more soluble, and therefore harder to extract from water.

In two interviews it was mentioned that environmental (bio)degradability could clash with the need to design for API stability against enzymes in the human body. However, in four interviews it was argued that the different conditions affecting the API along its lifecycle should be considered in detail when evaluating the potential of environmental (bio)degradability. It would be interesting to find some kind of 'window of opportunity' for compounds to be both sufficiently stable in the human body and (bio)degrade in the environment: the compound could be made so that it is sufficiently stable to have an effect in the human body, and later degrade relatively quickly in the environment.

Another concept for APIs designed to be only as stable as necessary was the antedrug[1] approach. Antedrugs could result in molecules that are smaller, more polar, more easily excreted and probably also readily biodegraded in the environment. An interviewee proposed that data on a molecule's behaviour in the patient (e.g. molecule clearance from the body) could be made available for environmental studies. In this context, it was recognized that modifying lead structures using certain functional groups to block metabolism (e.g. groups containing fluorine) could hinder biodegradation in the environment.

3.2.3.3. No/reduced adverse environmental effects. The aim of no/reduced adverse environmental effects in the environment would be strongly compatible with several established R&D practices, in the opinion of interviewees of six of the seven participating companies.

In particular, interviewees mentioned medicinal chemistry's aim of very high target specificity of their active substances – high specificity being an established strategy to reduce off-target (side-) effects in the patient. High specificity is synergistic with reducing off-target effects in the environment, as specific drugs, once excreted, might be expected to show fewer off-target environmental effects than less specific drugs dosed at similar levels (due to the high specificity to the drug target in the human body).

Several interviewees suggested that *in silico* testing with mouse enzymes or human enzymes could possibly inspire the testing with fish enzymes (off-targets). Off-target assay panels for human safety existing in pharmaceutical R&D could be of great interest to integrate targets of environmental species that are associated with adverse effects.

At the moment of this study, no concrete off-targets in environmental species (e.g. concrete enzymes) were known to the interviewees. If one would know concrete off-targets one could look at structural similarities and build selectivity into the molecule. However, this could be contradictory to environmental biodegradability and no/reduced undesirable moieties (next paragraph). To overcome such a consequence of increased specificity, targeted delivery and pro-drugs were mentioned as further strategies to reduce adverse effects. An interviewee mentioned ecotoxicity screens, developed by OECD (e.g. OECD test 201, 210, 211), the use of which could be generally plausible.

3.2.3.4. No/reduced undesirable moieties. Many interviewees saw high potential for implementing the criterion *no/reduced undesirable moieties* in future R&D, based on lists of moieties of concern. They suggested it should be considered as a parameter which weighs decisions in favour of molecules without problematic moieties, or a parameter which flags particular concerns and increase awareness, rather than a hard rule or 'blacklist' of forbidden structural elements, as a 'blacklist' could hinder the ability of the pharmaceutical industry to treat diseases (e.g. in the case of a complete ban on fluorinated groups).

A computational chemist highlighted the potential of *in silico* approaches for developing databases of matched molecular pairs that could, for a given molecule, on the one hand identify undesirable structural moieties in it, and on the other suggest better structural moieties for it. Pooling industry data and resources for a common large database with this purpose was seen as a great opportunity.

3.2.4. Key attributes of environmental models/assays (Q9)

Future integration of environmental criteria in the pharmaceutical R&D process requires that models and assays be developed that predict or assess environmentally relevant properties of APIs. Interviewees identified key attributes of these potential environmental screening

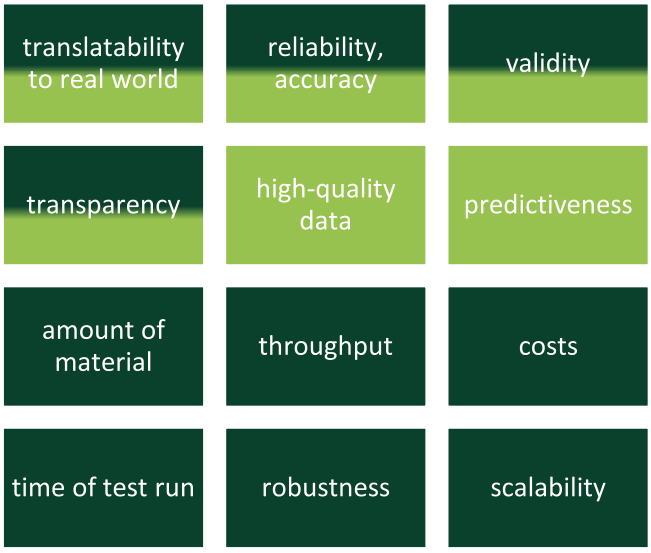

Fig. 2. Key attributes for environmental *in silico* screening models (light green) and *in vitro* assays (dark green). Note, high-quality data is a key attribute to build/improve *in silico* models, but comes from *in vitro* tests. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

[1] An antedrug is defined as an active synthetic derivative that is designed to undergo biotransformation to the readily excretable inactive form upon entry in the systemic circulation (Khan et al. (2005)).

models and assays, which would facilitate their future integration into the R&D process (Fig. 2).

Two attributes often mentioned, both for *in vitro* and for *in silico* tests, are reliability and translatability to the real world, i.e. the natural environment. Assays need to be reliable and sufficiently reflective of the natural environmental to play a role in an R&D project's TPP. Attributes mentioned for *in vitro* tests are the amount of substance needed for the assay, throughput, and test costs. An aspect of special relevance for *in silico* tests is high-quality experimental data as the basis for the models. It remained unclear to which extent companies already have such data and whether they would share it.

3.2.4.1. In silico models. Most interviewees stressed the very high potential of *in silico* models: they were considered enormously helpful, and quite an "easy sell", due to time and cost savings when compared to *in vitro* or *in vivo* assays. *In silico* models also have high potential because they would make it as easy as possible for medicinal chemists to include environmental considerations in their workflows and decision-making. Computational screening is well implemented for a range of desirable API parameters, from physicochemical properties to target binding. *In silico* models based on large compound databases are already available for physicochemical properties (e.g. lipophilicity, solubility, vapour pressure), which are also relevant for environmental properties.

The importance of the size of the applicability domain of the model as well as good predictiveness was stressed – qualities based on the availability of high-quality data for a wide variety of compounds. Reaching the necessary level of model predictiveness would just be a matter of time, hinging on the database eventually incorporating sufficient data on new compounds also from different sources (i.e. companies' and public data). Several companies expressed interest in developing *in silico* models for environmentally relevant parameters, required for environmental endpoints where high-quality data are missing. Three companies emphasized that they are open-minded regarding a cooperation across the pharmaceutical industry and academia on such models. Furthermore, the possibility of using already generated data for existing drugs was highlighted, including the environmental test data generated in the past 15 years in the context of EU authorisation.

3.2.4.2. In vitro assays. Aspects highlighted by interviewees as important for *in vitro* assays, independent of the phase in which an *in vitro* assay is implemented, were reproducibility and reliability, as well as its being well-defined, standardized and validated. Having defined cut-off criteria for environmental parameters, i.e. a concrete value for what would be acceptable, would be helpful.

Complexity, throughput, and costs of an assay as well as the amount of material required will influence where the assay is placed in the screening cascade along the drug discovery pathway. This means that there is not one set of attributes that *in vitro* assays for environmental properties need to meet for their being taken up in the R&D process; rather, environmental screen/assay characteristics will determine in which phase uptake could take place.

Interviewees highlighted the benefits of using models and assays at several different points along the R&D process, for example, during hit selection to get an idea on the environmental impact, and during lead optimization using a high-quality test to drive the molecule design with data allowing for a better understanding of the property. Repeating the evaluation of an environmental criterion by a high-quality test can also be desirable at a later stage to feed back into the models applied in earlier stages.

3.2.5. Incentives for the design of greener APIs (Q10)

Incentives in fostering *the design of greener APIs* are viewed by interviewees as potentially helpful in stimulating efforts in research and development in the field of greener APIs.

Incentives identified by interviewees can be described as either hard or soft (Fig. 3). Regarding hard incentives, proposals put forward often related to policy incentives with economic implications, such as fast-track approval in the authorisation process, and increased patent lifetime exclusivity for medicines that meet specific environmental criteria. It was speculated (by non-legal experts) that regulated patentability for reduced environmental impact as novelty could be helpful. Furthermore, a green label (which builds trust with society) could encourage companies to invest in environmental considerations. It would create a social incentive[2] as it may project a positive reputation in society. Financial support, e.g. by subsidizing testing for environmental impact, was also suggested as a viable incentive.

Interviewees observed that regulations in many fields are becoming more stringent; some saw the possibility that in future there could be "*a must*" around this topic for pharmaceuticals. Companies would thus have an intrinsic incentive for acting sooner rather than later, as they should be prepared for upcoming regulatory developments.

Soft incentives include creating awareness and motivation within teams of medicinal chemists. Awareness of a compound's environmental impact, based on in-depth knowledge (regarding environmental issues, and tools to avoid those), would be important to make good decisions in the R&D process. An interviewee mentioned that so as to increase their intrinsic motivation, companies should be exposed to and familiarized with test methods. Once this exposure has taken place, acceptance for integration would increase. Another soft incentive would relate to reputational issues, as implementing environmental considerations would be beneficial for the reputation of pharmaceutical companies.

Some interviewees suggested no policy incentives would be needed, as the direction society is travelling in was already creating 'soft' incentives; it generates public pressure, company willingness, and even personal interest of medicinal chemists. Personal interest and intrinsic motivation were indeed noticeable in each of the conducted interviews.

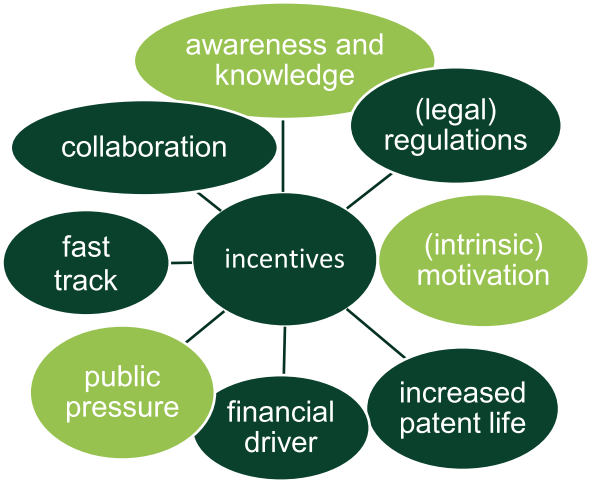

Fig. 3. Soft incentives (light green) and hard incentives (dark green) mentioned in the interviews (Q10). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

[2] Social incentives cover a wide spectrum of interpersonal rewards and motivations, encouraging people (here, companies' actors) to behave in a socially valued and approved manner. Examples are projecting a positive social image and reputation and gaining social acceptance (Dorfman and Grossmann (2020)).

4. Discussion

Current application of green chemistry principles by pharmaceutical companies interviewed is limited to the manufacturing process, e.g. using fewer and safer raw materials, or reducing a product's carbon footprint. Reducing the environmental impact of an API itself by minimizing its environmental exposure and adverse effects does not play a role in their drug discovery and development. This finding is in line with the study by Di Soete et al. (2017) who intensively discussed the application of green chemistry principles by the pharmaceutical industry beyond *the design of greener APIs*. However, Di Soete et al. (2017) highlighted the need to evaluate the feasibility of *the design of greener APIs* from the perspective of R&D experts, including needs and incentives. Our interview study should lead to a better understanding of the feasibility of this concept according to the medicinal chemists and other experts who work on discovering and developing APIs.

4.1. The R&D process and environmental considerations

No significant, company-specific deviations from the general approach to the pharmaceutical R&D process were identified when interviewing experts of seven global pharmaceutical companies. Differences in nomenclature and intermediate decision points exist, but are not necessarily linked to a governance process and can be neglected. As the overall process in different companies is largely the same, the potential for uptake of environmental parameters in the pharmaceutical R&D process identified in this paper can be generalized to other research-based pharmaceutical companies.

The finding that the pharmaceutical R&D process is highly flexible is consistent with literature data. Companies try to operate in a very nimble, agile nature to improve R&D productivity (Berggren et al., 2018), for example applying an agile, iterative test-and-learn approach (Gyurjyan et al., 2017). A study of the operational practices of more than 25 global pharmaceutical companies found that all companies had launched lean or Six Sigma projects in the recent past (Cremer et al., 2009). Thus, the R&D culture in pharmaceutical companies is familiar with highly dynamic processes and continuous changes. This process flexibility and flexible R&D culture show that there are no intrinsic barriers to the uptake of novel criteria of a different type to the criteria already playing a role in pharmaceutical R&D decision-making.

The interviewees saw numerous opportunities for uptake of environmental parameters in different phases of the R&D process. Environmental parameters could already play a role in the earliest phases of R&D, for example in hit identification approaches based on *in silico* screens and *in vitro* high throughput screens, all the way up to the final stages of lead optimization. With candidate selection the molecular structure is established, and environmental parameters will not lead to structural changes after that point. After candidate selection, relevant environmental data could however still be collected to improve the scientific knowledge base and the predictive capability of *in silico* models.

The opinions of medicinal chemists differed when it came to their preference for including environmental criteria earlier or later within phases of drug discovery. Different opinions can be explained by a lack of clarity on how strategies for decision-making could look like, including an approach on how to weigh parameters of different nature (pharmaceutical and environmental aspects). A strategy for decision-making to include environmental parameters in overall compound scores, or as flags, was seen as more promising by medicinal chemists than strict cut-off limits or go/no-go criteria.

4.2. Environmental parameters

According to many interviewees, some environmental properties can go hand in hand with common desirable API properties, leading to potential win-wins. For instance, physicochemical or fate properties such as plasma solubility or permeability also influence the uptake by environmental organisms, required for biodegradation by intracellular enzymes. Environmental bioaccumulation is often associated with high lipophilicity of the compound, combined with lack of enzymatic clearance in the non-target species. The current trend in drug design is toward lower lipophilicity (to enable better plasma solubility and minimize off-target binding, amongst other things) and thus only very few newly developed APIs should show bioaccumulative properties. Conversely, hydrophilic APIs and their transformation products that are persistent and mobile in the aquatic environment are more likely to be a concern for the future (Hale et al., 2020).

Furthermore, compounds that are highly selective in the human body may also cause fewer adverse effects on non-target species. Effects based on a mode of action needed for applications, e.g. of antibiotics, might be challenging to avoid in the environment. Orthology predictions of drug targets in the human body and environmental organisms can be obtained from the database Ecodrug.com (Verbruggen et al., 2018). Options to mitigate risks of adverse effects based on a mode of action are largely limited to reducing environmental exposure e.g. through degradation.

There was less consensus on the compatibility of the criteria 'no/reduced environmental exposure', 'no/reduced undesirable moieties', and 'environmental (bio)degradability'. Regarding environmental exposure, low dose drugs (leading to reduced excretion by patients into the environment) potentially go hand in hand with desirable environmental considerations. However, some interviewees highlighted that approaches aiming for lower dosing (which reduces exposure) are limited due to therapeutic dose requirements. Moreover, strategies of dose reduction require a more precise examination. Lower dose due to higher potency may also result in greater ecotoxicological potency in case of greater potency enhancement than dose reduction. A well-known example are endocrine disrupters, which are potent at low environmental concentrations (Vandenberg, 2014). This is possibly the reason for frequent hazard- and risk-based prioritization for environmental assessments but no consideration by exposure-based methods (Burns et al., 2018). Lower dose due to better bioavailability is considered beneficial from an environmental perspective not only because of reduced emissions, but also because of potentially better bioavailability for bacteria, which is required for biodegradability in the environment (Boethling et al., 2007; Daughton, 2003). The latter is true only if bioavailability is improved by optimizing the API structure, not the drug delivery system (Baron, 2012). Higher on-target specificity to enable a lower dose and reduced off-target effects in the patient will likely result in fewer effects on related targets conserved in environmental organisms (Moermond et al., 2022). Environmental adverse effects based on interaction with more distant targets are still possible, especially for bioaccumulating substances.

Regarding no/reduced undesirable moieties, it was not covered during the interviews which ones are undesirable. Regardless of this, an early flagging was considered generally feasible, keeping in mind that the importance of such moiety for the bioactivity is rather unclear at early stages. For the classification as undesirable moiety, a starting point might be looking at regulations for other product categories such as cosmetics, plant protection products, and chemicals in general. A highly topical example is the proposal by the Netherlands, Germany, Norway, Denmark and Sweden to restrict per- and polyfluoroalkyl substances (PFAS) under REACH. It covers a wide range of PFAS uses (ECHA, 2022). Even if the restriction proposal does not apply to pharmaceuticals, the criteria for a greener API can be guided by it.

At first glance, environmental (bio)degradability does not seem compatible with the required chemical stability for an API. However, in some interviews it was discussed that conditions such as pH, access to light and bacteria concentration and diversity (and therefore the set of enzymes that interacts with the compound) change continually during the API's lifetime (i.e. from synthesis to excretion and presence in the environment), possibly creating significant windows of opportunity for

compounds to be both sufficiently stable in the human body and (bio) degradable in the environment. Environmental conditions changing along the lifetime of a compound are described in literature in the context of 'benign by design' as an opportunity (Kümmerer, 2007; Puhlmann et al., 2021). For example, a compound could be designed that, after use, will be cleaved by light to TPs, which are then mineralizable by enzymes in the natural environment. Such consideration requires expertise in environmental processes within the teams of drug designers or by close collaboration with environmental scientists. At the moment, most R&D experts interviewed seem to not be aware of the debate of fate and effects of pharmaceuticals, leading to e.g. scepticism about aiming for degradability of APIs, not because of doubts about the feasibility but because of concerns of supposedly unknown consequences regarding TPs. However, research on TPs has been going on for more than 25 years, e.g. TPs of pesticides, demonstrated by search results using e.g. the literature data base 'Web of Science' and keywords "transformation products" combined with "environment".

Furthermore, the growing API complexity mentioned by an interviewee can hinder complete (bio)degradability (e.g. in case of carbon chain branching or amine functional groups carrying further substituents), but not necessarily, as (bio)degradability depends on the specific structural units and the molecule as a whole. Nonetheless, this knowledge could serve as rough guiding principles ('rules of thumb') for the design for environmental biodegradation (Boethling et al., 2007). For example, biomolecules such as the group of monoclonal antibodies can be very complex (with repeating structural features) and biodegradable at the same time as long as they carry natural function groups and are attached in a natural manner (Website Roche). Thus, it is too early for a final conclusion at this point in time. Environmental (bio) degradation has to be better understood.

The ideal outcome from an environmental perspective would be ready and complete environmental biodegradation of an API to inorganic compounds such as carbon dioxide and inorganic salts, i.e. complete mineralization. This would mean that neither parent compound nor any TPs are persistent and there would therefore be no risks in the environment, as there would be no meaningful exposure (DeVito, 2016; Kümmerer, 2010; Kümmerer and Hempel, 2010; Taylor, 2015).

Complete mineralization is probably not feasible for all APIs, but could be aspired to for a share of new APIs. According to publicly available ERA data from pharmaceutical companies, there are examples of APIs on the market which are unintentionally (i.e. without targeted design) biodegradable in the environment, e.g. rosiglitazone (inherently), interferon alfa-2a (readily), and enfuvirtide (readily), as well as monoclonal antibodies (readily), such as emicizumab and ocrelizumab (Websites GSK; Roche). Examples of high-volume marketed APIs which are readily biodegradable include several β-lactams (such as Penicillin V), valproic acid, and acetylsalicylic acid (Kümmerer and Al-Ahmad, 1997; Längin et al., 2009; Puhlmann et al., 2021). But overall, many of registered pharmaceuticals are persistent. For example, Schwarz et al. found that approx. 50% of APIs are persistent according to ERA datasets of around 300 APIs (Schwarz et al., 2021).

Interviewees would like to have a set of clearly defined, measurable and optimizable parameters for greener APIs, either related to ecotoxicity or to fate endpoints. Exchange of knowledge between R&D experts and environmental scientists is needed to clarify how to measure and optimize environmental parameters in practice. The GREENER approach by Moermond et al. (2022) proposing environmental criteria is a good starting point for such an exchange.

4.3. Needs of models and assays and incentives for this process modification

Interviewees often argued that incorporating environmental considerations into R&D is possible when suitable models or assays are available for clearly defined parameters. Standardized assays for ecotoxicity or environmental fate, such as OECD tests, are well established, e.g. as part of the marketing authorisation procedure (EMA, 2006, 2016, 2018). However, these are low-throughput assays and less suitable for uptake in early R&D processes. Approaches are needed to screen a higher number of compounds at an earlier phase of R&D, e.g. *in silico* tools for predictions or *in vitro* medium- or high-throughput screens. For chronic ecotoxicity, using less complex test systems than whole organisms enables shorter time of test run. Examples are enzymes (as a panel of targets related to effects in environmental species), cells (including bacteria) and embryos (e.g. embryo-larval zebrafish).

Development of *in silico* tools could play an important role in considering environmental parameters due to e.g. high throughput and comparatively low costs, and the possibility of prioritizing compounds for synthesis. The last years have seen a growing role of *in silico* approaches in different areas of pharmaceutical R&D and this growth is expected to continue (Leveridge et al., 2018; Trenfield et al., 2022; Wang et al., 2021), a development that underscores their potential for environmental parameters. Environmental *in silico* models could help e.g. predict a compound's binding affinity to relevant off-targets from wildlife species, or predict its environmental (bio)degradability and resulting transformation products and their properties. Furthermore, structural moieties (i.e. desired / undesired including rules of thumb from an environmental perspective) could be identified.

In silico tools like QSARs (Quantitative Structure-Activity Relationships) have been used to predict toxicity of industrial chemicals to algae, daphnia and fish, e.g. through ECOSAR (US EPA, 2012), with varying results. Predictions are best for neutral compounds in acute studies. However, many pharmaceuticals are ionisable rather than neutral, and concern regarding their environmental effects relates to chronic toxicity at low environmentally relevant concentrations rather than acute toxicity at high concentrations. Prediction of presence or absence of drug targets in the most susceptible species was found to be possible (Gunnarsson et al., 2019). Within the VEGA platform (https://www.vegahub.eu/), there are several *in silico* models to predict persistence of chemicals in soil, sediment and water, including ready biodegradability. Using the prediction system JANUS, transformation products formed in the environment can be evaluated. JANUS is also available within VEGA. Further examples for models to predict biodegradability are Biowin1–7 of the US EPA, CASE Ultra from Multicase, Model Applier from Leadscope, VEGA, and the OECD QSAR Toolbox (Rücker and Kümmerer, 2012). However, the applicability domain of *in silico* methods for environmental (bio)degradability is generally limited for pharmaceuticals as experimental data is missing to build (better) models with an applicability domain of considerable size. A practical *in-silico* workflow addressing such limitations was suggested by Lorenz et al. (2021) to support the design of new environmentally mineralizing compounds.

Generally, chemical and taxonomic read-across approaches may overcome the limited applicability domain of QSARs, and other future *in silico* approaches of artificial intelligence could also help, but their use requires great expertise, standardization, and rigorous validation (Benigni, 2019; ECHA, 2017; Fenner et al., 2020).

Attributes of the screening tools (throughput, costs, etc.) will decide for which phase in R&D a particular tool or assay is suitable. For instance, new tools and assays with lower throughput and more complexity will be helpful in later stages of drug discovery (e.g. lead optimization), as well as help improve knowledge and validate models used in the earlier stages. New tools with higher throughput and lower complexity could in turn prove helpful in the earlier stages of drug discovery (e.g. hit selection and optimization). In the long-term, a standard toolbox that is suitable for routine screens in analogy e.g. to the OECD QSAR toolbox (OECD, 2021) would be helpful for successful application of environmental considerations in R&D processes.

Efforts to develop screening tools are needed to close the identified gaps in methodology. Collaborations within and across pharmaceutical companies and between industry and academia on these topics was seen as highly promising. The incorporation of environmental parameters

into early R&D would benefit from an earlier involvement of environmental experts (including industry's own environmental experts) in the R&D process. Currently, these experts become involved around preclinical studies only (cf. Fig. 1). Including environmental expertise in the earlier phases could be beneficial, in a similar fashion to previous process transformations in pharmaceutical R&D in which other areas (e.g. formulation) were addressed earlier in the R&D process, as it was realized that an earlier evaluation of these topics was potentially profitable in the long term. R&D experts need to become aware of the issue of pharmaceuticals in the environment and how they can use models and assays. This will facilitate further discussions on the feasibility and follow up developments of incorporating *the design of greener APIs* in the R&D process. Furthermore, pharmaceutical companies could cooperate to pool data for a common large database needed for development of prediction models. Moreover, exchange with other sectors facing the same challenge of designing more environmentally friendly products, e.g. agrochemical or detergent industries, could also support efficient research and development of tools.

Important for the addressing of identified needs in research and development are financial, social, and regulatory incentives. Interviewees proposed a number of soft and hard drivers that would be reasonable 'carrots and sticks' to encourage industry investment: creating awareness and knowledge, (intrinsic) motivation, and public pressure, as well as pharmaceutical sector internal standards, increased patent life, fast track approval, financial drivers, and regulations. The European Union's 'Strategic Approach to Pharmaceuticals in the Environment' highlighting "the green pharmaceutical design" (European Commission, 2019) might generate pressure on the research-based pharmaceutical companies. According to Ågerstrand et al. (2015) an inclusion of environmental risks into the benefit-risk assessment of a medicinal product under consideration for marketing authorisation would consequentially lead to an early consideration of environmental aspects.

Based on the identified needs for process modifications, we propose a roadmap with sequential actions that would facilitate industry moving towards *the design of greener APIs*. It consists of four phases: i) uptake of novel approaches, ii) data gathering, iii) toolbox development and iv) ongoing tasks (Fig. 4). Especially for step i) and ii), collaboration across pharmaceutical industry, academia, authorities and all other stakeholders is essential. These changes would profit from guidance and regulation.

4.4. Discussion of the study design

Due to the COVID-19 pandemic the interviews could not be conducted face-to-face, leading to a loss of personal atmosphere and body language on the one hand. On the other hand, this format allowed for more interview participants, as no travel time was required. Interviews resulted in fruitful discussions between the interviewees of the same company. The different numbers of participants per interview (2 - 7) might have led to losses in comparability between the seven interviews due to differences in the diversity of opinions and dynamics of the discussions between company members. However, the different numbers of participants per interview is considered to have less impact on the data evaluation, as interview data was evaluated qualitatively. The same applies to differences in statements between companies due to corporate culture and policy.

Since all interviewed experts work for major pharmaceutical companies, the perspective of smaller pharmaceutical companies and biomedical start-ups was not captured by this study. It would be desirable to elicit the perspective of smaller companies in future research, given their significant share of newly approved and marketed drugs (Ioannou, 2018).

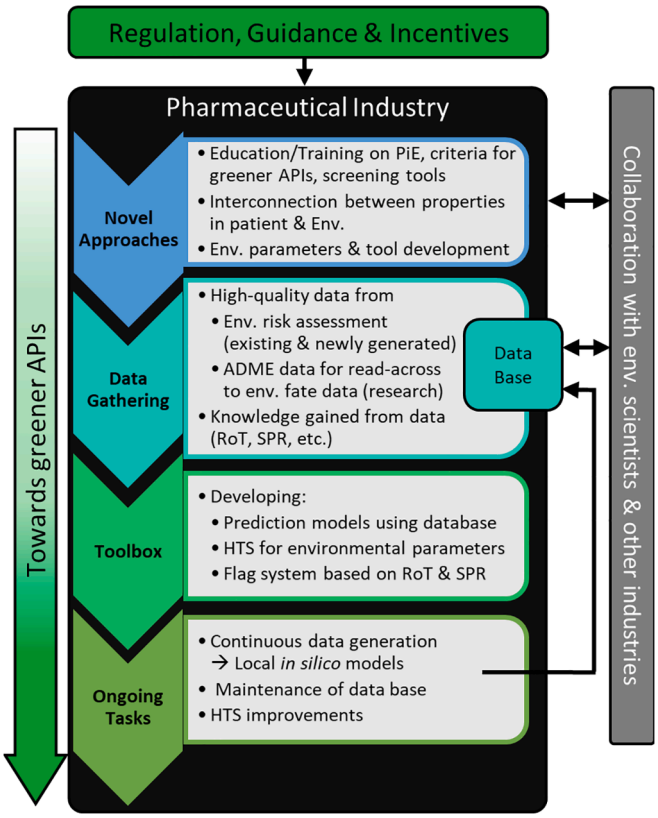

Fig. 4. Proposal of a roadmap towards *the design of greener APIs*.
Abbreviation: Env.: environment(al), PiE: pharmaceuticals in the env., HTS: high throughput screen, RoT: rules of thumb, SPR: structure-property relationship.

5. Conclusion

This study breaks new ground in that it managed to elucidate state, needs and opportunities for *the design of greener APIs* within pharmaceutical industry, based on in-depth interviews with medicinal chemists and other experts of major global pharmaceutical companies. Information on the technical feasibility of implementing *the design of greener APIs* was gained. Needs (e.g. tools and their attributes) and incentives for this process modification were identified.

The results indicate that including environmental considerations in pharmaceutical R&D is possible and could take place at several stages in the R&D process, from the hit selection phase to late stages of lead optimization. In addition, it could also result in new business opportunities, both in general terms and against the background of on-going policy developments e.g. in the EU. Environmental properties that are determined by the molecular structure should be considered early during R&D. The latest opportunity would be at late stages of lead optimization, as the molecular structure of an API candidate will no longer be subject to change thereafter. Uptake of environmental considerations is possible due to the highly flexible nature of the R&D process (with processes adapting continuously over time, but also individual projects updating continuously their approaches) and due to the fact that medicinal chemists are highly experienced in optimizing numerous interdependent parameters in parallel. Moreover, some properties that reduce environmental risks were considered compatible with common desirable API's properties in the human body, which are already optimized for in pharmaceutical R&D.

However, barriers exist to actually including environmental considerations in pharmaceutical R&D. Most significant are the challenges related to the current state of knowledge and the availability of appropriate *in silico* models and *in vitro* assays that can help evaluate

environmental parameters. For their uptake in R&D, environmental parameters should ideally be clearly defined, measurable and relevant. Furthermore, identified interdependencies between environmental aspects and the desirable API parameters need to be further investigated to understand exactly how any molecular modification affects its environmental behaviour and impact in order to identify synergies and possible hurdles. Collaboration between R&D experts and environmental scientists within and outside pharmaceutical companies and authorities would help R&D experts to better understand needs and possible future drivers on the one hand and existing tools to assess environmental properties and how to include environmental data into the decision-making during drug discovery on the other hand. For the development of new environmental assessment tools, financial, social, and regulatory incentives would be beneficial.

In future research, the scope of *the design of greener APIs* should be expanded to i) greener pharmaceutical products, i.e. considering excipients, and ii) further principles of green chemistry, e.g. reduced carbon footprint.

CRediT authorship contribution statement

Neele Puhlmann: Conceptualization, Methodology, Investigation, Writing – original draft, Visualization. **Rodrigo Vidaurre:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Klaus Kümmerer:** Conceptualization, Writing – review & editing, Supervision.

Data availability

The data that has been used is confidential.

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Supplementary materials

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Review

Transformation products of sulfonamides in aquatic systems: Lessons learned from available environmental fate and behaviour data

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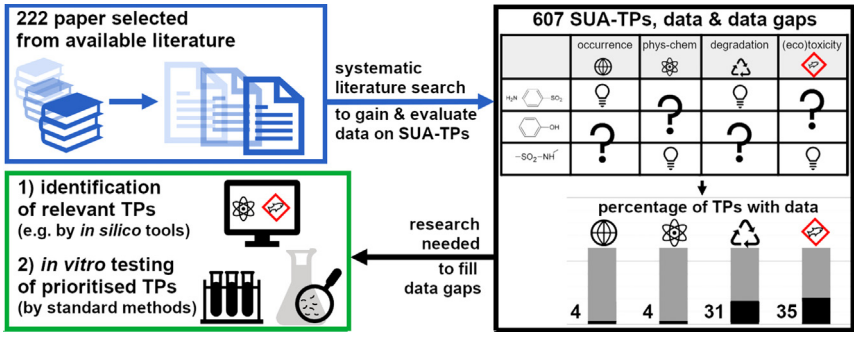
HIGHLIGHTS

- 607 transformation products (TPs) of sulfonamides reported in 222 paper
- Data on occurrence, phys-chem, degradation, (eco)toxicity for 4%, 4%, 31%, 35%
- TP-mixtures can be more toxic than the single parent compounds.
- Lack of data of high informative value by standard methods
- Mineralisation of TPs often unknown due to data gaps especially for individual TPs

GRAPHICAL ABSTRACT



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ABSTRACT

Sulfonamides (SUAs) and their transformation products (TPs) contribute to environmental pollution. Importance of research on TPs' properties has been emphasised, e.g. allowing a comprehensive environmental risk assessment of their parent compounds. However, TPs' properties have been discussed in reviews on SUAs only marginally, if at all. For the first time, a scientific literature review aims to discuss the current state of knowledge on SUA-TPs including research gaps, and commonalities of SUA-TPs and TPs in general.

Literature on SUA-TPs was consulted systematically to collect data on occurrence, physicochemical properties, degradability, and (eco)toxicity. TPs of 14 SUAs were reviewed, and aspects applicable for TPs in general were identified to guide future handling of TPs as a complex category of compounds. The data of sulfamethoxazole (SMX), the main representative, was analysed in more detail to discuss insights on a chemical level.

Literature search resulted in 607 SUA-TPs reported in 222 publications. Only for 4%, 31%, and 35% of these TPs, data on occurrence in aquatic systems, on degradation, and (eco)toxicity, respectively, was found. Several mixtures of SUA-TPs were more ecotoxic than their parent compounds, e.g. 10 of 15 mixtures of SMX-TPs. Only few TPs were tested as single substance. Although several TPs could be eliminated experimentally, their mineralisation rate remained often unknown. Thus, further transformation to persistent TPs could not be ruled out. Standardised biodegradability tests of individual TPs would monitor their mineralisation rate, but are almost completely lacking. Reasons are likely poor availability of TPs, but also the focus on abiotic water treatment.

Data assessment demonstrated that data of high significance according to standard methods, e.g. OECD methods for chronic (eco)toxicity and ready biodegradability, is needed to assess environmental risks of prioritised TPs, but also to redesign their parent pharmaceutical for complete environmental mineralisation in a long-term (*Benign by Design*).

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Contents

1. Introduction

The globally increasing use of pharmaceuticals comes along with ubiquitous and increasing pollution of the environment by their active ingredients (Wilkinson et al., 2022). As other active pharmaceutical ingredients (APIs), antibiotic sulfonamides (SUAs, effective against bacterial infections e.g. of the urinary tract) are excreted after human or veterinary administration either in their parent or metabolised form. They can enter the environment through effluents of wastewater treatment plants (WWTP), as a result of incomplete retention, through untreated wastewater, including stormwater overflow, or through the application of manure on soils of agriculture (Kümmerer et al., 2018; Tian et al., 2020). Contaminated manure is a relevant path of SUAs into the environment. With 544.1 t of SUAs (active ingredient weight only) sold in 31 European countries in 2018, SUAs account for the third-largest amount of sales of veterinary antibiotics after tetracyclines and penicillins (European Medicines Agency, 2020). In recent years, the ubiquitous environmental occurrence of APIs, including SUAs, in the ng L^{-1}–μg L^{-1} range has been reviewed extensively (Charuaud et al., 2019; Fatta-Kassinos et al., 2011a; Felis et al., 2020). Transformation products (TPs) of APIs result from (bio)chemical transformation of APIs within the technical systems, such as WWTP, and in the environment after their release. TPs contribute to environmental pollution (Fatta-Kassinos et al., 2011b; Haddad et al., 2015; Klavarioti et al., 2009; Kümmerer et al., 2019). The occurrence of SUA-TPs is discussed in very few reviews only as an aside, e.g. with a focus on manure (Spielmeyer, 2018; Wohde et al., 2016).

SUAs and related TPs can affect the environment and human health, e.g. by influencing the composition of microbial communities (Cycoń et al., 2019), generation of antibiotic resistance (Baran et al., 2011; Ezzariai et al., 2018; Felis et al., 2020), or by phytotoxicity (Christou et al., 2018). However, properties of SUA-TPs are mostly unknown, if these TPs are known and identified at all (Kümmerer et al., 2019). Even more, adverse effects of known TPs and compound mixtures, including metabolites and TPs, are difficult to predict. This is not only due to a lack of reference standards but also due to a large number of endpoints to be investigated (Vasquez et al., 2014). As a reaction to this situation and the European Green Deal (European Commission, 2019), the EU has developed the "Strategic Approach to Pharmaceuticals in the Environment" recently (European Commission, 2020c). This strategy announces upcoming changes in the legislation of pharmaceuticals to tackle environmental pollution.

In the context of the removal of SUAs from sewage or the aquatic environment, considering only removal rates or half-lives of parent compounds (PC) is not sufficient: Information on TP formation and their environmental fate is required (Cycoń et al., 2019; Ezzariai et al., 2018; Spielmeyer, 2018). TPs of the main representative sulfamethoxazole (SMX), such as *N4*-acetyl-SMX, are often only mentioned in the context of environmental processes (Bílková et al., 2019; Felis et al., 2020) as well as within abiotic or biotic wastewater treatment (Charuaud et al., 2019; Tian et al., 2020; Wang and Wang, 2018). Pathways of a few SUAs based on several intermediates via different microorganisms were summarised (Chen and Xie, 2018; Wang and Wang, 2018). Among these bio-TPs are 3-amino-5-methylisoxazole, aniline, *N4*-hydroxy-SMX, and *N4*-acetyl-SMX (Chen and Xie, 2018). However, a comprehensive overview of TPs of diverse SUAs is missing, let alone their properties.

Although the importance of research on properties of the variety of TPs has been emphasised already in 2011 (Fatta-Kassinos et al., 2011b), the most outcome of the reviews found with information on properties of SUA-TPs was only general or based on assumptions since the SUA-TPs were not in focus (Charuaud et al., 2019; Ezzariai et al., 2018; Spielmeyer, 2018; Tian et al., 2020; Wang and Wang, 2018; Wohde et al., 2016). General statements were that chlorinated TPs may be toxic and that TPs containing the pharmacophore may be still active (Charuaud et al., 2019), whereas more polar *N4*-OH-sulfadiazine and TPs by cleavage of the heterocycle should be less active (Spielmeyer, 2018). There were no validations of these general statements and assumptions by (eco)toxicity studies of single TPs. However, if TPs are not considered, the fact that *N4*-acetylated SMX transfer back to SMX, for example, demonstrates the potential underestimation of the environmental effects of SUAs (Ezzariai et al., 2018; Wohde et al., 2016). Recent reviews paid more attention to transformation processes and toxic effects of SUA-TPs than before. Core element of provided information is the elimination of SUAs to support the development of water treatment technologies rather than data on SUA-TPs needed for their environmental risk assessment (Hu et al., 2022; Li et al., 2021).

Properties of SUA-TPs, including their environmental fate, have so far only been marginally discussed. This is the first time that a literature review aims to put the TPs instead of the PC in the spotlight. We discuss data that provide information on occurrence, degradability, and (eco)toxicity of SUA-TPs. Such data is needed for a comprehensive environmental risk assessment of SUAs. We aim to find common features and dissimilarities of SUA-TPs and identify knowledge gaps as guidance for future research. Aspects for TPs in general are of special interest in guiding the future handling of this complex category of compounds.

Considering these objectives, literature on SUA-TPs was searched systematically to collect data on their occurrence and properties. Although studies focused very often on the removal of the PCs only, secondary data on the behaviour of TPs could be obtained from such studies. All properties of

reviewed TPs were included in our growing dataset together with information on their formation type (abiotic/biotic), structure, detection, and identification method, and the corresponding confidence level of the structure. The numbers of TPs in total, and for each SUA respectively, were determined to show the diversity and identify more or less studied SUAs. The proportion of TPs with data entries on occurrence and properties out of all collected TPs was extracted from the created dataset to identify knowledge gaps and further need for research. Data on the TPs of SMX (the most investigated SUA) was elucidated in more detail to understand their behaviour better, and based on this, to derive common ground for SUA-TPs.

2. Methods

2.1. Literature selection and description of publication pool

The literature was screened and selected systematically (Fig. 1). Based on an initial literature search using also the *snowball* principle (Greenhalgh and Peacock, 2005), the following SUAs were selected: sulfachloropyridazine (SCP), sulfadimethoxine (SDM), sulfadiazine (SDZ), sulfaguanidine (SGU), sulfisoxazole (SIX), sulfamethoxypyridazine (SMP), sulfamerazine (SMR), sulfamethizole (SMT), sulfamethoxazole (SMX), sulfamethazine (SMZ, synonym sulfadimidine), sulfapyridine (SPY), sulfasalazine (SSZ) and sulfathiazole (STZ). The database Web of Science Core Collection was used to search for publications with the name of the selected SUA and "Degrad* OR Transformation" in the topic. Besides Web of Science, the database SciFinder was used for the systematic search of publications on SMX-TPs. References containing the two concepts "transformation products" and "sulfamethoxazole" closely associated with one another were selected for further screening. To cover a broader part of the literature and not to miss significant literature the *back-snowball* principle (Greenhalgh and Peacock, 2005) was applied.

The time frame (2000 till August 2021) was set based on an initial literature search regarding the development of research on TPs over time. The procedure and results of this first search can be found in Section 1 of Supplementary information. A number of search hits ≥70 were refined using the keyword "product". For the selection of suitable publications, the following excluding criteria were applied: The title is not within the subject; the abstract is not about identified TPs, but rather about the removal of SUAs or analytical method development; in the text, no TPs are mentioned (at least by giving molecular formula).

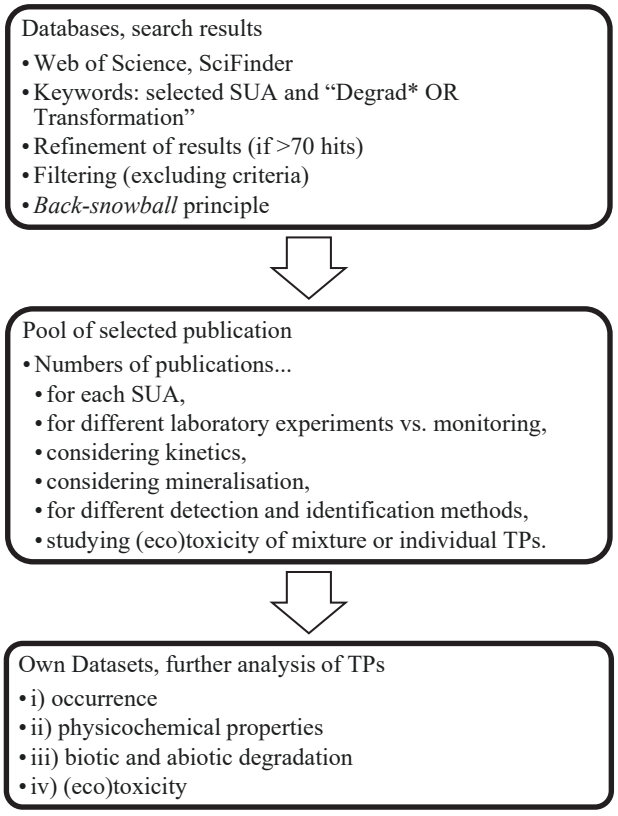


Fig. 1. Workflow from literature databases to own datasets.

Thereafter, the pool of selected publications was described and evaluated by the following parameters: Number of publications…

a) …for each SUA,
b) …for different laboratory experiments of treatment processes (simulation of a treatment stage in waste water or potable water treatment plants (WTP), or processes in the environment, abiotic or biotic) vs. monitoring in WTP or the environment,
c) …considering kinetics of TPs during the treatment process,
d) …considering mineralisation during the treatment process, e.g. by total organic carbon (TOC),
e) …for different detection and identification methods, and
f) …studying the (eco)toxicity of the mixture or individual TPs.

2.2. Creation of datasets and further analysis of TPs

Own datasets were created with information about TPs focussing on their i) occurrence in WTP or the environment, ii) physicochemical properties, iii) biotic and abiotic degradation and iv) (eco)toxicity (Fig. 1). Thereby, the data was pre-assessed and grouped regarding the following procedure:

i) Data on the TP's occurrence in WTP, e.g. influent or effluent, or the environment, e.g. surface, ground, or spring water, was taken from literature if the method, including sampling and TP detection, was well described. Thereby, the information describing the sample was important for the datasets. Since reference standards are most often not available for TPs their use was no prerequisite. Therefore and because of an occurrence in low concentrations, other data than values for concentration, e.g. peak areas or the simple detection without any quantification, was also stored.
ii) Data on physicochemical properties via well-described calculation or experimental determination was collected. Among them were octanol-water partition coefficients expressed as logarithm (log P) and acid/base dissociation constants for the sulfonamide (*N1*) group (pK_{a2}). In natural waters, only the deprotonation of this NH-group in SO_2NH was relevant since the pK_{a1}-values for the *p*-amino (*N4*) group of SUAs are generally below pH 2 (Bonvin et al., 2013).
iii) To receive data for degradation, the kinetics of TPs during the degradation of the SUA, TP-mixtures, or the TP itself were surveyed concerning the concentration-time curve to evaluate the elimination kinetics. Thereby, data was collected in dependence on the type of degradation test, i.e. biotic or abiotic degradation according to a) standard methods, such as OECD, or other methods simulating b) the (advanced) water treatment or c) processes in the environment. The various test designs (e.g. regarding concentration of SUA, pH, oxygen, catalyst, treatment period), especially if not standardised, needed to be described in the study and saved together with the received data. Data on degradation based on in silico predictions was not part of the screened publications and thus not considered.

To evaluate the data entry/entries per TP, we have classified into "not eliminated", "eliminated", and "varying results" in dependence on the type of degradation test (6 types: a–c, biotic or abiotic each). TPs of the class "not eliminated" were not or partially eliminated (<65%) until the end of the test period under the given conditions, whereas "eliminated" TPs were completely or almost completely eliminated (≥65%) within the test period under the given conditions. This classification was simple for TPs with a single data entry but less meaningful, compared to TPs with more than one data entry of similar elimination rate (within the same

Table 1
Number of publications classified by their test methods of mineralisation.

Mineralisation	total	TOC	DOC	NPOC	N&S	N	^{14}C	^{13}C
Total	**76**	**55**	**11**	**2**	**16**	**3**	**4**	**1**
TOC	**55**	42	1		11	1		
DOC	**11**	1	8		1	1		
NPOC	**2**			1	1			
N&S	**16**	11	1	1	3			
N	**3**	1	1			1		
^{14}C	**4**						4	
^{13}C	**1**							1

category for the type of degradation). Otherwise, i.e. when the data entries did not indicate similar elimination rate (e.g. due to different test conditions), data entries were classified as "varying results".

iv) Data on (eco)toxicity was collected in dependence on the test approach, i.e. in vitro using TP-mixtures or the single substance or tested in silico. Thereby, the received data was saved together with the selected endpoint, e.g. growth inhibition of bacteria for ecotoxicity or mutagenicity for human toxicity. Combining test results on (eco)toxicity of TP-mixtures with kinetics for the TPs, such data could give hints for further in silico or in vitro testing of individual TPs.

2.3. Tools for in-depth evaluation of data on SMX-TPs

A more detailed study on SMX-TPs was conducted as representative for SUAs to derive generic conclusions. SMX was chosen because it was found to be the most investigated SUA, e.g. due to the highest number of publications and of TPs described in our publication pool. SMX-TPs were described in detail via post-processing. The data was evaluated as follows: The groups of data on the occurrences of SMX-TPs in WTP and the environment were subclassified by sample types, e.g. WTP influent or effluent and surface water or groundwater. Data of these subgroups was reviewed.

As an extension of the classification into "not eliminated", "eliminated" and "varying results", the elimination rate of TPs with at least 3 data entries was assessed taking the test conditions into account to demonstrate the complexity of degradability due to its strong dependence on diverse biotic and abiotic test conditions. For this discussion, the mineralisation rate of the organic substances during the treatment was considered when available.

Data on (eco)toxicity (grouped by test approach, i.e. in vitro using TP-mixtures or the single substance, or in silico) was reviewed regarding different endpoints (initially without any exclusion of endpoints to get an overview). Where possible, TPs were classified as "toxic" and "not toxic" for a given endpoint. Thereby, (eco)toxicity was assessed in comparison to that of SMX. "Toxic" means similar or more toxic than SMX, and "not toxic" less toxic than SMX. In the case of different toxicities per TP, due to the dependence on different endpoints, the result of (eco)toxicity was classified as "depends". There, a closer evaluation was done.

3. Results

3.1. Selection and description of publications by their analytical methods (kinetic, mineralisation, and detection/identification)

In total, 222 publications were selected based on method 2.1 that has defined excluding criteria. Most of them correspond to SMX (88), SMZ (60), and SDZ (54), whereas only a few or no references were selected for SMP (5), SMT (5), SSZ (3), and SGU (1). A huge impact on the nature of data has the type of treatment of the PC. The focus in research providing information on TPs was on the abiotic advanced (waste)water treatment: 111 references, i.e. half of all selected publications. Especially the photocatalysis or (advanced) oxidation processes were studied to understand the removal of the PCs. The research extent of other categories (see method 2.1, lit b: simulation of biotic treatment stages in WTP, of abiotic or biotic processes in the environment, and monitoring in WTP or the environment) with around 25 references each was similar (Fig. S3).

Kinetics of generation and possible elimination of TPs were reported in 80 publications. 41 references of these are dealing with the treatment and removal of SMX. Data on kinetics together with the degree of mineralisation can help to distinguish transient and persistent TPs. Mineralisation was investigated by 76 publications to characterise the efficiency of PC's treatment. Among them are 56 references, i.e. more than two-third, that correspond to experiments investigating advanced treatment (abiotic) in WTP, showing that improving abiotic treatment is the focus of research. In Table 1 the publications are classified by their test methods of mineralisation. Among 76 publications investigating mineralisation, 55 publications used the measurement of TOC. There are publications with combined methods. For example, 11 publications investigated kinetics of TOC in the treatment system together with kinetics of nitrite/nitrate and sulphate (N&S) to receive more information on mineralisation. The degree of mineralisation ranged from 0% to 100%, indicating that it strongly depends on test conditions. In general, drastic conditions (e.g. ozone concentration, catalysts) and a longer treatment period lead to a higher mineralisation rate. For example, 95% up to 100% mineralisation was reached in 11 studies mostly via electro-Fenton reaction (up to 400 mA, up to 8 h treatment) (e.g. Dirany et al., 2012; El-Ghenymy et al., 2013), or photocatalysis (up to 24 h treatment) (e.g. Fukahori and Fujiwara, 2015; Guo et al., 2013), and in one case even biotically using an isolated and acclimated SMX-degrading stain (*Acinetobacter* sp.) (Wang et al., 2018).

In 192 publications that deal with the TP formation and identification the data quality depends mainly on the sensitivity of detection and identification methods. Mass spectrometry (MS) plays a major role. Especially, tandem mass spectrometry (MS/MS) was most frequent (118 of 192 publications) used for the detection and identification of TPs. Others, such as the DAD-, UV- or conductivity detector play a minor role (Table 2). NMR, suitable for structure elucidation, was used rarely (10 publications), likely because it is very demanding (e.g. on amount of material).

In only 26 publications all detected TPs, and in further 31 publications, a part of TPs was identified by reference standard. This means that in 135

Table 2
Number of publications classified by detection or identification method. Abbreviation: tandem mass spectrometry (MS/MS), high-resolution mass spectrometry (HR-MS), mass spectrometry (MS), diode array detector (DAD), ultraviolet/visible light detector (UV), nuclear magnetic resonance spectroscopy (NMR).

Detection/ identification	Total	MS/ MS	HR - MS	MS	DAD	UV	Radio	Conduct-ivity	NMR
Total	**192**	**118**	**29**	**38**	**9**	**2**	**2**	**2**	**10**
MS/MS	**118**	110						2	6
HR-MS	**29**		27						2
MS	**38**			30	6				2
DAD	**9**			6	3				
UV	**2**					2			
Radio	**2**						2		
Conductivity	**2**	2						0	
NMR	**10**	6	2	2					0

publications (70%) no reference standard was used. One reason is that TPs are often new compounds that cannot be bought and have to be synthesised (associated with high costs) which in turn needs a safe assessment of the molecular structure.

The results indicate that data on TPs' (eco)toxicity is not very extensive, particularly for other SUAs than SMX. In around one-fourth of publications (59 publications) (eco)toxicity of TPs is discussed. Among these are 23 publications corresponding to SMX. Typical endpoints of in vitro testing were growth and/or luminescence inhibition against bacteria, such as *Vibrio fischeri* and *Escherichia coli* (28 bacteria test studies). It must be stressed that mainly mixtures of TPs (received from SUA treatment) were investigated (41/59 publications; entire distribution of (eco)toxicity tests given in Table S1), leading to low significance for a single TP. In mixtures, adverse effects are difficult to predict, due to the mutual influence of the components. Furthermore, the effect concentration relation is specific for each compound. Thus, even when the TP composition of tested mixture, including quantity ratios, is known, it cannot be concluded with certainty that the TP of the highest concentration is most or solely responsible for the toxic effect.

Summarising, we found that there is an imbalance between the extent of research on different SUAs with SMX as most investigated SUA. Reasons could be different medical consumption and therefore different expectations for the input into the environment associated with possible environmental risks, which result in prioritised research. SMX is the most prominent SUA antibiotic. For example, the combination of SMX and trimethoprim, in contrast to other SUAs, was among the antibiotics that contributed to 75% of the total oral antibiotic consumption in 16 out of 65 countries and areas in 2015 (World Health Organization, 2018). Analytical challenges play a further role. For example, most SUAs are water-soluble, except SSZ (Baran et al., 2011). This could be a reason for fewer publications on SSZ. A less scientific reason, but a typical phenomenon familiar to many researchers, is *me-too* research that builds on available knowledge, tools, and methods, including materials and chemicals.

Furthermore, most studies of our publication pool aimed to improve advanced treatment of SUAs, mostly through drastic conditions. It seems that the focus in research is on the development of treatment in WTP, less on environmental behaviour and assessment. However, costly advanced treatment cannot be the sole measure to avoid environmental micro pollution since only 80% of wastewater is treated at all (UN Water, 2017). Keeping the description of our publication pool in mind, the TP data described in the following can be analysed better.

3.2. Evaluation of data on TPs reported

`The systematic literature research resulted in 607 reported SUA-TPs. The distribution of these TPs on corresponding PCs is shown in Fig. 2. The number of TPs per SUA is linked to the number of studies on the respective SUA. Main representatives are TPs of SMX, SDZ, and SMZ with 124, 119, and 103 TPs, respectively, whereas TPs of SMT, SMP, and SGU are less represented with 15, 10, and 8 TPs, respectively.

3.2.1. Chemical analysis and structure elucidation of TPs

Among the total of 598 TPs formed in degradation tests, there are 431 abiotic, 102 biotic, and 65 both, abiotic and biotic TPs. Furthermore, the SUA-TPs are classified according to their detection and identification method to support the assessment of the confidence in the proposed or elucidated structure. Fig. 3 demonstrates that MS/MS is the most often applied detection method and reflects the foregoing publication description. Structures are proposed based on fragmentation patterns. High resolution MS techniques that detect the precision mass (monoisotopic mass with 4 decimal places) improve the determination of the formulas of the ions. However, without further elucidation e.g. via NMR spectroscopy the precise identification of isomers, for example, is almost impossible, if no reference standard is used to confirm the proposed structure (used for only 12% of 598 TPs (72/598)). Therefore, in many cases isomers cannot be discerned as the accurate position of the hydroxy group at the benzene ring, for example, remains unclear. Structures of only 4% (26/598) were elucidated by NMR. UV and DAD techniques are useful to observe kinetics of the PC and TPs. However, they are not suitable for TP structure elucidation; Reference standards are essential for TP identification: 6 TPs were detected using a UV or DAD method; All of them were identified with a standard.

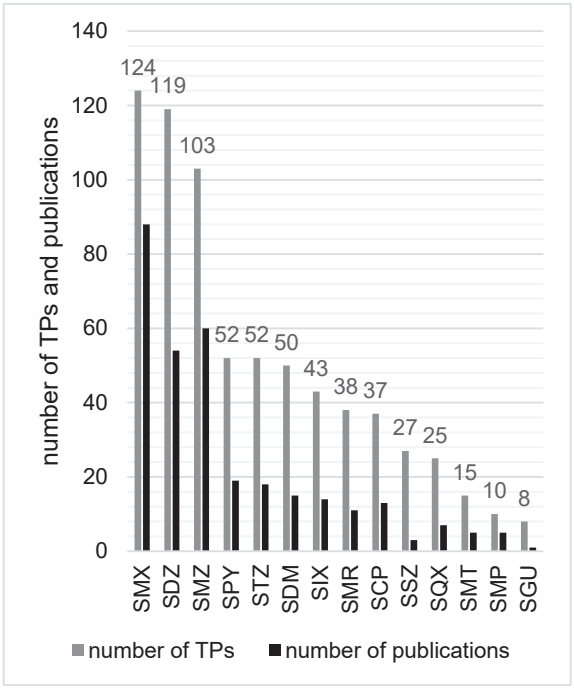


Fig. 2. Number of TPs reported in the selected publications for each SUA of in total 607 SUA-TPs.

Datasets were created particularly to gather information on occurrence and properties of the described TPs. An overview of found data is given in Fig. 4. Data entries regarding occurrence in WTP and/or the environment are made only for 23 TPs, i.e. nearly 4%, relating to 13 TPs with data for WTPs and environment, 4 TPs only for WTPs, and 6 TPs only for the environment.

Based on physicochemical properties of compounds, e.g. log P, their environmental fate and behaviour can be assessed. However, there is almost no data for selected TPs. Only for 21 TPs (4%), the log P (predicted or experimentally determined) was found in considered literature. Among them are

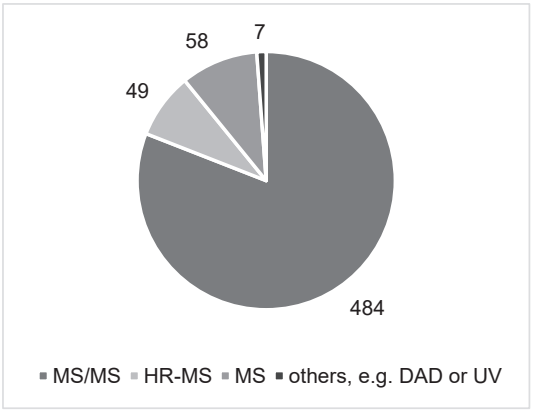


Fig. 3. Number of TPs classified by their detection or identification method out of 598 SUA-TPs in total. In case of more than one data entry, the most sensitive method was considered. Abbreviation: tandem mass spectrometry (MS/MS), high resolution mass spectrometry (HR-MS), mass spectrometry (MS), diode array detector (DAD), ultraviolet/visible light detector (UV).

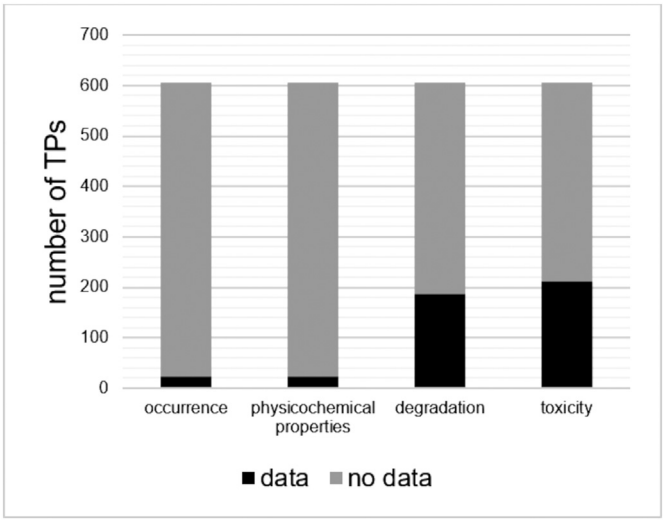


Fig. 4. Overview of TPs with data entries regarding occurrence and properties.

8 TPs, for which the log P is discussed together with the bioconcentration factor to make statements regarding their fates (Zhang et al., 2020).

Data on behaviour during abiotic or biotic degradation was extracted for 187 TPs. Data is based mainly on kinetics under given test conditions. Studies on degradability of individual TPs, taking their mineralisation rates also into account, are very rare. The data evaluation leads to classifying TPs into "eliminated", "not eliminated" and "varying results" depending on type of degradation test (Fig. 5). TPs with "varying results" are mainly TPs studied more often. Consequently, the results differ depending on test methods. This group demonstrates that a rough grouping of degradation tests is a simplification. It cannot reflect the wide range of test conditions during experimental tests simulating technical treatment processes or the environment. Furthermore, data according to standard test methods, e.g. OECD, with clearly defined conditions are very rare. There are data entries only for 21 and 5 TPs resulting from biotic and abiotic degradation, respectively. Additionally, among them, data of 10 TPs reflects biotic degradation of two TP-mixtures, that were both more biodegradable than their PCs (Gao et al., 2013; Sun et al., 2019). Such results are not very meaningful for a single TP as TP's mineralisation remains unclear, and give only hints for further investigation.

Data on (eco)toxicity were found for 212 TPs (Fig. 4). However, the data is based mainly on tests of TP-mixtures (data for 172 TPs). Thus, a clear indication of (eco)toxicity of individual TPs is hardly possible. Even data by in silico methods, where the substance does not have to be available, is rare (data for 68 TPs). Only for 17 TPs, data on (eco)toxicity from in vitro testing of the single compound was found.

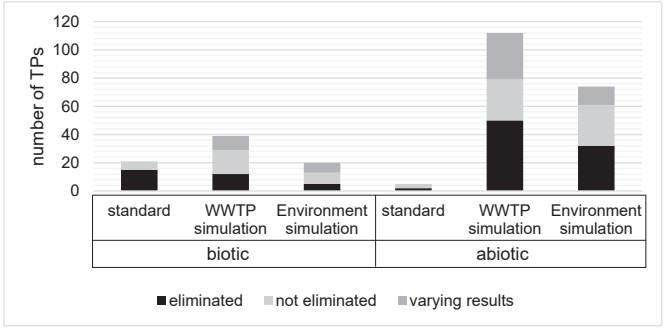


Fig. 5. Number of TPs with data on degradation during different degradation tests with further classification, i.e. "not eliminated", "eliminated" or "varying results" during degradation tests, mostly derived from kinetics. It depends strongly on the test conditions.

Summarising, more than 600 SUA-TPs were found in the reviewed literature. Most of them were detected and their structures proposed using MS/MS and HR-MS. Only a few SUA-TP structures were further elucidated by NMR, e.g. to distinguish isomers. The main reason is likely that the focus of most of reviewed studies was on the elimination of the SUA with TP detection in passing. Furthermore, TPs were present in low concentrations (as often several TPs are resulting from one PC) and would have required elaborate isolation for structure elucidation by NMR. This is in line with the findings of other authors: Non-targeted MS/MS and HR-MS would be suitable for a more sophisticated TP analysis. They are increasingly used (Chibwe et al., 2017; Hernandez et al., 2014; Wohde et al., 2016).

Data on occurrence and physicochemical properties of SUA-TPs are very rare. The evaluation of data on degradation shows that tests of environmental degradation according to standard methods, e.g. OECD, are underrepresented, although they would allow a more reliable and comparable assessment of degradability. Moreover, they provide a data basis to develop or improve in silico models. The (eco)toxicity entries are dominated by data on TP-mixtures. Data on single TPs are rare.

One of the reasons for these data gaps is likely that TPs in general are not intentionally formed for any use as it is for the PCs, and therefore not or hardly regulated by law. Even TPs from pesticides, which are subject to requirements for ecotoxicology in contrast to pharmaceuticals (European Union, 2009), are still neglected in current proposals for future risk assessment of pesticides (Schäfer et al., 2019). However, TPs are likely to get more attention, e.g. due to the European Green Deal and the follow-up EU strategies, e.g. for a toxic-free environment (European Commission, 2020a, 2020b, 2020c, 2020d). Associated with the aspect of unintentional formation, is the low commercial availability of TPs, complicating individual testing. Commissioning an elaborate synthesis would be related to high costs (Menz et al., 2017). This is seen as another reason for the data gaps.

3.3. In-depth evaluation of the data on SMX-TPs

The dataset evaluation revealed that SMX is the most investigated representative (88 publications). In total, 124 SMX-TPs were described in the analysed literature. Among them are constitutional isomers that are counted in our dataset as one TP if the detailed structure was not elucidated (e.g. position of a hydroxyl group at a benzene ring).

Detection via MS/MS accounts for the largest part of SMX-TPs (Table S3), analogous to all SUA-TPs. 22 of 118 TPs were identified using a reference standard. 9 of 118 TPs could be identified by NMR, four of them also by a reference standard: 3A5MI, *N4*-chloro-*p*-benzoquinone imine, desamino-SMX, and *p*-nitro-SMX. Based on this evaluation regarding the detection and identification methods as well as on the comparison with literature data, the confidence level is set for the proposed or elucidated structures of the SMX-TPs (Table S3). For this purpose, the classification of Schymanski et al. (2014) is extended by level 3c and 3d: probable structure detected by MS/MS or single MS, respectively, but missing comparison with literature data (Table S2).

70% of described SMX-TPs (87/124) were formed under abiotic conditions, particularly via photolysis and photocatalysis. Among them are highly hydroxylated TPs and dimers. Further 13% (15/124) are formed also under biotic conditions, e.g. *p*-nitro-SMX (TP283c), 3-amino-5-methylisoxazole (3A5MI, TP-99) and *N4*-acetyl-SMX (Ac-SMX, TP295a). The large number of abiotic TPs (87 + 15 = 102) is the consequence of the publication pool with predominantly abiotic treatments of SMX, as shown above. The low-selective attack by ubiquitous OH-radicals during advanced oxidation and photolysis is another reason for the diversity of abiotic TPs. For example, in the abiotic treatment experiment from Hu et al. (2021), using photocatalysis, and Zhu et al. (2021), using electro-Fenton, 17 and 18 TPs, respectively, were detected just for one single PC. It must be stressed that there are 55 abiotic TPs that were described only within one study each. However, several degradation methods, e.g. photo(cata) lysis or oxidation by ozone or chlorine can also lead to the same products. The SMX-TPs 3A5MI (TP98b), *p*-nitro-SMX (TP283c), an X-OH-SMX isomer (OH at benzene, TP269b), the SMX isomer (TP253), sulfanilic acid

(TP173b), and desamino-SMX (TP238b) are generated and detected by at least 10 different studies. Different test conditions regarding the kind of catalyst, initial concentration of SMX, and pH value, for example, can also result in the same TPs. For instance, the oxidation of SMX by Gao et al. (2014) using chlorine, permanganate, and ozone led to 7, 5, and 5 TPs each, and in total 11 different TPs. Thus, among them are identical TPs formed by different oxidants: 3A5MI, *p*-nitro-SMX, and an X-OH-SMX isomer (OH at benzene, TP269b) (Gao et al., 2014).

17% (21/124) were generated only under biotic conditions, e.g. *N4*-formyl-SMX, *N4*-pterin-SMX, and SMX-*N1*-Glucuronide. Most of them were described only within one study each. The distribution indicates a gap in research on biotic processes, whether in the environment or technical treatment. Nevertheless, even with increased research efforts, lower diversity of biotic TP would be expected, as specific enzymes lead to fewer biotic TP, in contrast to abiotic processes driven by highly reactive and therefore less selective OH-radicals.

3.3.1. Occurrence of SMX-TPs in WTP and the environment

Information about the occurrence of SMX-TPs in WTP and/or the environment is available for 10% of identified TPs (12/124) (Table 3). These are all TPs most often described (at least by 10 references), apart from the SMX isomer (TP253) and sulfanilic acid for which information on occurrence is missing in our dataset. Most data entries were collected for Ac-SMX with 22 data entries since it is also a well-investigated metabolite and appeared already in some reviews (Ezzariai et al., 2018; Fatta-Kassinos et al., 2011a; Felis et al., 2020; Wohde et al., 2016). *p*-Nitro-SMX, desamino-SMX, and SMX-glucuronide (SMX-Glu) are investigated in at least 4 studies, all others in only one study.

The analysis of influent and effluent is a typical test setup for the investigation of samples from WTPs. Such an influent-effluent study was conducted by more than the half of analysed studies (12/20). Influent-effluent comparison enables conclusions regarding the fate of TPs. More specific is the test setup by Deeb et al. (2017): The sampling was before and after ozonation, at the effluent after biological treatment, and finally from surface water loaded by wastewater. For example, 3A5MI was detected after ozonation, however was not detected before ozonation as well as after biological-post-treatment and in the corresponding surface water (Deeb et al., 2017). Thus, 3A5MI can be formed through ozonation, but eliminated or retained during biological treatment. To understand the behaviour during sedimentation, the occurrence in the sewer (pre-wastewater) and influent WW was compared by Mamo et al. (2018). The initial concentration of Ac-SMX was reduced by more than 50%. Desamino-SMX and *p*-nitro-SMX could not be detected in sewer or influent WW (Mamo et al., 2018). A fate study with conventional activated sludge treatment vs. a membrane bioreactor is also suitable to conclude on properties, in this case on biodegradability, where absorption on sludge or the membrane cannot be ruled out (García-Galán et al., 2016).

Environmental samples were most often surface water. In many cases, the circumstances of the sampling site (e.g. distance to the entry of wastewater) are regarded for the interpretation of data in terms of amount and diversity of TPs. The highest concentrations of Ac-SMX were detected in three rivers in China impacted by WWTP with max. 356 ± 30 ng L^{-1} (Zha et al., 2017). In groundwater, Ac-SMX was detected from different districts in Catalonia (Spain), e.g. with max. 6.2 ng L^{-1} in groundwater impacted by effluents from WWTP entering the Besòs river (López-Serna et al., 2013). Desamino-SMX and *p*-nitro-SMX were detected in groundwater in concentrations of max. 8 ± 1 ng L^{-1} and 54 ± 5 ng L^{-1}, respectively, at a watershed in Bolivia with high impact of wastewater (Brienza et al., 2017). The occurrence of TPs in spring water was pointed out in one study with 4 and 6 positive results out of 62 samples for desamino-SMX and *p*-nitro-SMX, respectively (Nödler et al., 2012).

Summarising, the occurrence of 12 SMX-TPs was investigated. All of them, except X-OH-SMX isomers (TP269b), were found in WTP and/or the environment, even sometimes in groundwater. This indicates mobility in the aquatic environment and therefore high polarity and at least temporary stability. TPs in general tend to be more polar than their PC due to

Table 3
Occurrence of SMX-TPs in WTP and the environment.

	WTP Eff: effluent Inf: influent d: detected nd: not detected	Reference	Environment SW: surface water GW: groundwater SpW: spring water nd: not detected	Reference
3A5MI	After ozonation: d	Deeb et al., 2017	SW: nd	Deeb et al., 2017
Desamino-SMX	1/7 Eff: d	Osorio et al., 2016	SW: ng L^{-1} range	Brienza et al., 2017; Osorio et al., 2016
	Inf and Eff: nd	García-Galán et al., 2016; Mamo et al., 2018	GW: max. 8 ± 1 ng L^{-1}	Brienza et al., 2017
			SpW (4/62): d	Nödler et al., 2012
X-OH-SMX isomer	Inf and Eff: nd	Deeb et al., 2017	SW: nd	Deeb et al., 2017
N4-formyl-SMX	8/9 Eff: d	Achermann et al., 2018		
p-Nitro-SMX	1/7 Inf, and 1/7 Eff: d	Osorio et al., 2016	SW: nd	Brienza et al., 2017; Osorio et al., 2016
	Inf and Eff: nd	García-Galán et al., 2016; Knopp et al., 2016; Mamo et al., 2018	GW: 54 ± 5 ng L^{-1}	Brienza et al., 2017
			SpW (6/62): d	Nödler et al., 2012
p-Nitroso-X-OH-SMX, COOH-SMX, or *p*-nitro-SMX	After ozonation and after biological post-treatment: d	Deeb et al., 2017	SW: d	Deeb et al., 2017
6,7-$(OH)_2$-SMX, reduced	After ozonation and after biological post-treatment: d	Deeb et al., 2017	SW: nd	Deeb et al., 2017
Ac-SMX	Numerous tests, recently reviewed	Felis et al., 2020	SW: max. 356 ± 30 ng L^{-1}, frequency 100% (n = 14)	Zha et al., 2017
			GW: nd - 6.2 ng L^{-1}, with frequency 25% (n = 21)	Jurado et al., 2020
			GW: d	López-Serna et al., 2013
			GW: nd	Archundia et al., 2017; Brienza et al., 2017
N4-HAc-SMX	1/9 Inf, and 8/9 Eff: d	Achermann et al., 2018		
N4-pterin-SMX	5/9 Eff: d	Achermann et al., 2018		
N4-PtO-SMX	3/9 Inf, and 7/9 Eff: d	Achermann et al., 2018		
SMX-Glu	Eff: d	Archundia et al., 2017	SW: ng L^{-1} range	Archundia et al., 2017; Brienza et al., 2017
			GW: nd	Archundia et al., 2017; Brienza et al., 2017
			GW: 9.66–22.9 ng L^{-1}	López-Serna et al., 2013

introduction of more polar groups (Chibwe et al., 2017), as is the case with investigated SMX-TPs of Table 3. If the concentration could be determined (i.e. the TP was available as an analytical standard), it was in the low ng L^{-1} range – typical for TPs in the environment (Fatta-Kassinos et al., 2011b; Kümmerer, 2010). No information about all the other SMX-TPs (112 TPs) is available in our dataset. The data gap for TPs of other SUAs is even larger. For TPs of pesticides, unlike pharmaceuticals, the data situation can be somewhat better since they are at least partially subject to an environmental risk assessment. For example, about 33% of 45 TPs (of six pesticides, potentially formed in the environment) have been already detected in the aquatic environment (Hensen et al., 2020).

3.3.2. Properties of SMX-TPs

3.3.2.1. Physicochemical properties. Log P and the pK_a are important substance properties that determine the fate in the environment as well as (eco)toxicity. However, only a few studies provided these substance properties (Bonvin et al., 2013; Majewsky et al., 2014; Nödler et al., 2012; Zha et al., 2017; Zhang et al., 2020). Log P and pK_{a2} of 17 TPs and 9 TPs, respectively, are given in Table 4. Achermann et al. (2018) found that bio-SMX-TPs such as *N4*-pterin-SMX and other pterin-conjugates occur mainly in the aqueous fraction (as findings on occurrence already suggested). Therefore, a lower log P is expected compared to SMX as is the case for hydroxylated SMX-TPs. Such compounds could be rather mobile in the aquatic environment compared to TPs with higher log P than SMX. TPs with higher log P, e.g. chlorinated or brominated SMX-TPs, might be more likely to bioaccumulate (Zhang et al., 2020). Majewsky et al. (2014) investigated the ecotoxicity of SMX-TPs about pK_{a2}. They concluded increased ecotoxicity with increased pK_{a2} using the EC_{50} for growth inhibition of *Vibrio fischeri* as endpoint (Majewsky et al., 2014).

Summarising, we found log P and pK_{a2} values only for a part of reviewed SMX-TPs, although physicochemical properties would be useful to dictate the behaviour of compounds in the environment. Physicochemical properties are already routinely predicted, e.g. log P within drug discovery due to time and cost savings (Lombardino and Lowe, 2004). Computational prediction, e.g. via ChemSpider or EpiSuite, can fill data gaps, but needs expertise and eventually an in vitro confirmation.

3.3.2.2. Degradability. Data about abiotic and biotic degradability were found for more than 50% of reported SMX-TPs (64/124), mainly extracted from kinetic studies considering concentration-time curves (kinetics of 47 TPs). Information for 48 (75%), 13 (20%), and 3 (5%) of these 64 TPs originates from abiotic, abiotic & biotic, and biotic tests, respectively.

The distribution of the classification for 64 SMX-TPs in dependence on type of degradation (standardised method, WTP or environmental simulation; biotic vs. abiotic) is nearly the same (Fig. 6) as for all SUA-TPs (Fig. 5). The lack of data according to standard methods is not that high as it is for the other SUA-TPs. However, the few data entries on biotic and abiotic degradation according to a standard method originate from two studies only, using OECD method 308 (modified water-sediment test setup) (Radke et al., 2009; Su et al., 2016). The comparison of rate constants for active and inactive sediments led to dissipation half-life (DT_{50}, all processes) and biodegradation half-life ($DegT_{50}$, biodegradation only) of TPs within a mixture after photolysis of SMX (Su et al., 2016), and of Ac-SMX and SMX-Glu (Radke et al., 2009). In the mixture, 3A5MI, *p*-nitro-SMX, the SMX isomer (TP253), and TP271c (SMX opened isoxazole ring) showed evidence of biodegradation (with DT_{50} of 39.7 d, 12.7 d, 7.6 d, and 2.4 d, respectively), whereas sulfanilic acid and 5-methylisoxazole-3-yl-sulfamate were eliminated abiotically (DT_{50} of 31 d and 74.9 d, respectively). Desamino-SMX and an X-OH-SMX isomer (OH at benzene ring) were recalcitrant (Su et al., 2016). Ac-SMX (DT_{50}: 8.5 d) and SMX-Glu (DT_{50}: 11.7 d) were removed from test system by biodegradation via sediment. Due to potential of back transformation into SMX under environmental conditions (Radke et al., 2009), they cannot be classified as biodegradable (Achermann et al., 2018). The fact that *N4*-acetyl-SUAs cleavage back into the PC was reviewed already (Ezzariai et al., 2018; Wohde et al., 2016). Back transformation was found in the selected literature also for *p*-nitro-SMX (Nödler et al., 2012; Su et al., 2016) and *p*-nitroso-SMX (Bonvin et al., 2013).

Data based on WTP and environment simulations is more complex since the fate of TPs depends strongly on the various test methods and conditions.

Table 4
Log P and pK_{a2} of SMX-TPs reported in references. SMX is listed for comparison.

TP	log P	Reference	pK_{a2}	Reference
SMX	0.89	Zhang et al., 2020	5.89 ± 0.07	Bonvin et al., 2013
3A5MI	0.3[a]	Majewsky et al., 2014		
HSO_2-3A5MI	<0[d]	Zhang et al., 2020		
SAA	−0.25[a]	Majewsky et al., 2014		
Sulfanilic acid	−2.04[a]	Majewsky et al., 2014		
Desamino-SMX	1.34 ± 0.40[b]	Nödler et al., 2012	6.92 ± 0.5[b]	Nödler et al., 2012
p-OH-SMX	0.67[a]	Majewsky et al., 2014	4.89, 5.97[a]	Majewsky et al., 2014
p-Nitroso-SMX	0.96[a]	Majewsky et al., 2014	4.71 ± 0.1,	Bonvin et al., 2013
			5.74[a]	Majewsky et al., 2014
X-OH-SMX (at isoxazole)			Not determined (rapidly converted to NO-SMX)	Bonvin et al., 2013
N4-OH-SMX	0.53[a]	Majewsky et al., 2014	4.51, 6.07[a]	Majewsky et al., 2014
p-Nitro-SMX	1.22[d],	Zhang et al., 2020,	3.66 ± 0.01,	Bonvin et al., 2013, Majewsky et al., 2014,
	0.81[a],	Majewsky et al., 2014	5.70[a],	Nödler et al., 2012
	1.27 ± 0.41[b]	Nödler et al., 2012	5.65 ± 0.4[b]	
6,7-$(OH)_2$-SMX, reduced	<0[d]	Zhang et al., 2020		
X-Cl-SMX	1.13[d]	Zhang et al., 2020		
Ac-SMX	0.86,	Zha et al., 2017,	5.90,	Zha et al., 2017,
	0.86[c],	Jurado et al., 2020,	5.07 ± 0.08,	Bonvin et al., 2013
	0.18[a]	Majewsky et al., 2014	5.88[a]	Majewsky et al., 2014
N4-(HO-acetyl)-SMX	−0.64[a]	Majewsky et al., 2014	5.28, 5.88[a]	Majewsky et al., 2014
Br-SMX (at benzene)	1.37[d]	Zhang et al., 2020		
Br-Cl-SMX (at benzene)	2.02[d]	Zhang et al., 2020		
Br-Br-SMX (at benzene)	2.26[d]	Zhang et al., 2020		
SMX-Glu			No pK_{a2}	Bonvin et al., 2013
Dimer of SMX	2.93[d]	Zhang et al., 2020		

Computed values reported in literature without further information on accuracy.
[a] MarvinSketch.
[b] SciFinder.
[c] ChemAxon.
[d] Unknown software used for prediction.

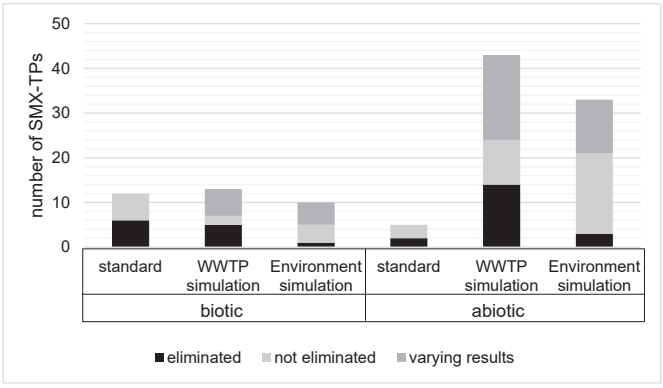


Fig. 6. Number of SMX-TPs with data on degradation during different degradation tests with further classification, i.e. not eliminated or eliminated during degradation tests, mostly derived from kinetics. Different conditions of several tests lead to varying results.

Albeit diverse test conditions even within one type of degradation test (WTP or environment simulation, abiotic or biotic), there are 10 SMX-TPs with nearly all and at least 3 data entries of similar elimination rate: 6 TPs that were eliminated are listed in Tables 5, and 4 TPs that were not eliminated are listed in Table 6. An eliminated TP may be a precursor of further degradation products. For example, the X-OH-SMX isomers were completely or at least almost completely eliminated within the test period. However, since PC's mineralisation was not complete as far as observed (Gómez-Ramos et al., 2011; Hu et al., 2007; Ioannidou et al., 2017), transformation to further, eventually persistent TPs cannot be ruled out.

In contrast to the presented 10 SMX-TPs with data entries of similar elimination (Tables 5, 6), there are 5 SMX-TPs with at least 3 data entries of varying elimination rate: Ac-SMX, 6,7-$(OH)_2$-SMX, SAA, sulfanilic acid, and the SMX isomer. Examination of such data can be found in the Supplementary information (Section 5). Compared to other SMX-photo-TPs, sulfanilic acid, as well as 3A5MI, and the SMX isomer (TP253), are likely the most abundant and persistent TPs during photolysis (Guan et al., 2019; Trovó et al., 2009b).

Summarising, knowledge about degradability of TPs in the environment is crucial to understand their environmental fate. It is limited due to missing data from standardised methods, provided e.g. by OECD.

Data from not standardised tests under very different conditions is less comparable since degradability as non-intrinsic property depends strongly on conditions, such as the kind of catalyst and light spectrum. This makes the evaluation of data of not standardised tests more difficult. Through comparison of several data entries per TP from different tests, we could at least tentatively conclude the degradation behaviour. For example, X-OH-SMX isomers were not detectable anymore after abiotic treatment (mainly photocatalysis, simulating conditions in WTP). However, they displayed persistence under biotic environmental conditions in the dark according to OECD method 308. The intact isoxazole ring of these isomers, (5-methylisoxazole-3-yl) sulfamate, and desamino-SMX prevents their biotic transformation. Respectively, the TP271c, which is formed via ring-opening, biodegraded during the same study (Su et al., 2016). Biodegradation rates depend tremendously on the inoculum (density and diversity, i.e. source and concentration). An isolated and acclimated SMX-degrading stain (*Acinetobacter* sp.) can mineralise SMX almost completely (Wang et al., 2018). Compounds that are mineralised by a specialised inoculum would not necessarily mineralise in the environment, where the specialised strains are often out-competed in the complex bacterial composition. In contrast, conditions are set in WTP that favour nitrification, for example (Kassotaki et al., 2016). Furthermore, not-standardised data is of lower coverage and usability on the one hand and lower quality on the other since validity criteria are not defined. Methods such as OECD tests include criteria for a toxicity and a quality control. The endpoint for biodegradability is clearly defined. OECD method 301 F requires also a sterile control to distinguish abiotic from biotic processes (OECD 301 F). This also demonstrates the importance of laboratory methods as a complement to real-world methods for an understanding of complex processes by reducing the number of variables under standardised conditions.

3.3.2.3. (Eco)toxicity. Information on (eco)toxicity was found for more than half of selected SMX-TPs (64/124). The simplified classification of these SMX-TPs according to the test approach (i.e. tested as mixture, single substance in vitro, or in silico) is shown in Fig. 7. Compared to Fig. 4, the distribution is nearly the same for all SUA-TPs: The conclusions drawn

Table 5
TPs that were eliminated in nearly all and at least 3 studies within one type of degradation test (WTP or environment simulation, abiotic or biotic). Simple conclusions are drawn in the last column.

TP	Degradation method	Result	Reference	Conclusion
p-Aminophenol	Microbacterium sp. strain BR1	Transient metabolite, mineralisation unknown	Ricken et al., 2013	Biodegradable in WTP simulations
	Microbacterium sp. strain BR1, TP treated individually	Almost eliminated, mineralisation unknown	Ricken et al., 2015	
	Electro-Fenton pre-treatment, activated sludge	Total TOC removal after 2 weeks with activated sludge	Mansour et al., 2014	
Formic acid, acetic acid, oxalic acid	Well investigated compounds; an example for checking databases, e.g. ECHA, 2021			Readily biodegradable
X-OH-SMX isomers (at benzene)	Photocatalysis (TiO_2-SG, TiO_2-SG or Ce-ZnO, 5–8 h)	Eliminated after max. 60 min, mineralisation unknown	Fabbri et al., 2019	Predominantly eliminable in WTP simulations (abiotic), Further TPs expected since mineralisation is not complete Position of hydroxy group affects the elimination rate
	Photocatalysis (TiO_2, pH 3, 1 h)	Almost eliminated completely, mineralisation: 80% (DOC) of SMX	Hu et al., 2007	
	Photocatalysis (H_2O_2, pH 6 or 9, H_2O_2/NH_3, pH 9)	Depends on conditions and OH-position: Isomer 270-1 was not eliminated (H_2O_2), isomer 270-2 degraded (H_2O_2, only pH 9; H_2O_2/NH_3) but not *via* H_2O_2 at pH 6, mineralisation unknown	Zhang et al., 2016	
	Photocatalysis (WO_3-modified TiO_2)	Eliminated, mineralisation: 28% (TOC) of SMX	Ioannidou et al., 2017	
	Oxidation (ozone, 10 min)	Eliminated almost completely, mineralisation: 0–25% (TOC) of SMX	Gómez-Ramos et al., 2011	
X-OH-SMX isomers (at isoxazole)	Oxidation (Fe(VI))	Eliminated, mineralisation unknown	Kim et al., 2015	Eliminable in WTP simulations (abiotic), Further TPs expected due to low or unknown mineralisation
	Photocatalysis (TiO_2-SG, TiO_2-SG or Ce-ZnO, 5–8 h)	Eliminated after max. 60 min, mineralisation unknown	Fabbri et al., 2019	
	Photo-Fenton (H_2O_2, pH 3)	Eliminated, mineralisation unknown	Hong et al., 2020	
	Photocatalysis (WO_3-modified TiO_2)	Eliminated, mineralisation: 28% (TOC) of SMX	Ioannidou et al., 2017	

Table 6
TPs that were not eliminated in nearly all and at least 3 studies of any category (WTP or environment simulation, abiotic or biotic). Conclusion is drawn in the last line.

TP	Degradation method	Result	Reference
3A5MI	Photolysis (4 h)	Not eliminable	Bonvin et al., 2013
	Photolysis (1 week)	Not eliminable	Gmurek et al., 2015
	Photolysis (24 h)	Not eliminable	Periša et al., 2013
	Photolysis (30 min)	One of the most abundant and persistent intermediates	Trovó et al., 2009b
p-Nitro-SMX	Photolysis (4 h) of *p*-nitro-SMX	Photo-stable, environmental $t_{1/2}$: 337 ± 30 h, 1515 ± 135 h (summer, winter)	Bonvin et al., 2013
	Photolysis (1 week)	Not eliminable	Gmurek et al., 2015
	Photolysis (nitrate-, nitrite-sensitised, 8 h)	Not eliminable	Scholes et al., 2019
p-Nitro-X-OH-SMX	Photolysis (4 h) of *p*-nitro-SMX	Photo-stable	Bonvin et al., 2013
	Photolysis (1 week)	Not eliminable	Gmurek et al., 2015
	Photolysis (nitrate-sensitised, 8 h)	Not eliminable	Scholes et al., 2019
p-OH-X-nitro-SMX	Photolysis (4 h) of *p*-nitro-SMX	Photo-stable	Bonvin et al., 2013
	Photolysis (1 week)	Not eliminable	Gmurek et al., 2015
	Photolysis (nitrate-, nitrite-sensitised, 8 h)	Not eliminable	Scholes et al., 2019

Conclusion: These TPs are expected to be photo-stable in the environment.

already that the data based mainly on tests of TP-mixtures is valid for SMX-TPs, too.

Starting with tests of TP-mixtures, they are predominantly received after photo(cata)lysis or oxidation (e.g. by H_2O_2 or ozone). Data of TP-mixtures which is linked to nearly all TPs of the (eco)toxicity dataset (61/64) can be considered first hints. In many cases, TP-mixtures were more toxic than the PC related to the same endpoint (10 of 15 studies, e.g. by Gmurek et al. (2015), Qi et al. (2014), and Abellán et al. (2008)). Section 6 of the Supplementary information provides further information on monitored endpoints of (eco)toxicity of SMX-TPs.

A typical procedure to gain first hints is the testing of mixtures along the treatment period of SUAs with changing compositions of compounds. Changes in toxicities for the samples along the treatment period can be linked to the concentration curves of these compounds, or in the case of unknown concentration, to relative changes of the intensity of the detection signal (typically as peak area). For example, when the (eco)toxicity was significantly reduced, TPs that are still present were considered possibly not toxic, e.g. TP201 ($C_6H_3NO_5S$) and 3A5MI, whereas TPs that were lost at the time of reduced (eco)toxicity could be toxic, e.g. desulfonated SMX or TP155 (Mirzaei et al., 2018). Thereby, the impact on reduced (eco)toxicity by the removal of the PC needs to be considered. Based on such a kinetic approach, potential toxic candidates should be further investigated as an individual compound in vitro (Trovó et al., 2009b) or at least in silico tests (Hong et al., 2020; Yang et al., 2017; Zhang et al., 2016).

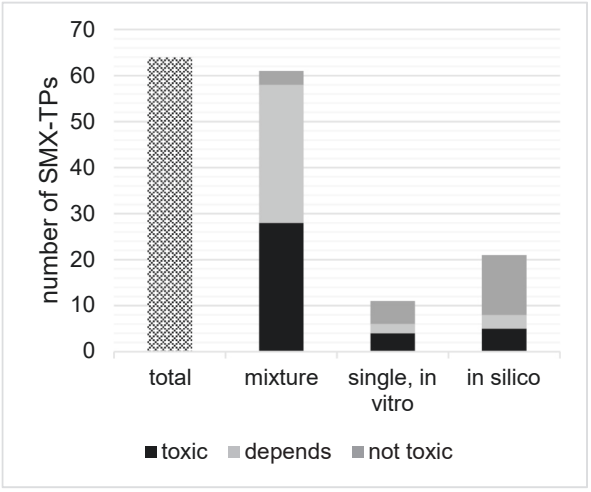


Fig. 7. Number of SMX-TPs with data entries on (eco)toxicity, grouped by test approach (test of mixture, of single substances in vitro or in silico) subdivided according to the classification "toxic", "depends", and "not toxic". The result was classified as "depends" in the case of different (eco)toxicities per TP, due to the dependence on different endpoints.

In total, information about (eco)toxicity by in silico, in vitro, and both in silico and in vitro tests was found for 21, 7, and 4 TPs, respectively. Focus of data was on ecotoxicity, similar to studies on TP-mixtures. For example, acute and chronic ecotoxicity for fish, daphnia, and green algae was predicted using ECOSAR (Guan et al., 2019; Yang et al., 2017; Zhang et al., 2016), or growth inhibition of bacteria was investigated (Białk-Bielińska et al., 2017; Eguchi et al., 2004; Majewsky et al., 2014; Osorio et al., 2016).

More than half of investigated TPs (13/21) were predicted as less toxic than SMX. Among them are sulfanilic acid (Yang et al., 2017) and *N4*-acetyl-SMX (Jurado et al., 2020), which are also less ecotoxic according to in vitro daphnia and algae tests, e.g. using *Daphnia magna*, *Limnic green algae*, and *Lemna minor* (Białk-Bielińska et al., 2017; Eguchi et al., 2004; Grabarczyk et al., 2020; Trovó et al., 2009b). Although their chemical structures still include the pharmacophore they are also less toxic against *Vibrio fischeri* (Majewsky et al., 2014). Presumably, these TPs had lower binding affinity than SMX to inhibit the dihydropteroate synthase, central for the bacteriostatic effect, and did also not react via another mode of action.

5 TPs were predicted to be similar or more toxic for fish, daphnia, and green algae than SMX. Among them is *p*-nitro-SMX (Yang et al., 2017), which is also similar or more toxic as SMX to *Vibrio fischeri* and *Daphnia magna* according to in vitro tests (Majewsky et al., 2014; Osorio et al., 2016). Among them is also 3A5MI (Guan et al., 2019; Yang et al., 2017; Zhang et al., 2016), which is by contrast less toxic according to in vitro bacteria and daphnia tests (Majewsky et al., 2014; Trovó et al., 2009b). The prediction for 3A5MI contradicts the experimental result likely because 3A5MI was out of the applicability domains of the model. The class of anilines (unhindered) was used for prediction although the structure of 3A5MI does not belong to this class (Guan et al., 2019; Yang et al., 2017). Therefore, we consider only the in vitro result for 3A5MI, i.e. less toxic, for the dataset. In in vitro studies, additional 3 TPs, such as *p*-nitroso-SMX, showed similar or higher ecotoxicity compared to SMX (Majewsky et al., 2014; Osorio et al., 2016).

For 5 TPs (3 and 2 TPs from in silico and in vitro datasets, respectively) endpoints were very different. Therefore, they are classified as "depends". For example, desamino-SMX was found to be not harmful to *Daphnia magna* but harmful to *Vibrio fischeri* (Osorio et al., 2016).

Combination of in silico and in vitro test results for ecotoxicity (i.e. very few results for endpoints of human toxicity such as mutagenicity excluded) leads to an initial simplified statement for 24 TPs, displayed in Fig. 8: More than half might be less ecotoxic than SMX (15/24), whereas almost one third is of similar or even higher ecotoxicity (7/24) compared to SMX: the SMX isomer (Yang et al., 2017; Zhang et al., 2016), SMX-dimer, OH-SMX-dimer (Yang et al., 2017), as well as *p*-OH-SMX, *p*-nitroso-SMX, *N4*-OH-SMX, and *p*-nitro-SMX. The study of the letter 4 TPs against *Vibrio fischeri* showed that structures without the *p*-amino group can also be bacteriostatic (Majewsky et al., 2014).

Summarising, the finding of toxic TP-mixtures demonstrates the need for further data on individual TPs to enable a comprehensive environmental risk assessment. However, experimental or computational data of individual

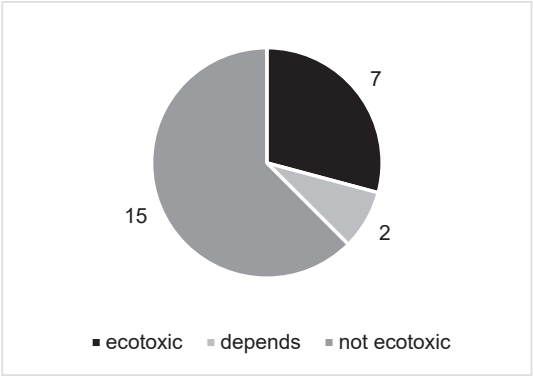


Fig. 8. Number of TPs that displayed ecotoxic or not ecotoxic via in vitro and/or in silico tests. In total, data for 24 TPs was found. The result was classified as "depends" in the case of different ecotoxicity per TP, due to the dependence on different endpoints.

TPs was found only for a quarter of SMX-TPs, despite the great advantage of in silico prediction not requiring elaborate synthesis of non-available TPs. On the other hand, in silico prediction has limitations. Fitting into the applicability domain and sufficient reliability are required for successful prediction (Lorenz et al., 2021) as discussed above for 3A5MI. Such information was missing in the publications of in silico data, although it would be essential to assess their validity. We could use in vitro data of the same endpoint to assess if/how often in silico predictions could be confirmed. However, there is not enough in vitro data to do this assessment. In vitro data on SMX-TPs is limited. Moreover, data entries belong to multiple endpoints, which are difficult to compare. Ecotoxicity alone as a single branch of toxicity depends very much on the endpoint, including the species. For example, SAA is classified as ecotoxic to plants and harmful to green algae (Białk-Bielińska et al., 2017), but less ecotoxic to *Vibrio fischeri* than SMX (Majewsky et al., 2014).

Possible reason for the identified gap of in vitro data – besides the aforementioned poor availability of TPs – is the diversity of TPs, which makes simultaneous detection and identification difficult. It might be impossible to select an ecotoxicity assay based on a specific mode of action, that is suitable for the diversity of TPs (Toolaram et al., 2014). Furthermore, ecotoxicity caused by another mode of action than that of the pharmaceutical should not be ruled out. For example, in vitro tests showed that TPs such as *p*-OH-SMX, *p*-nitro-, and *p*-nitroso-SMX with modified pharmacophore at the *p*-amino position can still be ecotoxic for bacteria.

Thus, a reasonable selection of methods is needed but difficult due to diverse TPs and multiple existing endpoints. Methods should be standardised to ensure comparability and quality as discussed already for degradation methods.

3.3.3. Common features and differences of SUA-TPs

SUA-TPs with common features based on the same mechanism of transformation are presented in Table 7. Thereby, it is differentiated between TPs that are identical for all SUAs ("non-specific TPs"), e.g. SAA, and TPs with different SUA-specific moieties (R) but identical transformed backbone of SUAs ("SUA-specific TPs"), e.g. desamino SUA. Common features that were frequently found are identified and highlighted in bold letters. Among them are the non-specific TPs SAA and sulfanilic acid (for 10 and 11 SUAs, respectively). Although SAA was found for almost all SUAs, the counterpart of the ε-bond cleavage (Fig. 9) was less often detected, probably because of its reactivity or analytical challenges of a screening method. All observed bond cleavages are marked in Fig. 9.

Among common SUA-specific TPs are *p*-nitroso- and *p*-nitro-SUA (for 10 SUAs each), and TPs formed via δ-bond cleavage, desulfonation, and hydroxylation especially at the aniline moiety (for all 14 SUAs each). In laboratory studies, hydroxylation as initial transformation step occurs very often via reactive OH-radicals formed in aqueous solutions under various conditions (e.g. oxidations), in contrast to more specific reactions, such as chlorination and glycosylation, that require the addition of further reactants and are less frequently studied. Thus, for all SUAs a hydroxylated SUA was described, while Cl- or glycosyl-SUAs were less often found. Additionally, the lack of typical biotic formyl-, glycosyl-, and pterin-TP for many SUAs confirms that biotic processes are less studied resulting in missing data for biotic degradation.

Desulfonation via Smiles rearrangement (γ-bond opening), which leads to an imine, was observed for six-membered SUAs during direct and indirect photolysis by Boreen et al. (2005) as a difference to five-membered SUAs (Boreen et al., 2004). Results of the literature search confirm Boreen et al.'s observation since TPs resulting from Smiles rearrangement were found only for six-membered SUAs, but not for five-membered SUAs. For five-membered SUAs, desulfonation leads to an amine. As an enhancement of the collected SUA-TPs, desulfonation via Smiles rearrangement (imine) can be expected also for 6-membered SMP (Fig. 10). Thus, comparison of known TPs and identification of typical transformation mechanisms give guidance to define, search for and identify unknown but expected TPs. This would facilitate their detection and identification.

This approach, i.e. identifying expectable TPs based on general organic chemistry knowledge, has already been applied by Majewsky et al. (2015). A generic scheme was developed to predict possible TPs of SPY and SDZ. For this purpose, major transformation reactions, e.g. acetylation, hydroxylation, and desulfonation were identified. Before HPLC-QTOF screening, 5 corresponding product ions of each SUA were selected. The prediction of these product ions facilitated the tentative identification of numerous formerly unknown TPs: *p*-Nitro-SDZ, desamino-SPY, and *N4*-formyl-SPY were reported for the first time (Majewsky et al., 2015). However, even small structural changes can lead to different reactivities and therefore to different TPs. For example, Wang and Helbling (2016) observed the same reaction types for similar SUAs (SMX, STZ, SDM): chlorine substitution, δ-bond hydrolysis, and desulfonation, but γ-bond cleavage, oxidation/hydroxylation, and conjugation reactions were observed only for one or two SUAs. Therefore, laboratory testing is needed to confirm predicted TPs. For a reasonable laboratory effort, strategies are important to limit the number of TPs to the relevant ones (Hensen et al., 2020; Menz et al., 2017).

4. Discussion of insights gained from the reviewed literature data

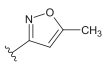
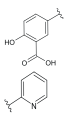
We have seen, that the diversity of TPs even within the group of SUAs and SMX as the main compound is tremendous: The dataset of SUA-TPs includes more than 600 different structures. However, data on their occurrence in the technosphere and the environment, degradability, and (eco) toxicity are scarce even for SMX, the most investigated SUA. Few data entries on occurrence suggest that SUA-TPs can be widespread in the environment, indicating this as a general feature. TPs are often more polar than respective PC. According to data of SMX-TPs, we expect SUA-TPs that have not been eliminated in WTP to be mobile in the aquatic environment at concentrations in the low ng L^{-1} range. Thus, they can often be considered persistent, mobile, and toxic (PMT) substances (Rüdel et al., 2020). To fill identified gaps of data on occurrence, tremendous laboratory work would be needed.

Only relevant TPs should be part of monitoring programs to reduce laboratory work, as discussed for TPs of pesticides (Escher et al., 2014; German Federal Environmental Agency, 2019; Hensen et al., 2020). An assessment scheme and criteria, as well as data, are needed to identify relevant TPs, i.e. TPs that might be ecotoxic and/or (very) persistent, and (very) mobile (Cousins et al., 2019). Compounds, especially small TPs such as organic acids, aniline, or sulfanilic acid, could be already registered in databases such as PubChem and SciFinder. In those cases, renewed investigation regarding their characteristics is not needed.

To assess, if TPs would be persistent or completely biodegradable in WTP or the environment, data from degradation tests of individual TPs, that investigate also their mineralisation, is needed. However, such data is almost completely absent for SUA-TPs. Most data on SUA-TPs was

provided by elimination studies of their PC (simulation of WTP or environment). Data depend very much on different test conditions. Even though PC's mineralisation rate was monitored in many studies, the mineralisation rate of each formed and eliminated TP remained unknown. Eliminated TPs may further transform to persistent TPs, possibly ecotoxic. To assess ecotoxicity, data from significant testing of individual TPs is needed. However, such data is lacking for most SUA-TPs in reviewed literature. Many ecotoxicity tests were performed using mixtures of SUA-TPs. They indicated less or more ecotoxic TPs compared to the PC. Thus, for both degradability and ecotoxicity assessment, standardised testing of individual TPs is

Table 7
TPs with common features, separated into non-specific and SUA-specific TPs for each SUA. The molar mass [g/mol] is given. TPs described for most SUAs are in bold type.

SUA M [g/mol]	SMX 253	SDZ 250	SMZ 278	SDM 310	SPY 249	STZ 255	SMR 264	SCP 285	SIX 267	SSZ 398	SQX 300	SMP 280	SMT 270	SGU 214
Specific rest R														
Non-specific TPs M [g/mol]	Hocks (✓) show for which SUAs the non-specific TPs were found in the literature.													
Organic acids	✓	✓	✓			✓	✓	✓		✓	✓			
Aniline (γ-bond cleavage) 93	✓	✓	✓	✓	✓	✓	✓							
p-Benzoquinone imine 107	✓				✓								✓	
p-Aminophenol 109	✓	✓	✓			✓	✓	✓	✓					
Hydroquinone 110		✓	✓		✓		✓	✓						
Benzenesulfonic acid 158	✓	✓				✓								
SAA (ε-bond cleavage) 172	✓	✓	✓	✓	✓	✓		✓	✓				✓	✓
Sulfanilic acid (δ-bond cleavage) 173	✓	✓	✓	✓	✓	✓	✓	✓	✓			✓	✓	
SUA-specific TPs	Molar masses M [g/mol] show for which SUAs the SUA-specific TPs were found in the literature. M (TP) = M (SUA, see first row) −/+ M (common feature, see first column)													
Specific rest R (ε-bond cleavage) −170		80	108					115						
NH_2R (δ-bond cleavage) −155	**98**	**95**	**123**	**155**	**94**	**100**	**109**	**130**	**112**	**−14 = 94**	**145**	**125**	**115**	**59**
H(*O*)SO_2-NHR (γ-bond cleavage) −91 (−75)	162		187	235	174	180			189					
Desulfonated SUA (Imine, I or Amine, A) −64	**189** (A)	**186** (I)	**214** (I, A)	**246** (I, A)	**185** (I, A)	**191** (A)	**200** (I, A)	**221** (I)	**203** (A)	**334** (I, A)	**236** (I, A)	**216** (A)	**206** (A)	**150** (I ↔ A)
Desamino SUA (β-bond cleavage) −15	238	235	263	295	234					−149 = 234				
4-*ipso*-OH-SUA +1	254	250	279	311	250	256	265			−149 = 248			287 (incl. X-OH)	
p-Nitroso-SUA +14	**267**	**264**	**292**	**324**	**263**	**269**			**281**			**294**	**284**	**228**
X-OH-SUA +16	**269**	**266**	**294**	**326**	**265**	**271**	**280**	**301**	**283**	**−149 = 265**	**316**	**296**	**286**	**230**
At aniline	**269**	**266**	**294**	**326**	**265**	**271**	**280**	**301**	**283**	**414**		**296**	**286**	**230**
At R	269	266	294	326	265	271			283		316			
N4-formyl-SUA +28	281	278	306		277	283					328			
p-Nitro-SUA +30	**283**	**280**	**308**	**340**		**285**	**294**	**315**	**297**		**330**		**300**	
X, Y-$(OH)_2$-SUA +32	285	282	310	342	281	287	296	317	299					
X-Cl-SUA +34	288		312	344		289					334			
N4-acetyl-SUA +42	295	292	320	352	291	297	306		309		342			
Glycosyl-SUA +162	415	412	440		411	417								
Pterin-SUA +175	428	425	453		424	430								
Dimer of SUA 2*M(SUA) -2,-4	502	496	552	632, 648 (incl. X-OH, 2OH)		508			530	792			538	

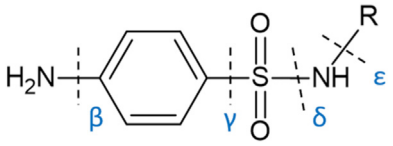


Fig. 9. Bond cleavage in sulfonamides (adapted from Boreen et al. (2004)).

urgently needed to enable a comprehensive environmental risk assessment of the PC and the TPs themselves.

Poor commercial availability is a likely cause of data gaps. An approach to overcome the challenge of not buyable TPs (or being too expensive to synthesise) could be the use of in silico tools. TPs could be predicted using in silico models such as the EAWAG-BBD Pathway Prediction System (Fenner et al., n.d.), the photodegradation model META (Sedykh et al., 2001), and PathPred predicting enzyme-catalysed pathways (PathPred, n.d.). Prediction can facilitate the detection of expected TPs. Properties of predicted structures (ideally analytically confirmed after abiotic or biotic generation) can be computed using (quantitative) structure-activity relationships ((Q)SARs) or other approaches, such as read-across. These tools are already well established for physicochemical parameters, such as pK_a, log P, and water solubility (Cumming et al., 2013; Lombardino and Lowe, 2004), but also toxicity such as mutagenicity (Ames). In silico models have limitations, e.g. regarding predictivity in general and applicability domain due to missing experimental data to develop/improve models (Lorenz et al., 2021). Therefore, extensive laboratory experiments to study the formation of TPs and further mechanisms cannot be circumvented for all substance classes. Furthermore, predictions for prioritised structures need to be confirmed by experimental tests (Hensen et al., 2020).

Another approach to overcome the challenge of not buyable TPs, and also limitations of in silico tools, would be the fast and simple generation of TPs, e.g. by photolysis, and subsequent biodegradability testing, teasing e.g. by OECD 301 D, OECD 308, or other standardised tests. OECD tests would be preferred as they are widespread and needed for the authorisation of chemicals and pharmaceuticals. This approach was done already by Rastogi et al. (2014, 2015a, 2015b) using β-blockers as an example and shortly after by Su et al. (2016) using SMX. Their studies have provided useful knowledge on TP-mixtures due to the use of standardised biodegradation tests. Then, TPs of interest need further individual investigation.

Similarly, ecotoxicity of TP-mixtures was tested in some reviewed studies to identify possible ecotoxic TPs for further investigation. For example, the formation of TP-mixtures in different matrices, e.g. distilled water vs. surface water, led to different TP compositions linked to different toxicities. Identified differences in composition could be used to derive potentially toxic TPs (Trovó et al., 2009a). Another example is a treatment study to improve the TOC removal, e.g. by using an appropriate catalyst. At the same time, ecotoxicity of the remaining TP-mixture was monitored and found to be reduced. In this way, TPs eliminated by improved test setup might be ecotoxic, whereas TPs that are still present in the mixture might not be ecotoxic (Mirzaei et al., 2018). These examples are comparable to experimental effect-driven approaches to determine the ecotoxicity of TPs, proposed already by Hensen et al. (2020) and Menz et al. (2017). According to an effect-driven approach, TP-mixtures along a degradation process (e.g. photodegradation) are screened to get first hints on ecotoxic TPs by the observation of effect changes compared to their kinetics. These TPs should be structural elucidated, isolated or synthesised, and then tested individually (Escher and Fenner, 2011). We want to highlight that finding no toxic mixture does not mean that compounds are not toxic as one PC often results in many TPs of different share, i.e. at lower concentrations. To illustrate, if there would be 10 TPs, the (eco)toxicity of one TP in the mixture needs to be 10-fold compared to the PC to reach the same toxic effect (simplifying that each TP has the same probability to be formed).

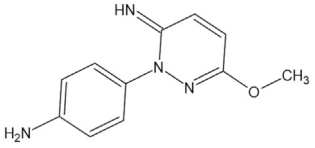


Fig. 10. Desulfonated SMP (imine), expected for SMP as 6-membered SUA *via* Smiles rearrangement.

Besides the limitations of individual testing, the selection of endpoints can be challenging since ecotoxicity can be defined by many endpoints. The ecotoxicity standard within the environmental risk assessment of pharmaceuticals is the testing of chronic effects on algae, daphnia, and fish (European Commission, 2004; European Medicines Agency, 2018). As a result of taxa sensitivity comparison to reduce vertebrate testing, tests of antibiotics should focus on algae and cyanobacteria, whereas fish testing is unnecessary. For endocrine-active substances it would be the other way around: fish tests are necessary, whereas algae are not sensitive (Schwarz et al., 2021). Although there is this common practice of testing, ecotoxicity is still very complex. It can be tested and monitored on different levels (receptors, cells, organs, organisms, populations, ecosystems). There may be further, quite different environmental effects than currently known. Moreover, it might be impossible, as mentioned above, to select an ecotoxicity assay that is suitable for diverse TPs even if they stem from the same PC (Toolaram et al., 2014). Thus, multitude and diversity of TPs seem to be another big challenge of TP assessment, especially in terms of ecotoxicity.

Ultimately, the risk of any environmental effect posed by (potentially) ecotoxic TPs would be absent for those APIs that mineralise completely in the environment. No laborious testing would be needed. Therefore, the concept *Benign by Design* (Kümmerer, 2007), based on the 10th principle of green chemistry *Design for Degradation* (Anastas and Warner, 1998), should be applied to the API discovery process to consider complete environmental degradability from the very beginning (Puhlmann et al., 2021). Further measures at the source to minimise emissions and levels of APIs and their TPs in the environment are improved prescribing practices, reduction of package sizes, and separate collection of household hazardous waste including pharmaceuticals (European Commission, 2020c).

5. Conclusion

The focus of this review was on fate and effects as well as properties of TPs, and their assessment including possible knowledge gaps. Pharmaceutical sulfonamides (SUAs) have been used as an illustrative group of substances as there are many similar PCs of high usage with many related publications, but also still many knowledge gaps.

This review indicates that further extensive research is needed to fill identified knowledge gaps: Despite many studies are dealing with SUA-TPs only a few data entries are available according to standardised tests related to environmental fate and toxicity. Thus, the environmental fate of most SUA-TPs is still unclear. Additionally, ecotoxicity data for the single TP is scarce, although potential ecotoxicity is indicated by many studies on TP-mixtures. The collected and systematised data together with future research can contribute to a comprehensive risk assessment of SUAs in the environment to avoid an underestimation of their environmental effects.

Based on findings for SUAs and data published for other pharmaceuticals and chemicals, some conclusions can be drawn that apply to TPs in general: There is a high number of compounds and an even higher number of TPs. Analysis of TPs is challenging, e.g. due to poor availability of TPs for testing, leading to large gaps of data, especially of high-quality data. Back transformation into the PC or further transformation to ecotoxic TPs poses a risk for the environment. Thus, complete elimination of the PC, e.g. in WTP, does not mean that no environmental pollution and related risks can occur, as elimination does not indicate complete mineralisation.

According to greener chemistry and pharmacy, measures should be taken to reduce or even avoid the input of chemicals and pharmaceuticals into the environment. The share of compounds whose input is unavoidable by essential use (e.g. excretion of APIs) should completely mineralise at the end of their life in the environment. In this context, data on TPs will be very important for future work on the redesign of SUAs for complete

environmental mineralisation (*Benign by Design*): A TP that is pharmacologically active and also readily biodegradable in the environment would present a promising candidate for the redesign of pharmaceuticals including SUAs.

CRediT authorship contribution statement

Neele Puhlmann: Conceptualization, Methodology, Investigation, Writing-Original draft preparation. **Oliver Olsson**: Conceptualization, Methodology, Writing - Review & Editing, Supervision. **Klaus Kümmerer**: Conceptualization, Writing - Review & Editing, Supervision.

Declaration of competing interest

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.154744.

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Anhang der Publikation 3

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How data on transformation products can support the redesign of sulfonamides towards better biodegradability in the environment

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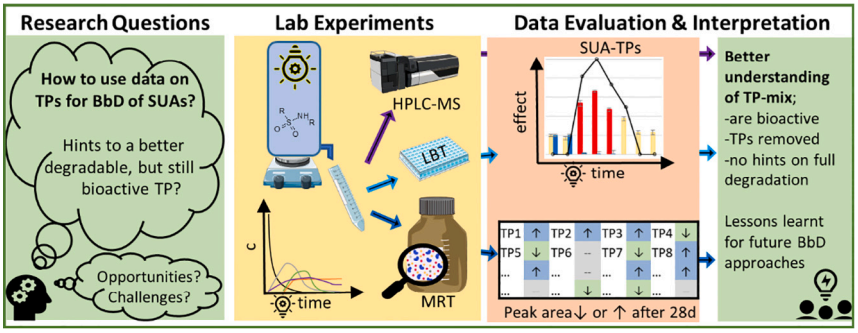
HIGHLIGHTS

- Transformation products (TPs) of sulfonamides (SUAs) inhibited bacterial luminescence.
- SUA-isomers & hydroxylated SUAs were eliminated in biodegradation test of TP-mixtures.
- Tested hydroxylated derivatives of sulfanilamide are not readily biodegradable (OECD 301F).
- No photo-TP was promising as a candidate for a better biodegradable SUA.

GRAPHICAL ABSTRACT



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ABSTRACT

Sulfonamide antibiotics (SUAs) released into the environment can affect environmental und human health, e.g., by accelerating the development and selection of antimicrobial resistant bacteria. *Benign by Design* (BbD) of SUAs is an effective risk prevention approach. BbD principles aim for fast and complete mineralization or at least deactivation of the SUA after release into the aquatic environment. Main objective was to test if mixtures of transformation products (TPs) generated via photolysis of SUAs can be used as an efficient way to screen for similarly effective but better biodegradable SUA alternatives.

Six SUAs were photolyzed (Hg ultraviolet (UV) light), and generated UV-mixtures analysed by high performance liquid chromatography coupled to an UV and tandem mass spectrometry detector. UV-mixtures were screened for antibiotic activity (luminescence bacteria test, LBT, on luminescence and growth inhibition of *Aliivibrio Fischeri*) and environmental biodegradability (manometric respirometry test, MRT, OECD 301F) using untreated parent SUAs in comparison. Additionally, ready environmental biodegradability of three commercially available hydroxylated sulfanilamide derivatives was investigated.

SUA-TPs contributed to acute and chronic bacterial luminescence inhibition by UV-mixtures. LBT's third endpoint, growth inhibition, was not significant for UV-mixtures. However, it cannot be excluded for tested TPs as concentrations were lower than parents' concentrations and inhibition by most parental concentrations tested was also not significant. HPLC analysis of MRT samples revealed that one third of SUA-TPs was reduced during incubation. Three of these TPs, likely OH-SIX, OH-SMX and OH-STZ, were of interest for BbD because the sulfonamide moiety is still present. However, hydroxylated sulfanilamide derivatives, tested to investigate the effect

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of hydroxylation on biodegradability, were not readily biodegraded. Thus, improving mineralization through hydroxylation as a general rule couldn't be confirmed, and no BbD candidate could be identified. This study fills data gaps on bioactivity and environmental biodegradability of SUAs' TP-mixtures. Findings may support new redesign approaches.

1. Introduction

Residues of sulfonamide antibiotics (SUAs) including their metabolites and transformation products (TPs) occur in the environment as a result of excretion by patients and treated animals and insufficient removal in wastewater treatment plants (WWTPs) (Baran et al., 2011; Felis et al., 2020; García-Galán et al., 2016; Spielmeyer, 2018). A recent study on pharmaceutical pollution in the world's rivers showed that the sulfonamide sulfamethoxazole (SMX) belongs to the contaminants with the highest concentrations out of 61 targeted active pharmaceutical ingredients. At 140 monitoring sites, the SMX concentration was above the predicted no-effect concentration, which may be of concern (Wilkinson et al., 2022).

Residues of SUAs can affect the environment and human health. Examples are the influence on the composition of microbial communities (Cycoń et al., 2019), phytotoxicity (Christou et al., 2018), and the development and selection of antimicrobial resistance especially in WWTP (Baran et al., 2011; Ezzariai et al., 2018; Felis et al., 2020). The latter is a serious global threat to human health. According to a risk-based assessment approach for WWTP effluents, SMX belongs to the top risk group using hazard units (Finckh et al., 2022). In order to reduce the development of resistance in patients, SUAs are often administered in combination with trimethoprim (e.g. as cotrimoxazole). Due to their inhibition of bacterial folic acid synthesis at two different sites, the antibacterial effect is improved.

Benign by Redesign of SUAs aims to develop a fast mineralizing SUA-derivative to avoid occurrence of SUA residues in the environment and associated effects. Like the *De-Novo Design* approach, *Benign by Redesign* belongs to the *Benign by Design* (BbD) concept (Kümmerer, 2007, 2010; Lorenz et al., 2021), which is based on the 10th principle of Green Chemistry, *Design for Degradation* (Anastas and Warner, 1998). BbD and other measures to tackle environmental pollution are proposed in the EU's Chemicals Strategy for Sustainability (European Commission, 2020a) and Pharmaceutical Strategy for Europe (European Commission, 2020b).

Lorenz et al. (2021) proposed a workflow to support the application of BbD, which begins with literature search on potential benign candidates. Useful literature data for the redesign of SUAs may be data on SUA-TPs that still contain the pharmacophore, i.e., the sulfanilamide structure. Reason is that slight structural transformation of the aniline ring could improve biodegradability, while preserving the bacteriostatic effect of SUAs due to sufficient structural similarity (Puhlmann et al., 2021). More than 600 SUA-TPs generated in laboratory experiments, WWTPs or the natural environment have been reported in literature (Puhlmann et al., 2022). However, no compound was identified to be of interest for BbD, mainly because of missing data for antibiotic activity and biodegradability.

Some hints taken from literature serve as good starting point for SUA's redesign. For example, *p*-OH-SMX, *p*-nitroso-SMX, *N4*-OH-SMX, and *p*-nitro-SMX were found to be bacteriostatic against *Aliivibrio fischeri* (Majewsky et al., 2014). This finding questions the necessity of the *p*-amino group of the pharmacophore for the bacteriostatic effect and expands the possibilities for BbD. During incubation of a photo-TP-mixture with sediment, *p*-nitro-SMX, the SMX-isomer, and a SMX-TP with opened isoxazole ring showed evidence of primary environmental biodegradation (Su et al., 2016). However, more data on mineralization rates is still needed to exclude the formation of environmentally persistent, possibly bioactive TPs, or even back-transformation to SMX. There are hints that *p*-nitro-SMX transforms back to SMX, like acetyl-SMX and glucuronide-SMX (Achermann et al., 2018; Radke et al., 2009; Su et al., 2016).

More data on a large pool of SUA-derivatives is needed to screen for a better biodegradable SUA. An effect-driven approach, starting with the synthesis of a pool of molecules within one step, provides a remedy for costly synthesis of SUA-derivatives, which are hardly commercially available, and their individual laborious testing. Such an approach consists of an *in vitro* testing of mixtures, generated e.g., by UV-irradiation, and focusing on those showing the desired effect (see e.g., Hensen et al., 2020; Rastogi et al., 2014). Suitable tests to screen UV-mixtures for antibiotic activity and environmental biodegradability are the 24 h luminescence bacteria test (LBT) according to Menz et al. (2013) and ready biodegradability tests according to the OECD series 301 (OECD, 2006). The 24 h LBT is typically used as ecotoxicity test, but also suitable to screen for antibiotic activity of SUAs' UV-mixtures. The reason is that Menz' 24 h LBT analyses not only acute ecotoxicity (luminescence inhibition, LI_{30min}) but also chronic ecotoxicity (LI_{24h}) and growth inhibition (GI_{14h}) of the gram-negative bacterium *Aliivibrio fischeri* (Menz et al., 2013). SUAs are administered also against gram-negative bacteria. Antibiotic activity tests may seem more suitable (e.g. Balouiri et al., 2016; Wiegand et al., 2008). However, they are using pathogenic bacteria, and this can be avoided for a first step of an effect-driven approach.

Given the state of research and methodology, primary aim of this study was to search for a TP that might be antibiotically active like the parent compound (PC) but better environmentally biodegradable than the PC. Secondary aim was to fill data gaps on the behaviour of SUAs and their TPs in biodegradability and ecotoxicity tests. Overall aim was to create a better understanding of possible opportunities and challenges of BbD. For this purpose, six SUAs (Fig. 1) were photolyzed as a means of non-targeted synthesis (cf. Lorenz et al., 2021) and generated UV-mixtures analysed by high performance liquid chromatography coupled to an UV and tandem mass spectrometry detector (HPLC-UV-MS^n). UV-mixtures were screened for bioactivity against *Aliivibrio*

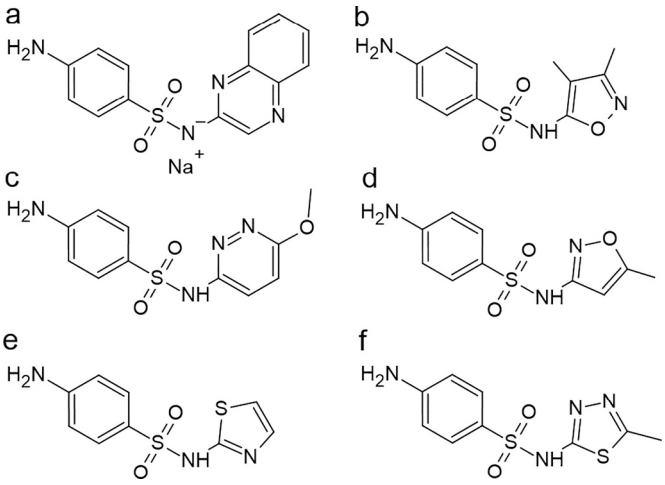

Fig. 1. Selection of parent compounds for the redesign of sulfonamides. a) sulfaquinoxaline-Na (SQX), b) sulfisoxazole (SIX), c) sulfamethoxypyridazine (SMP), d) sulfamethoxazole (SMX), e) sulfathiazole (STZ), f) sulfamethizole (SMT). The specific functional groups are: a) quinoxaline, b) 3,4-dimethyl-isoxazole, c) 6-methoxy-pyridazine, d) 5-methylisoxazole, e) thiazole, and f) 5-methyl-1,3,4-thiadiazole.

fischeri (luminescence bacteria test, LBT) and environmental ready biodegradability (manometric respirometry test, MRT, OECD 301F). In addition, sulfanilamide (SAA) and commercially available hydroxylated SAA-derivatives (Fig. 2) were tested for ready biodegradability to examine the rule of thumb that hydroxylation favours biodegradability (Boethling et al., 2007).

2. Method

2.1. Test compounds and other chemicals

Six SUAs (Fig. 1, $c_0 = 50$ mg L^{-1}) were photolyzed to create diverse TP-mixtures with fast decrease in concentration of the PC: sulfaquinoxaline-Na (SQX), sulfisoxazole (SIX), sulfamethoxypyridazine (SMP), sulfamethoxazole (SMX), sulfathiazole (STZ), sulfamethizole (SMT). Selection of SUAs covered various specific functional groups at the pharmacophore, i.e., the SAA structure, with regard to the size of the aromatic rings, heteroatoms and substituents at the rings (Fig. 1).

Three hydroxylated SAA-derivatives (Fig. 2) were selected for additional biodegradability testing (MRT). All chemicals used in this study are listed in the supporting information (SI; Table S1).

2.2. Stock solution and dilution series

To prepare a SUA stock solution of 50 mg L^{-1}, 50 mg SUA were solved in about 800 mL ultrapure water. Then, pH was increased up to pH 10 using NaOH solution (0.1 M) to facilitate the dissolution of SUAs in water. Once the solution was homogenous, pH was set to pH 7 with HCl solution (0.1 M) before filling up to 1 L. SUAs remained in solution. The stock solution was diluted with water in 8 steps for later use in the LBT: 50, 40, 30, 20, 10, 5, 2.5 and 1.25 mg L^{-1}. The dilution was stored at −20°C until the use in the LBT (see Section 2.5).

2.3. Generation of SUA-TPs by photolysis

SUAs were photo-transformed according to the method described by Rastogi et al. (2014). 800 mL stock solution (see Section 2.2) were irradiated for 256 min with a medium pressure mercury UV light source (TQ 150; UV lamp, Consulting Peschl, Mainz, Germany; λ = 200–600 nm) at 20 ± 1°C and pH 7 ± 1. To ensure a stable temperature during the experiment, the lamp was housed in a water-cooled quartz glass tube (cooling system WKL230, LAUDA, Berlin, Germany). pH was kept constant using NaOH solution (0.1 M). While stirring continuously, samples were drawn from the reactor in an exponential series (0, 2, 4, 8, 16, 32, 64, 128, 256 min; samples SUA-X, with X = irradiation time) and used for HPLC-UV-MS^n analysis and the LBT. Samples were stored at −20°C until the HPLC-UV-MS^n analysis and LBT.

2.4. HPLC-UV-MS^n analysis

Removal rates of the PC and related concentration-time courses (c-t courses) of the formed photo-TPs were monitored. Typical for TPs are c-t courses that show first a formation phase and then further transformation/degradation or constant concentration while the parent compound is primarily degraded. Thus, a peak is considered a SUA-TP in case of monitored peak areas that are increasing and then decreasing or are constant along the irradiation time. The used analytical system was an Agilent 1100 series HPLC system (Agilent Technologies, Waldbronn, Germany) coupled to an UV–Vis detector and a Bruker Daltonic Esquire 6000 + ion-trap Mass Spectrometer with electrospray ionization interface (Bruker Daltonics, Bremen, Germany). Reversed phase (Accucore C18–2.6, 100/2.1, Thermo Scientific) gradient method was applied using 0.1% formic acid and acetonitrile (gradient see SI, Section 2). Column oven temperature was set to 40°C, the injection volume to 10 μL of 1:10 diluted samples, and flow rate to 0.25 mL min^{-1}. MS^n was operated in full-scan mode.

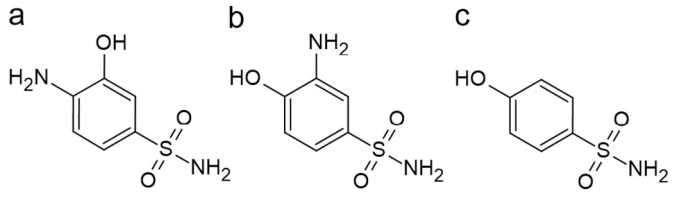

Fig. 2. Commercially available sulfanilamide-derivatives. a) 4-amino-3-hydroxybenzenesulfonamide (4A3OH-BS), b) 2-aminophenol-4-sulfonamide (2AP4-S), c) 4-hydroxybenzenesulfonamide (4OH-BS).

TPs were named according to their retention time and *m/z* in brackets. No brackets indicate that the TP was detected solely by UV. Due to the lack of TP-standards, quantification was not possible. Amount was expressed as relative peak area (rel. PA; $PA_{TP}/PA_{PCinSUA-0}$ or $PA_{TP}/PA_{TP,max}$).

Structures were proposed provisionally based on MS^1 and MS^2 data, including differences in m/z to PC and isotope peaks (particularly ^{34}S indicating presence of SUA moiety) and fragment ions. Supportively, literature data on TPs and transformation processes of PC was used (e.g., Bonvin et al., 2013; Boreen et al., 2004, 2005; Zhou and Moore, 1994). We need to stress that only BbD candidates would have been prioritized for structure elucidation for this BbD approach (see intro on effect-driven approach).

2.5. Luminescence bacteria test (LBT)

Based on EN ISO 11348, Menz et al. (2013) developed a special luminescence bacterium test (LBT) to assess not only acute but also chronic inhibition of bacterial luminescence (LI_{30min}, LI_{24h}). The growth inhibition after 14 h (GI_{14h}) can be evaluated by this method, which indicates bacteriostatic effects. Moreover, no pathogenic bacteria are used in contrast to typical activity tests for antibiotics (e.g., Balouiri et al., 2016; Wiegand et al., 2008). For these reasons and also because the test is quick and simple while having a high level of standardization, SUAs and UV-mixtures were investigated using Menz' 24 h test. Note, SUAs' half maximal effective concentration (EC_{50}) for GI_{14h} are known to be higher than SUA's concentration set in this study design. Nevertheless, we decided to use lower concentrations because preceding photolysis was more efficient at lower concentrations, water solubility was assured and fewer resources were consumed. Moreover, this endpoint served as a first screening to identify those TPs that are bacteriostatic at lower concentrations than the PC, i.e., more active. For example, EC_{50} of SMX and *p*-nitro-SMX for GI_{14h} in Menz' 24 h LBT performed by Majewsky et al. (2014) were 599 μmol L^{-1} (= 152 mg L^{-1}) and 28 μmol L^{-1} (= 5.8 mg L^{-1}), respectively. From pharmaceutical perspective, high activity enabling a lower dose which may reduce the risks for side effects can be advantageous.

To investigate if TPs of the UV-mixtures inhibit luminescence or growth of *Aliivibrio fischeri*, measured inhibition of UV-mixtures ($I_{measured}$) was compared to the theoretical inhibition attributed to the PC residue in the sample ($I_{theoretical}$). The theoretical inhibition was derived from dose-response relationship of measured dilution series of the SUA stock solution (see Section 2.2, with $n = 3$ replicates). TPs occurring in the UV-mixture were suspected to contribute to bacterial luminescence or growth inhibition if measured inhibition by this mixture was significant (≥20% according to Menz et al., 2013) and ≥ 6% higher than the theoretical inhibition by the PC residue (threshold of 6% set based on authors' experience):

$$\Delta\text{Inhibition } \Delta I = I_{measured} - I_{theoretical} \geq 6\%.$$

To investigate if the effects can be attributed to specific TPs the evolution of the inhibitory effect associated with TPs (when $\Delta I \geq 6\%$.) was compared to concentration-time courses of TPs determined by

HPLC-UV-MS^n.

For each SUA, the dilution series of the SUA stock solution (7–8 steps, see Section 2.2) and the UV-mixtures (8–9 irradiation time points, see Section 2.2) were tested in one test run. Eight (0–128 min) instead of nine UV-mixtures (0–256 min) were tested for those SUAs where no transformation was observed anymore from 128 to 256 min, i.e., SUA-256 equalled SUA-128. Before performing the LBT, samples were salted up to 2% NaCl to create right conditions for this marine bacterium. Their pH and peroxide content were controlled to be at pH 7 ± 1 and < 0.5 mL^{-1} (H_2O_2 equivalents), respectively.

The overnight culture of *Aliivibrio fischeri* NRRL-B-11177 (Hach-Lange GmbH, Düsseldorf) was prepared in culture medium (details in SI, Section 2.1) and incubated at 21°C and 150 rpm for 21 ± 0.5 h. The overnight culture was diluted with fresh culture medium to an initial density of 20 formazine turbidity units (FTU). Aliquots of 100 μL of this main culture were transferred into a 96-well microplate (100 mL of culture medium for negative controls, NC). Initial luminescence emission and optical density (578 nm) were measured after 30 min pre-incubation at 15°C. Subsequently, the test culture (or culture medium for NC) was supplemented with 100 μL of the respective sample (SUA test samples (n = 3), 2% NaCl for blanks (n = 16) and NC (n = 8), 2 positive controls (2*n = 2*4)). This resulted in a dilution factor of 2 for all test samples and 25 mg L^{-1} as the highest SUA concentration).

Continuously, luminescence and optical density were measured for 24 h at 15°C (Menz et al., 2013).

2.6. Manometric respirometry test (MRT)

The manometric respirometry test (MRT, OECD 301F) was performed using activated sludge taken from the municipal WWTP in Lüneburg, Germany in December 2022 and January 2023 to investigate biotic removal of SUA-TPs (OECD, 2006). TPs were tested as a UV-mixture of one specific UV-irradiation timepoint (see photolysis). The irradiation timepoint was selected based on a high number of photo-TPs and low PC residue to test as many TPs as possible at higher concentrations and less impacted by the PC. Biodegradability of the unirradiated solution (SUA − 0) was tested as well to serve as reference for later evaluation. Parallel determination was performed for all samples (n = 2). For this purpose, all reagents for the test preparation were combined in one bottle and only divided into two bottles at the start of incubation.

Sample concentration was adjusted to give a theoretical oxygen demand (ThOD) of approx. 30 mg L^{-1}. ThOD factors ($m_{oxygen}/m_{compound}$; for SUAs see SI, Section 2.2) of UV-mixtures cannot be calculated as the molecular formulas and composition in the mixture are unknown. Approximately, the same ThOD as the one of PC was taken. Preceding results of a photometric cell test on the chemical oxygen demand of five UV-mixtures (SIX-16, SMP-64, SMX-8, STZ-16, SMT-16) showed that this simpler and less resource intensive method is a good approximation (SI, Table S2).

Non-purgeable organic carbon (NPOC) and oxygen concentrations were measured, the latter continuously, to calculate mineralization rates based on NPOC removal and oxygen demand, respectively. NPOC was measured at d0 and d28 using a TOC-analyzer (TOC-V CPH/CPN, Shimadzu, Duisburg, Germany). The thresholds for ready biodegradability are 60% of ThOD or 70% removal of dissolved organic carbon (considered NPOC). Thresholds must be reached in a 10-days window within the incubation of 28 days (OECD test series 301). HPLC-UV-MS^n analysis (described above) of MRT samples from d0 and d28 of incubation was performed to identify TPs with constant, increased or reduced PA after incubation. Sterile control (no inoculum added) supported differentiation of biotic processes and sorption on sludge to abiotic transformation.

Analogously, SAA and hydroxylated SAA-derivatives (Fig. 2) progressed through the MRT. ThOD factors can be found in SI (Table S2).

2.7. Identifying TPs of interest for BbD

Structures of interest for BbD should be similar to the parent on the one hand, e.g., still contain the pharmacophore. On the other hand, a structure of interest should be modified sufficiently to result in better biodegradability. For example, modification of the SAA structure may improve biodegradability in contrast to slight modification of the specific functional group, e.g., resulting in isomers. The rational is that the entire group of SUAs with diverse specific functional groups at the sulfonamide group is not readily biodegradable in the environment (National Center for Biotechnology Information, 2023a, 2023b, 2023c; and own data not published), and a TP structure that is slightly modified only at the specific functional group is expected to belong to this group of not readily biodegradable compounds. Therefore, these structural features, indicated by MS^1 data, excluded a TP as candidate for BbD:

- lost pharmacophore, i.e., SAA, indicated by missing ^{34}S isotope peak for the sulfonamide moiety and m/z < 173 (m/z of SAA).
- same m/z as SUAs, i.e., SUA-isomers as they are likely to be similarly resistant to biodegradation as the PC.

Remaining structural-suitable TPs were of interest for BbD if they were identified in the LBT as possibly contributing to the inhibitory effect of their UV-mixture or detected with decreased PA after 28 days of MRT. TPs of interest were investigated and discussed in more detail to gain further insights. A BbD candidate would meet both criteria.

TPs detected only by the UV detector, i.e., not by MS, lacked structural information. They were prioritized for closer examination, only if both criteria, i.e., contributing to the inhibitory effect (LBT) and decreasing in PA (MRT), were met.

Independent of BbD interest, all TPs were considered to interpret MRT and LBT results of the whole UV-mixture.

3. Results and discussion

3.1. Generation of photo-TPs of sulfonamides

HPLC analysis of UV-mixtures showed that primary elimination of the parent compound was completed by 64 min UV-irradiation for all except SMP (256 min) and SQX (almost by 256 min) (SI, Fig. S1). In total 66 photo-TPs, 8–15 TPs per SUA, were detected in the UV-mixtures, some of them only by UV-detection, i.e. not by MS^n (Table 1). MRT samples of one specific irradiation timepoint (SQX: 64 min, SIX: 16 min, SMP: 64 min, SMX: 8 min, STZ: 16 min, SMT: 16 min) contained most of them (Table 1, first column). Concentration-time courses of TPs can be found in SI (Figs. S2–S7).

The mass spectrum of 19 TPs did not show the ^{34}S isotope peak of the sulfonamide moiety clearly indicating the loss of the pharmacophore for these TPs (Table 1).

Several detected TPs were in line with well-known transformation processes occurring during UV-irradiation of SUAs such as δ-cleavage, hydroxylation, desulfonation, oxidation, e.g., of the *p*-amino group, and isomerization (e.g., Boreen et al., 2004, 2005; Zhou and Moore, 1994). Among them were:

- amines of the specific functional group (NH_2-R; 2-amino-quinoxaline of SQX, 5-amino-3,4-dimethylisoxazole of SIX, 3-amino-6-methoxy-pyridazine of SMP, 3-amino-5-methyl-isoxazole of SMX, 2-amino-thiazole of STZ, 2-amino-5-methyl-1,3,4-thiadiazole of SMT),
- hydroxylated SUAs (OH-SQX, -SIX, -SMP, -SMX, -STZ, -SMT),
- desulfonated SUAs (desulfo-SQX, -SMP, -SMX, -SMT),
- nitroso-SUAs (NO-SQX, NO-SMP), and
- SUA-isomers (3 SIX-isomers, SMP-isomer, SMX-isomer, STZ-isomer).

To the best of our knowledge, detected SIX-isomers (m/z = 268; see SI, Figs. S8a,b and S9a,b) and the SMP-isomer (m/z = 281) are not yet

Table 1
SUA-TPs detected in UV-mixtures. Row on occurrence: timepoint of concentration peak in brackets. MRT: TP detected in UV-mixture used for MRT; na: not applicable for this TP as UV-detection only; $\Delta m/z$: m/z_{TP} - m/z_{PC}.

PC	TP named with retention time (and m/z detected by MS)	Occurrence during irradiation/min	^{34}S peak in MS^1 indicating intact SUA moiety	Literature describing the same m/z of the ion (MS^1)	Structure proposal based on MS^n (incl. fragments from MS^2) and literature
	TP-2.9 (146) MRT	8–256 (128)	no	Ji et al., 2017; Nassar et al., 2018; Qiu et al., 2019; Urbano et al., 2017b; Urbano et al., 2017a	2-amino-quinoxaline
	TP-7.3 (317) MRT	8–128 (16, 128)	yes	Liao et al., 2016; Qiu et al., 2019	OH-SQX
	TP-7.8 (237) MRT	2–256 (64)	no	Liao et al., 2016; Nassar et al., 2018	desulfo-SQX
	TP-10.3 (238) MRT	16–256 (16)	no		$\Delta m/z$: −63, desulfo-4-OH-SQX
SQX	TP-10.7 (315) MRT	16–256 (128)	yes		NO-SQX
	TP-3.2 MRT	8–256 (128)	na		na
	TP-4.7	64–128 (128)	na		na
	TP-9.5	4–128 (32)	na		na
	TP-14.1	16–128 (32)	na		na
	TP-15.2	16–128 (64)	na		na
	TP-16.0	32–256 (32)	na		na
	TP-16.2	16–256 (32)	na		na
	TP-1.2 (286) MRT	2–16 (4)	yes		Δm/z: +18 (1)
	TP-1.4 (173) MRT	8–256 (16)	yes	Many, e.g., Baena-Nogueras et al., 2017	SAA
	TP-1.5 (113)	64–256 (128)	no	Ge et al., 2019; Yang et al., 2010	5-amino-3,4-dimethylisoxazole
	TP-3.2 (286) MRT	2–32 (4)	yes		Δm/z: +18 (2)
	TP-6.2 (268) MRT		yes	cf. SMX-isomers by Palm et al., 2023	SIX-isomer (1)
	TP-7.0 (140) MRT	8–16 (8)	no	Wang et al., 2021	nitrophenol
	TP-7.0 (268) MRT	4 (4)	yes	cf. SMX-isomers by Palm et al., 2023	SIX-isomer (2)
SIX	TP-10.3 (284) MRT	32–256 (128)	yes	Baena-Nogueras et al., 2017; Ge et al., 2019; Yao et al., 2017	OH-SIX
	TP-10.9 (268) MRT	8–256 (16)	yes	cf. SMX-isomers by Palm et al., 2023	SIX-isomer (3)
	TP-12.3 (378)	2–4 (2)	yes		$\Delta m/z$: +110
	TP − 0.9 MRT	4–256 (8)	na		na
	TP-1.3	16–32 (16)	na		na
	TP-1.8 MRT	8–256 (32)	na		na
	TP-3.2 MRT	4–64 (4)	na		na
	TP-4.2	8–64 (16)	na		na
	TP-1.3 (126) MRT	4–256 (128)	no	Nassar et al., 2017	3-amino-6-methoxypyridazine
	TP-2.4 (233) MRT	16–256 (128)	no		OH-desulfo-SMP
	TP-3.2 (217) MRT	0–256 (64)	no	Gao et al., 2019	desulfo-SMP
	TP-3.9 (297) MRT	8–256 (32)	yes	Gao et al., 2019; Khaleel et al., 2013	OH-SMP (1)
SMP	TP-4.8 (245) MRT	128–256 (256)	no		$\Delta m/z$: −36
	TP-8.1 (297)	8–128 (32)	yes	Gao et al., 2019; Khaleel et al., 2013	OH-SMP (2)
	TP-9.1 (295) MRT	16–256 (64,128)	yes	Gao et al., 2019; Khaleel et al., 2013	NO-SMP
	TP-10.2 (281)	32–256 (128)	yes		SMP-isomer
	TP − 0.9 MRT	8–256 (64–256)	na		na
	TP-1.1 MRT	MRT only	na		na
	TP-1.0 (99) MRT	MRT only	No	Many; well elucidated by Zhou and Moore, 1994	3-amino-5-methyl-isoxazole
	TP-1.1 (190)	4–16 (8)	no	Baena-Nogueras et al., 2017; Bonvin et al., 2013; Liu et al., 2020	desulfo-SMX
	TP-1.3 (286) MRT	4–32 (8)	yes	Fabbri et al., 2019; Gómez-Ramos et al., 2011; Liu et al., 2020	OH-OH-SMX
	TP-1.5 (246)	4–8 (4)	no	Wang et al., 2020	Δm/z: −8
	TP-1.9 (216)	4–8 (8)	yes		Δm/z: −38
SMX	TP-1.9 (99) MRT	MRT only	no	Boreen et al., 2004; Zhang et al., 2016	2-amino-5-methyloxazole
	TP-4.1 (254) MRT	2–16 (4)	yes	Many; well elucidated by Zhou and Moore, 1994	SMX-isomer
	TP-9.5 (270) MRT	4–16 (8)	yes		OH-SMX
	TP − 0.9 MRT	4–256 (16)	na		na
	TP-1.1 MRT	4–256 (16)	na		na
	TP-2.1 MRT	4–16 (8)	na		na
	TP-2.4	4–8 (4,8)	na		na
	TP-10.3	4–8 (8)	na		na
	TP-1.1 (140)	4–32 (8)	yes		Δm/z: −116
	TP-1.1 (101) MRT	4–64 (16)	no	Baena-Nogueras et al., 2017; Boreen et al., 2004; Ge et al., 2019; Niu et al., 2017	2-aminothiazole
	TP-1.4 (94)	16–64 (32)	no	Deng et al., 2019; Hu et al., 2021	aniline
STZ	TP-3.6 (272) MRT	0–16 (8)	yes	Baena-Nogueras et al., 2017; Calza et al., 2004; Ge et al., 2019	OH-STZ
	TP-7.3 (256) MRT	4–32 (8)	yes	Spielmeyer et al., 2015	STZ-isomer
	TP-9.0 (170)	4–32 (4)	no		Δm/z: −86
	TP-1.1 MRT	0–256 (32)	na		na
	TP-1.3	8–16 (8)	na		na
SMT	TP-1.2 (116) MRT	2–256 (16)	no	Baena-Nogueras et al., 2017; Boreen et al., 2004; Klauson et al., 2010	2-amino-5-methyl-1,3,4-thiadiazole
	TP-1.7 (233)	16–256 (16)	yes		Δm/z: −38

(*continued on next page*)

Table 1 (*continued*)

PC	TP named with retention time (and m/z detected by MS)	Occurrence during irradiation/min	^{34}S peak in MS^1 indicating intact SUA moiety	Literature describing the same m/z of the ion (MS^1)	Structure proposal based on MS^n (incl. fragments from MS^2) and literature
	TP-2.0 (207) ^{MRT}	4–32 (8)	no	Baena-Nogueras et al., 2017	desulfo-SMT
	TP-3.5 (312) ^{MRT}	2–16 (4)	yes		Δm/z: +4
	TP-4.3 (158) ^{MRT}	8–64 (16,32)	no		4-aminobenzenesulfinate
	TP-7.7 (287) ^{MRT}	4–16 (8)	yes	Klauson et al., 2010	OH-SMT
	TP − 0.9 ^{MRT}	2–256 (16)	na		na
	TP-3.4	16–128 (64)	na		na

described in literature. Given structural similarity between SIX and SMX (both are isoxazoles) it is plausible that the isoxazole ring of SIX could isomerize to oxazole like SMX for which the photo-isomer 4-amino-*N*-(5-methyl-2-oxazolyl)benzenesulfonamide is well studied (Zhou and Moore, 1994). SMX and this photo-isomer can be present in amine or imine structure due to enamine-imine tautomerization, leading to four structures with the same molecular formula (Palm et al., 2023). Same tautomerization could also justify the detection of four structures with m/z of SIX = 268 (Fig. 3).

Many TPs (about 75%) that were detected by UV only are expected to be very polar because of short retention times on the reversed phase.

3.2. Luminescence bacteria test (LBT)

Menz' 24 h luminescence bacteria test (LBT) was suitable to determine differences between PCs and TPs in mixtures with respect to their acute and chronic luminescence inhibition (LI_{30min}, LI_{24h}).

Acute luminescence inhibition (LI_{30min}) was not significant for all tested SUAs even at the highest test concentrations of 25 mg L^{-1}. In contrast, UV-mixtures of SMP, SMT, and STZ showed significant LI_{30min} (≥ 20% inhibition) that can therefore be attributed to the TP portion of the UV-mixture (Fig. 4). The concentration-time courses of one SMP-TP and five STZ-TPs matched the evolution of LI_{30min}, indicating possible contribution of such TPs to the inhibitory effect. However, there was no match for a SMT-TP (Fig. 4, and Table 2, upper part).

Chronic luminescence inhibition (LI_{24h}) by PCs was in general much higher than LI_{30min}. Maximum LI_{24h} by all PCs was about 80% except SMT with 40% (dose-response curves in SI, Section 3.2). Significant LI_{24h} attributed to the TP portion was found for several UV-mixtures of all SUAs, namely SQX-32-256, SIX-16, SMP-64-256, SMX-32-128, SMT-8-128 and STZ-8-32. Several specific TPs that could have contributed to LI_{24h} due to their concentration-time courses were identified for all of these UV-mixtures, except those of SQX and SMT (Table 2, middle part, Fig. 5).

Growth inhibition (GI_{14h}) by PCs was very low (< 30% by highest c of 25 mg L^{-1}). GI_{14h} by SMT was not significant (< 20% by 25 mg L^{-1} SMT). Based on ΔGI_{14h}, no UV-mixture indicated highly active TPs that resulted in significant GI_{14h} (SI, Section 3.2). This doesn't signify that UV-mixtures didn't contain bacteriostatic TPs, considering very low GI_{14h} by PCs which were tested in higher concentration than their resulting TPs by default.

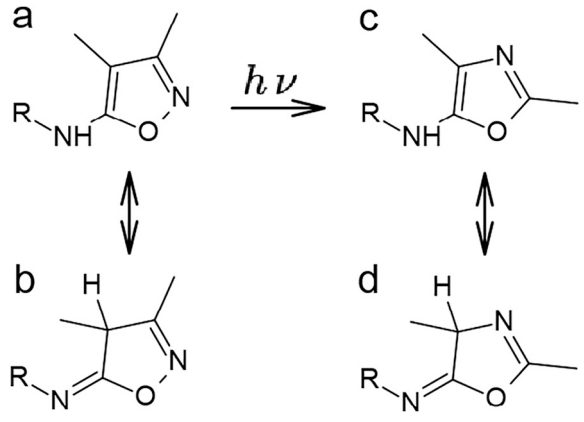

Fig. 3. SIX (a, b) and its photo-isomers (c, d) proposed as amine (a, c) or imine (b, d) structure (compare SMX-isomers by Palm et al., 2023), with R-NH_2 = Sulfanilamide.

3.2.1. Insights from LBT

UV-mixtures resulting from photolysis of SMP (256), STZ (4–16) and SMT (16, 128) showed significant acute luminescence inhibition (LI_{30min}) attributed to the TPs in the UV-mixture. 5 STZ-TPs and 1 SMP-TP were suspected being contributing to LI_{30min}. This includes the STZ-isomer TP-7.3 (256).

STZ-TP-7.3 (256) could be promizole, an antituberculotic, that was identified by Spielmeyer et al. (2015) as the main TP after UV-irradiation of STZ. Promizole was less microbially active than STZ in the Brilliant Black Reduction Maximum Residue Level test (EC_{50}: 250 μg/L vs 50 μg/L) (Spielmeyer et al., 2015). This is not in contrast to our findings as another mechanism of action for this bioactive compound could lead to LI_{30min}. Moreover, promizole lacks the sulfonamide structure because of a direct bond between the sulfonyl group and the thiazole ring, indicating another mechanism of action apart from the inhibition of the dihydropteroate synthase.

TP-4.8 (245) is the only SMP-TP identified as potentially bioactive. It is not reported in literature to the best of our knowledge. SMP-TP-4.8 (245) could also contribute to LI_{24h}. Missing ^{34}S isotope peak in the mass spectrum indicates the loss of the sulfonamide moiety and thus a mechanism of action apart from the inhibition of dihydropteroate synthase.

Chronic luminescence inhibition (LI_{24h}) by TPs in the UV-mixture was found for SQX-32-256, SIX-16, SMP-64-256, SMX-32-128, STZ-8-32 and SMT-8-128. In these UV-mixtures, 4 SIX-TPs, 1 SMP-TP (see previous paragraph), 2 SMX-TPs and 1 STZ-TP were identified tentatively being contributing to LI_{24h}. This includes the newly identified SIX-isomer TP-10.9 (268). Structural similarity to SIX (Figs. 1b, 3) hints at bioactivity due to same mechanism of action.

Among detected SMX-TPs, the only TPs that could contribute to LI_{24h} by mixtures SMX-32-128 were TP-0.9 and TP-1.1. Desulfo-SMX, the SMX-isomer and OH-SMX are considered bioactive according to literature, but were not detectable anymore in the bioactive UV-mixtures from 32 min onwards (Table 1, row "Occurrence during irradiation"). Desulfo-SMX was found to contribute potentially to the growth inhibition of *E. coli* by a TP-mixture generated by photocatalysis (catalyst Fe_3O_4-ZnO@g-C_3N_4, low energy-consuming UV lamp, pH = 7) (Mirzaei et al., 2018). The SMX-isomer was similarly bioactive compared to SMX in the Brilliant Black Reduction Maximum Residue Level test (Spielmeyer et al., 2015). *N4*-OH-SMX was found to be similarly bacteriostatic against *Aliivibrio fischeri* as SMX, showing an EC_{50} value of 200 mg L^{-1} (Majewsky et al., 2014). *p*-OH-SMX, *p*-nitroso-SMX, and *p*-nitro-SMX, that are bacteriostatic against *Aliivibrio fischeri* (Majewsky et al., 2014), were not detected in this study.

Comparing the c-t courses of STZ-TPs and ΔLI_{24h} development, indicated STZ-TP-1.1 (101) to contribute to ΔLI_{24h} in mixtures STZ-8-32 (max at 16 min) due to maximum PA at 16 min. However, this is a good example to stress that, albeit such identification based on c-t courses, the

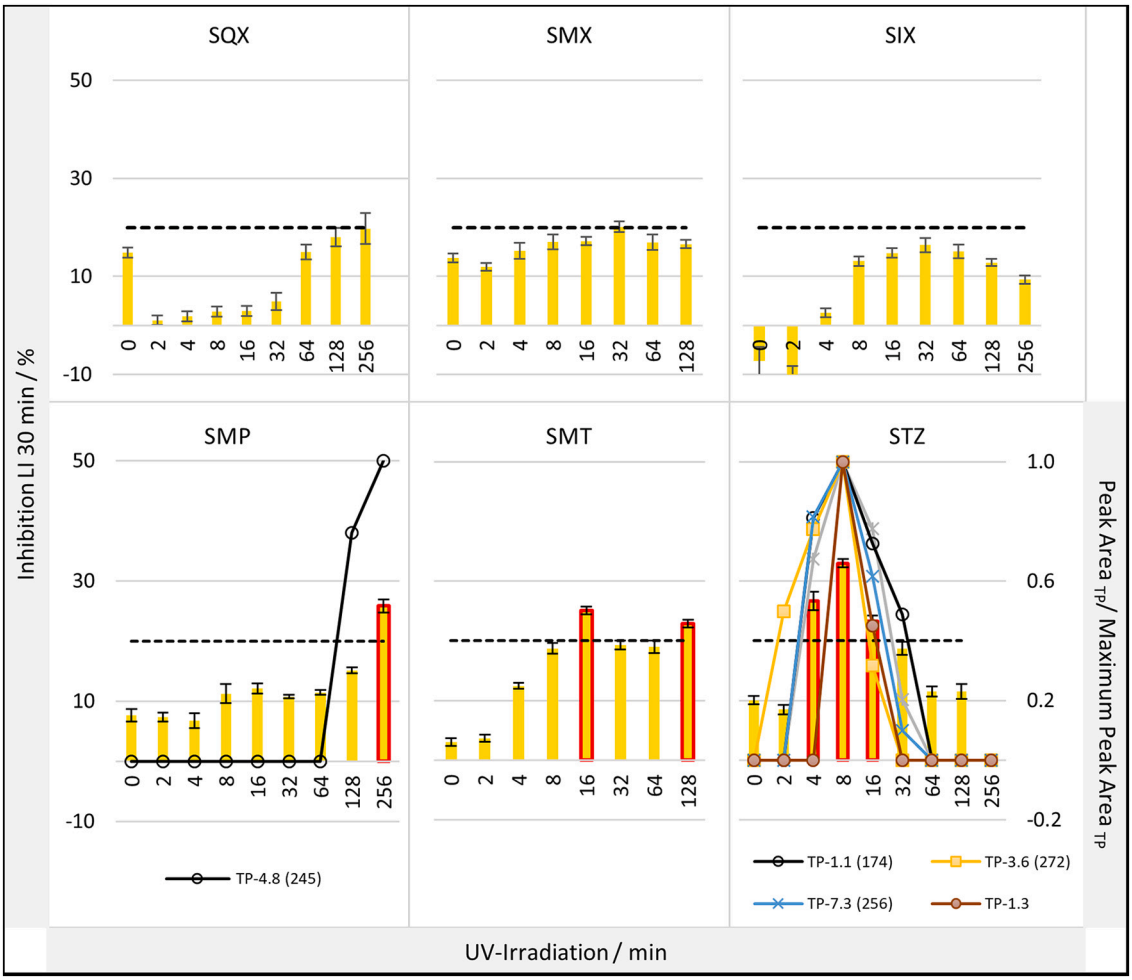

Fig. 4. Acute luminescence inhibition (LI_{30min}) by UV-mixtures of SUAs (yellow bars), and concentration-time courses of possibly bioactive TPs of SMP and STZ. Significant LI_{30min} by UV-mixtures of SMP, SMT, and STZ can be attributed to the TP portion of the UV-mixture (red bordered) because of non-significant initial LI_{30min} even by SUA-0 with highest concentration of 25 mg SUA L^{-1}. Threshold for significant inhibition is 20% (dashed line). Raw data can be found in SI (Section 3.2).

Table 2
TPs that could have contributed to luminescence inhibition (LI_{30min}, LI_{24h}). Reasoning was most often a c-t course of the TP with maximal concentration in the UV-mixture showing also maximal ΔLI (see*).

	Sample SUA-X, with X = irradiation / min	Possibly Contributing TPs		
		without SUA moiety	with SUA moiety, or an isomer	no data on structure
LI_{30min}	SMP-256	4.8 (245)*		
	SMT-16, −128	***		
	STZ-8	1.1 (140)*	1.1 (174)* 3.6 (272)* 7.3 (256)*	1.3*
	SQX-32-256	***		
	SIX-16		1.4 (173)* 10.9 (268)*	1.3* 4.2*
LI_{24h}	SMP-64-256	4.8 (245)*		
	SMX-32-128			0.9** 1.1**
	SMT-8-128	***		
	STZ-32-128	1.1 (101)*		
Reasoning	*max c in the UV-mixture of max ΔLI. **present in samples where ΔLIs were of constant magnitude. ***No TP was detected with a c-t course that could explain the evolution of ΔLI.			

entire UV-mixture needs to be reviewed, especially as additive, synergistic and other mixture effects can result in measured LI_{24h}. For example, TP-7.3 (256) may also have contributed to LI_{24h}, possibly even to higher extent than TP-1.1 (101), although its c-t course didn't match the development of ΔLI_{24h} (PA_{max} at 8 min and not 16 min). The reason for this consideration is that PA_{16min} of TP-7.3 (256) was still greater compared to other TPs ($PA_{16min}/PA_{STZ,0}$ = 3.9% versus < 1% for other TPs, see SI, Fig. S6), suggesting a high TP share. Moreover, TP-7.3 (256) is proposed to be promizole, the main photo-TP of STZ and a bioactive compound (see above).

In general, the identification of TPs that might contribute to the effect based on their c-t courses is challenging because of the variety of detected TPs. Non-detectable TPs could also contribute to the measured effect. Other effects than the inhibition of the dihydropteroate synthase can cause luminescence inhibition, especially by TPs that lost the sulfonamide moiety. In addition, effects can be additive, synergistic or antagonistic. Moreover, concentrations of TPs are unknown but expected to be rather low, especially when the PC is transformed into many different TPs to an equal extent. Therefore, indication of TPs that contributed to the bacterial inhibition need further proof to understand ecotoxicity of single TPs, but was supportive for the identification of TPs of interest for BbD (see Section 4).

Growth inhibition (GI_{14h}) by PCs was low or not significant as expected for tested concentration range because the concentration was not higher than 25 mg L^{-1} whereas, for example, the EC_{50} of SMX was 152

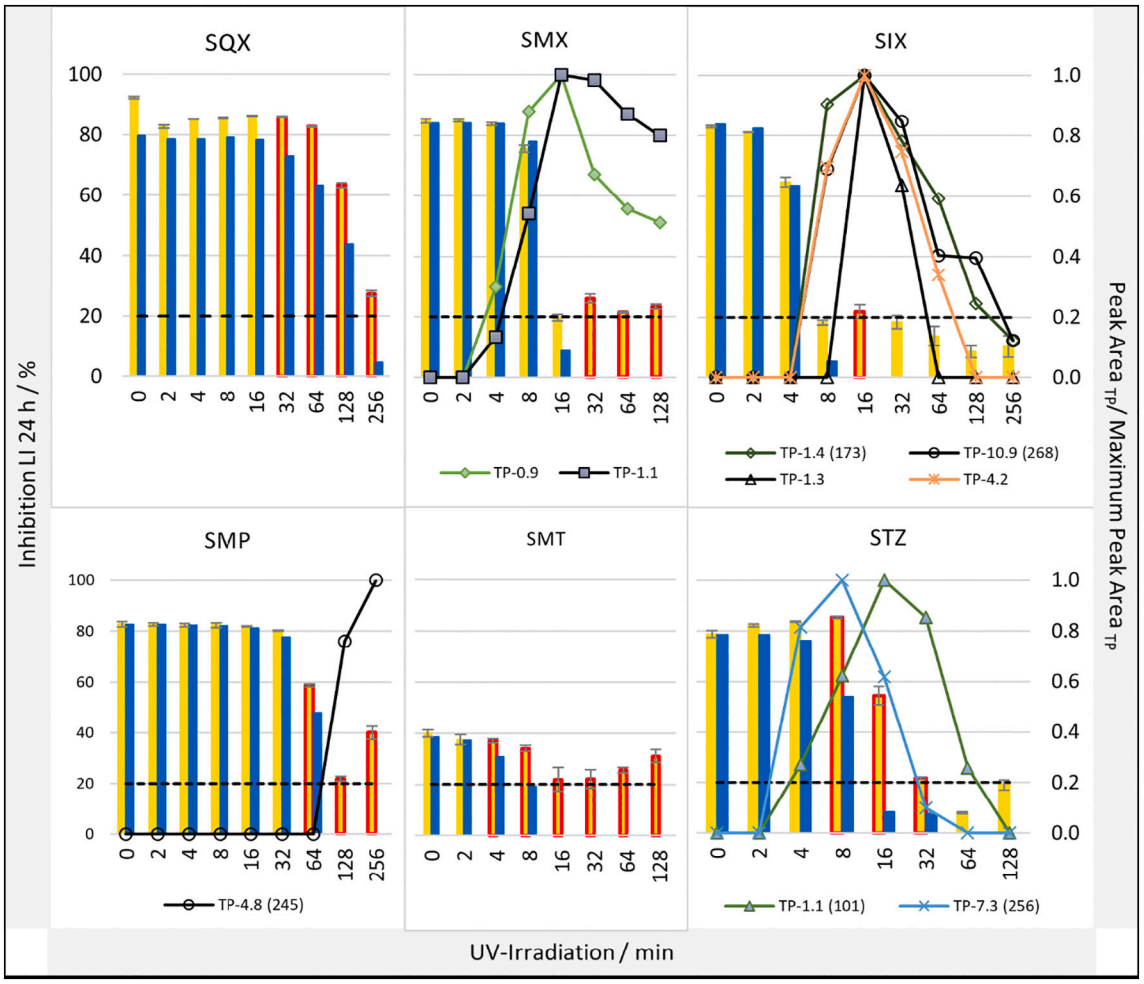

Fig. 5. Chronic luminescence inhibition (LI_{24h}) by UV-mixtures of SUAs (yellow bars), and concentration-time courses of possibly bioactive TPs. For several UV-mixtures, measured LI_{24h} can be attributed (partly) to the TP portion (red bordered yellow bares) because measured LI_{24h} was significant and ≥ 6% higher than the theoretical LI_{24h} by the PC residue (blue bars). Threshold for significant inhibition is 20% (dashed line). Raw data can be found in SI (Section 3.2).

mg L^{-1} in the LBT study by Majewsky et al. (2014). Since GI_{14h} was also low or not significant for tested UV-mixtures (with $c_{TP,i} << c_{PC,0min}$ by default; see Section 2.4), no TP was identified being significantly more active than the PC, i.e., bacteriostatic at significantly lower concentrations than PC's concentration. Note, the occurrence of TPs that are similarly bacteriostatic as the PC cannot be excluded with this study design. To refine the evaluation of the bacteriostatic effect of SUA-TPs a more sensitive test could be used in future, for example the cell proliferation inhibition test using *Escherichia coli* (MIC range: 2–512 mg L^{-1}, EUCAST database). Broth dilution methods using different pathogenic bacteria may also be suitable (e.g., Wiegand et al., 2008). Summarizing, ecotoxic effects by TPs in UV-mixtures but no bacteriostatic effects on *V. fischeri* were found.

3.3. Biodegradation test

Mineralization rates of tested SUAs and UV-mixtures, except SMX-0, were below the threshold of 60% ThOD, classifying them as not readily biodegradable (data in SI, Fig. S17a,b and Table S3). SMX-0 is considered not readily biodegradable as well although the mineralization rate was >60% ThOD but only slightly and carbon removal was significantly below the threshold of <70%. Note, when testing mixtures, the mineralization rates of a single compound remains unclear. However, rates of removal under biotic and abiotic conditions could be determined for the single compounds in the test samples based on HPLC analysis.

Starting with untreated PCs, SIX was slightly removed under biotic conditions, whereas SMP and SMX were completely removed under biotic conditions. SQX, STZ, and SMT were completely biotically removed in one test sample of the parallel determination. In the other sample, no removal of STZ and SMT, and only partial removal of SQX took place. These differences in samples A and B are also indicated by fluctuating mineralization rates.

Regarding UV-mixtures, 15 SUA-TPs were removed by >25% (i.e., PA_{d28}/PA_{d0}: 0–75%), 5 of them also under sterile conditions. The peak

Table 3
Common SUA-TPs and the parent detected in A and B samples of the parallel determination with decreased PA (green), increased PA (blue) or similar PA (grey) after 28 days, MRT. For SIX, 3 isomers were detected (1–3) showing different behaviour during incubation. nd: not detected.

Compound	SQX	SIX	SMP	SMX	STZ	SMT
NH_2-R	A B	nd	A B	A B	A B	A B
OH-SUA	A B	A B	A B	A B	A B	A B
SUA-Isomer	nd	1 2 3	nd	A B	A B	nd
Parent	A B	A B	A B	A B	A B	A B

Table 4
Mapping of data on structure, MRT and LBT of SUA-TPs. Highlighted in blue: TPs with the possibility of becoming a BbD candidate due to structure properties not contradicting BbD (yellow) and decreased peak area (↓PA, green) or possible contribution to luminescence inhibition (LI) (red). Within the group of TPs lacking structural information (see "na") there was no TP with ↓PAs (light green) and contribution to LI, thus, no TP of interest for BbD (see Section 2.7).

Sample	Name	Structure		MRT[i] PA_{d28}/PA_{d0}/%				LBT
		Structure proposal not applicable (na)	Structure properties not of BbD interest: no SUA moiety (1), SUA-isomers (2); not applicable (na)	MRT Test Sample A	B	sterile	Conclusion a: abiotic, b: biotic	LI_{30min} LI_{24h}
-0	SQX (11.6, 301)			67	0	100	↓PA (b)	
				5	5	75	↓PA (b)	
SQX-256	TP-1.8 (161)	OH-2-amino-quinoxaline	1	110	167	78	↑PA (b)	
	TP-2.9 (146)	2-amino-quinoxaline	1	222	260	109	↑PA (b)	
	TP-7.3 (317)	OH-SQX	BbD	148	182	129	↑PA (b,a)	
	TP-7.8 (237)	desulfo-SQX	1	4	4	0	↓PA (b,a)	
	TP-10.3 (238)	Δm/z: -63, desulfo-4-OH-SQX	1	754	826	455	↑PA (b,a)	
	TP-10.7 (315)	NO-SQX	BbD	108	110	91	-PA (b,a)	
	TP-3.2	na	na	98	98	87	-PA (b,a)	
-0	SIX (10.7, 268)			101	100	100	-PA (b,a)	
				not present in SIX-16				
SIX-16	TP-1.2 (286)	Δm/z: +18 (1)	BbD	65	62	191	↓PA (b)	
	TP-1.4 (173)	SAA	BbD	144	101	136	inconclusive	LI_{24h}
	TP-3.2 (286)	Δm/z: +18 (2)	BbD	124	118	103	-PA (b,a)	
	TP-6.2 (268)	SIX-isomer (1)	2	124	120	90	-PA (b,a)	
	TP-7.0 (140)	nitrophenol	1	385	371	nd	↑PA (b)	
	TP-7.0 (268)	SIX-isomer (2)	2	422	400	nd	↑PA (b)	
	TP-10.3 (284)	OH-SIX	BbD	0	0	0	↓PA (b,a)	
	TP-10.9 (268)	SIX-isomer (3)	2	1	1	6	↓PA (b,a)	LI_{24h}
	TP-0.9	na	na	56	52	101	↓PA (b)	
	TP-1.8	na	na	44	65	74	↓PA (b,a)	
	TP-3.2	na	na	107	129	123	-PA (b,a)	
-0	SMP (8.8, 281)			0	0	94	↓PA (b)	
				0	0	99	↓PA (b)	
SMP-64	TP-1.3 (126)	3-amino-6-me-thoxypyridazine	1	394	398	105	↑PA (b)	
	TP-2.4 (233)	OH-desulfo-SMP	1	38	40	114	↓PA (b)	
	TP-3.2 (217)	desulfo-SMP	1	102	102	102	-PA (b,a)	
	TP-3.9 (297)	OH-SMP (1)	BbD	96	98	121	-PA (b,a)	
	TP-4.8 (245)	Δm/z: -36	1	48	52	28	↓PA (b,a)	LI_{30min}, LI_{24h}
	TP-9.1 (295)	NO-SMP	BbD	99	104	87	-PA (b,a)	
	TP-0.9	na	na	86	111	100	-PA (b,a)	LI_{24h}
	TP-1.1	na	na	181	209	nd	↑PA (b)	LI_{24h}
-0	SMX (10.1, 254)			0	0	100	↓PA (b)	
				0	0	108	↓PA (b)	
SMX-8	TP-1.0 (99)	3-amino-5-methyl-isoxazole	1	116	111	91	-PA (b,a)	
	TP-1.3 (286)	OH-OH-SMX	BbD	211	217	287	↑PA (b,a)	
	TP-1.9 (99)	2-amino-5-	1	311	307	110	↑PA (b)	

		methyloxazole						
	TP-4.1 (254)	SMX-isomer	2	0	0	134	↓PA (b)	
	TP-9.5 (270)	OH-SMX	BbD	0	0	136	↓PA (b)	
	TP-0.9	na	na	58	101	116	inconclusive	LI24h
	TP-1.1	na	na	82	94	124	-PA (b,a)	LI24h
	TP-2.1	na	na	70	87	116	-PA (b,a)	
-0	STZ (4.5, 256)			0	104	99	inconclusive	
				0	0	116	↓PA (b)	
STZ-16	TP-1.1 (101)	2-aminothiazole	1	257	262	125	↑PA (b)	LI_{24h}
	TP-3.6 (272)	OH-STZ	BbD	36	0	160	↓PA (b), ↑PA (a)	LI_{30min}
	TP-7.3 (256)	STZ-isomer	2	39	21	110	↓PA (b)	LI_{30min}, LI_{24h}
	TP-1.1	na	na	50	60	nd	↓PA (b)	
-0	SMT (8.5, 271)			99	0	101	inconclusive	
				110	110	104	-PA (b,a)	
SMT-16	TP-1.2 (116)	2-amino-5-methyl-1,3,4-thiadiazole	1	106	104	103	-PA (b,a)	
	TP-2.0 (207)	desulfo-SMT	1	83	72	211	-PA (b), ↑PA (a)	
	TP-3.5 (312)	Δm/z: +41	BbD	91	93	83	-PA (b,a)	
	TP-4.3 (158)	4-amino-benzene-sulfinate	1	41	60	112	↓PA (b)	
	TP-7.7 (287)	OH-SMT	BbD	130	123	137	↑PA (b,a)	
	TP-0.9	na	na	42	41	114	↓PA (b)	

[i]MRT conclusion on decreased (↓), increased (↑), or constant (−) peak area (PA) after 28 days incubation is based on PA_{d28}/PA_{d0} quotient of test sample A, B and sterile control with 0–75% ↓PA, 76–125% -PA, or > 125% ↑PA.

areas (PAs) of 12 SUA-TPs increased by at least >25% (i.e., $PA_{d28}/PA_{d0} \geq 126\%$), for 4 of them also under sterile conditions. The PAs of 15 SUA-TPs kept constant (i.e., PA_{d28}/PA_{d0}: 76–125%) (Table 4, column "MRT").

Comparing HPLC results of UV-mixtures from different SUAs let to patterns for similar TPs (Table 3). Amines NH_2-R (R: specific functional group), detected for 5 SUAs, generally increased in PA during incubation, while hydroxylated SUAs (OH-SUA, OH position not clear) and SUA-isomers, detected for 3 SUAs, rather decreased. The latter are likely structural similar to the PCs which showed also the tendency to decrease in PA.

Mineralization rates of SAA and hydroxylated SAA derivates – tested to investigate the effect of hydroxylation on SAA's biodegradability – were ≤ 20%, all classifying as not readily biodegradable. Removal under biotic conditions only (considered primary biodegradation) was observed but was still incomplete. With about 50% primary degradation, 4A3OH-BS and 2AP4-S were better primarily biodegradable than 4OH-BS and SAA with about 10% and < 5%, respectively.

3.3.1. *Insights from MRT*

Mineralization of SUAs and their UV-mixtures as well as removal rates of detected compounds were investigated successfully by MRT and subsequent HPLC-UV-MS^n analysis. The finding that tested SUAs (SUA-0) were not readily biodegradable is in line with literature (Ingerslev and Halling-Sørensen, 2000). The sulfanilamide core structure as well as sulfanilic acid can be expected to degrade very slowly in water (National Center for Biotechnology Information, 2023b, 2023c). This demonstrates SUA's resistance to complete biodegradation (National Center for Biotechnology Information, 2023a, 2023b, 2023c).

SUAs showed the tendency of complete primary elimination under biotic conditions. Due to the observed incomplete biodegradation, the primary elimination should have led to not degraded TPs and/or adsorption on the sludge. The reason for primary elimination could be SUA-adapted bacteria due to exposure to SUAs in Lüneburg's WWTP leading to growth of specific SUA-degraders (García Galán et al., 2012; Ingerslev and Halling-Sørensen, 2000). The occurrence of specific SUA-degraders could also explain the disappearance of structurally similar TPs (OH-SMX, OH-STZ, SMX-, and STZ-isomer) under biotic conditions (Table 4) as it was found that enzymes involved in SUA's degradation are class-specific rather than compound-specific (Ingerslev and Halling-Sørensen, 2000).

Amines NH_2-R (R: specific functional group) of 4 SUAs were formed likely due to δ-cleavage of SUAs or SUA-related TPs, which is in line with literature describing the amines NH_2-R as dead-end TP of SUA's biodegradation (Ricken et al., 2013). Only for SIX, the corresponding NH_2-R (5-amino-3,4-dimethylisoxazole) was not detected in the UV-mixture used for the MRT (SIX-16). This can be explained by SIX-16 being free of PC in contrast to the UV-mixtures of the other PCs.

4. TPs of interest for BbD and future research

Based on LBT and MRT results and considering structural features not contradicting BbD (see method 2.7), five TPs were of interest for BbD due to removal in the MRT and/or possible contribution to luminescence inhibition, requiring a closer look (Table 4, blue highlighted). Among them were SIX-TP-10.3, SMX-TP-9.5, and STZ-TP-3.6, proposed to be hydroxylated (OH-SIX, -SMX, -STZ), due to differences in *m/z* to the PC of +16. Bioactivity wasn't concluded based on the comparison of LBT data and their c-t curves (see Section 3.2) but cannot be excluded because their concentrations in the UV-mixtures may have been too low to inhibit bacterial luminescence (cf. SI, Figs. S3, S5, S6). In addition, single hydroxylation of a SUA is only a slight structural change, and might not reduce the bacteriostatic efficacy of the PC significantly. For example, Majewsky et al. (2014) found *N4*-OH-SMX to be similarly bioactive as SMX. Hydroxylated TPs were assumed, to some extent, to decrease during incubation because of the rule of thumb that

oxygenation favours biodegradability. There is no generic rule for the number and position of a substituent, e.g., regarding hydroxy group(s) at a phenyl ring, but they are known to influence biodegradability depending on the base structure (Boethling et al., 2007). The subtle rule of thumb on oxygenation didn't apply to tested hydroxylated SAA-derivatives (Fig. 2), whose hydroxylation in terms of degree and position didn't result in increased mineralization rate. Therefore, disappearance of OH-SUAs (OH-SIX, -SMX, -STZ) during MRT (Table 4) was likely only due to further transformation and/or adsorption on sludge but not mineralization, excluding them as BbD candidate.

The remaining TPs of interest for BbD were SIX-TP-1.2 (286) and SIX-TP-1.4 (173) (Table 4, blue highlighted). The latter is proposed to be SAA. According to literature data (National Center for Biotechnology Information, 2023b), SAA is predicted not to biodegrade readily in the aquatic environment. This excludes TP-1.4 (173) as BbD candidate. A closer look at the mass spectrum of TP-1.2 (286) revealed that the fragments characteristic for the SAA core structure (m/z = 156, 113, 108) were still present. This indicates an unchanged SAA structure, but slightly modified specific functional group ($\Delta m/z = +18$; additional fragments with m/z = 241, 243), which is an excluding criterion for structures of interest for BbD (see Section 2.7). Thus, no BbD candidate was identified using this effect-driven approach.

When redesigning SUAs by generating derivatives through photo-transformation, improving environmental biodegradability was considered more challenging than retaining bioactivity. The MRT evaluation of UV-mixtures didn't result in the identification of a candidate for better biodegradability, whereas the LBT indicated bioactive TPs and also other studies identified bacteriostatic SUA-TPs (see LBT insights). Identifying improving biodegradability as the limiting factor of the BbD approach may help steer future research on the redesign of SUAs and BbD of antibiotics in general in the following way:

Primarily, future work should aim for better biodegradability by targeted modification of the sulfonamide structure. More specifically, modified SUA would first have to be deactivated by an abiotic transformation step (e.g., photolysis or hydrolysis; compare Leder et al., 2021). Generated inactive TPs should then mineralize readily by subsequent enzymatic processes in order to avoid chemical pollution of the environment. The reason for this stepwise approach is to avoid biodegradation acting as a mechanism of antibiotic resistance (Ricken et al., 2017; Vila-Costa et al., 2017).

Secondly, bacteriostatic efficacy could be tested on molecules that are degradable. Even a modification of the pharmacophore might be possible (cf. findings by Majewsky et al., 2014) as long as features relevant for pharmacological properties, e.g., the target binding (SUA-dihydropteroate synthase complex), remain.

An important lesson learnt from this study for BbD in general is that, although bioactive TP-mixtures and removal rates of single TPs can be determined via LBT and MRT, respectively, it is challenging to find any hints to specific benign TPs based on data from complex mixtures. This is where future work could start. For example, as a variation of the non-targeted design approach, UV-mixtures of interest could be fractionated chromatographically or using solid phase extraction so that selected fractions with significantly reduced TP diversity are screened for bioactivity and (bio)degradability. The targeted design approach (cf. Lorenz et al., 2021) could also be applied by modifying the PC based on expertise and then prioritizing a promising structure for synthesis through computational methods. In the targeted approach, the pharmacophore could also undergo a change (see paragraph above). For this purpose, collaboration between environmental scientists and drug design experts would be helpful.

5. Conclusion

The objective of this study was to broaden the understanding of the redesign approach within BbD using sulfonamide antibiotics (SUAs) as an example. Applied methodology was beneficial in terms of simultaneous generation and subsequent screening of many transformation products of six SUAs which are hardly commercially available. Searching for indications on possibly better biodegradable TPs, limited significance of mixture tests for a single compound was accepted. Based on biodegradability testing (manometric respirometry test, MRT) and screening of antibiotic activity needed for application (luminescence bacteria test, LBT), no photo-TP was indicated as a potentially greener alternative for SUAs.

Gained insights from MRT and LBT support the understanding of SUAs and SUA-TPs and future work on SUA's redesign. It was also found that photo-TPs can be ecotoxic while being not removable under biotic conditions. Even hydroxylated derivatives of SAA, assumed to be better biodegradable and therefore tested as a single compound, were not readily biodegradable. This demonstrates that known rules of thumb can give general guidance and have to be checked on a case-by-case basis. The specific results underline the high stability of the sulfonamide moiety against environmental biodegradation.

CRediT authorship contribution statement

Neele Puhlmann: Writing – original draft, Methodology, Investigation, Conceptualization. **Oliver Olsson:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Klaus Kümmerer:** Writing – review & editing, Supervision, Methodology, Conceptualization, Funding acquisition.

Declaration of competing interest

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

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.171027.

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Anhang der Publikation 4

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How data on transformation products can support the redesign of sulfonamides towards better biodegradability in the environment.

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