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Umweltverhalten und Verbleib von Transformationsprodukten ausgewählter Pflanzenschutzmittel und Biozide in aquatischen Systemen

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Zusammenfassung

Pestizide werden als Pflanzenschutzmittel im landwirtschaftlichen Bereich und als Biozide z. B. in der Industrie, in Haushalten und Kommunen eingesetzt. Bereits auf den behandelten Flächen (z. B. auf Äckern oder Hausfassaden) und in den angrenzenden Gewässern können Pestizide Abbauprozessen durch u. a. Photolyse unterliegen. Diese Prozesse führen zur Entstehung von Transformationsprodukten (TP), deren Berücksichtigung bei der Umweltrisikobewertung für ein umfassendes Risikomanagement von großer Bedeutung ist. Doch gibt es über die in der Umwelt vorkommenden Transformationsprozesse und die dabei entstehenden TP immer noch Wissenslücken. Darüber hinaus sind die Eintragswege von TP, vor allem von Biozid-TP, in die angrenzenden Gewässer zum Teil unbekannt. Da eine Vielzahl von TP mit unterschiedlich starken ökotoxikologischen Effekten bewertet werden muss, besteht ein großer Bedarf an schnellen und umfassenden Methoden, um die stetig wachsende Anzahl an Chemikalien auf dem Markt erfassen zu können. Das Ziel der vorliegenden Arbeit ist daher, das Verhalten und den Verbleib ausgewählter Pestizid-TP in der aquatischen Umwelt zu analysieren. Zu diesem Zweck wurden unterschiedliche Phototransformationsprozesse von Pestiziden sowie der Eintrag aus Fassaden über Regenwasserversickerungsanlagen (RVA) in angrenzenden Gewässern der Stadt Freiburg untersucht. Schlussendlich erfolgte die Identifizierung der ökotoxikologischen Eigenschaften von 45 Pestizid-TP in einem mehrstufigen Ansatz durch die Kombination experimenteller und computerbasierter Methoden.

Inwiefern unterschiedliche Phototransformationsprozesse zu unterschiedlichen TP führen, wurde im ersten Teil der Arbeit durch einen Vergleich der Entstehung von TP durch direkte und indirekte Photolyse der Substanzen Penconazol, Terbutryn und Mecoprop untersucht. Weiterhin wurde der Abbau durch die Bestrahlung mit unterschiedlichen Xenonlampen untersucht. Die Ergebnisse zeigen, dass unterschiedliche Phototransformationsprozesse zu unterschiedlichen TP führen können. So entstanden durch indirekte Photolyse von Mecoprop unterschiedliche TP im Vergleich zu den TP, die durch direkte Photolyse gebildet wurden. Wohingegen kein Unterschied der Entstehung der TP von Penconazol und Terbutryn festgestellt wurde. Der Vergleich von drei verschiedenen Xenonlampen zur Simulation von Photolyse im Labormaßstab zeigte, dass eine genaue Spezifizierung der Lampen hinsichtlich des emittierten Spektralbereich sowie der absoluten Photonenflussdichte notwendig ist. Auf diese Weise können künftig Fehler bezüglich der Geschwindigkeit des direkten Abbaus insbesondere von schwach absorbierenden Pestiziden vermieden werden.

Im zweiten Teil der Arbeit wurde der Eintrag von Bioziden, die in Fassadenanstrichen Anwendung finden, und deren TP über Regenwasserversickerungsanlagen in das Grundwasser untersucht. Dabei wurden qualitative und quantitative Target-Screening-Methoden zum Nachweis und zur Quantifizierung bekannter und unbekannter TP der Biozide Diuron, Terbutryn und Octhilinon (OIT) in der aquatischen Umwelt mittels Flüssigkeitschromatographie mit gekoppeltem Massenspektrometer (LC-MS) kombiniert. Die Untersuchung zeigt, dass der gewählte methodische Ansatz einen wichtigen Beitrag zur Identifikation von Eintragspfaden in Gewässer leisten kann. Auf diese Weise wurden erstmalig dezentrale Versickerungssysteme als Eintragspfad für biozide Wirkstoffe und insbesondere deren TP ins Grundwasser identifiziert. Weiterhin wurden Fassaden als Quelle von Biozid-TP durch die Ausgangssubstanz Diuron und des TP-219 anhand eines Beregnungsexperiments einer 14-jährigen Hausfassade festgestellt..

Die ökotoxikologischen Eigenschaften von 45 Pestizid-TP wurden im dritten Teil dieser Arbeit in einem mehrstufigen Ansatz untersucht. Dafür erfolgten auf der ersten Stufe eine Literaturauswertung und die Anwendung computerbasierter Methoden, um die bakterielle Ökotoxizität und Genotoxizität zu ermitteln. Im Fall von toxischen Hinweisen wurden photolytische Mischungen durch Photolyse der Ausgangssubstanzen hergestellt. Diese wurden auf der zweiten Stufe in einem Leuchtbakterientest hinsichtlich der akuten und chronischen Ökotoxizität und der Wachstumshemmung untersucht. Die Genotoxizität wurde in einem Umu-Test ermittelt. Bestätigten sich die positiven Befunde, erfolgten auf der dritten Stufe Einzeluntersuchungen der TP durch die zuvor genannten Tests. Die Ergebnisse legen nahe, dass mit Hilfe des mehrstufigen Verfahrens eine schnelle und umfassende Ersteinschätzung der Ökotoxizität von Pestizid-TP erfolgen kann. Dabei bietet vor allem die Kombination von computerbasierten Methoden und experimentellen Tests die Möglichkeit einer Vielzahl von Substanzen gerecht zu werden und auch schwer synthetisierbare und analysierbare Substanzen einzubeziehen. So konnten mit Hilfe des Ansatzes 96 % der TP bewertet werden.

Insgesamt zeigte sich, dass die Berücksichtigung von TP im Rahmen von Gewässerüberwachung und Risikobewertung eine genauere Abschätzung der Risiken durch Schadstoffe ermöglicht. Die in dieser Dissertation entwickelte Vorgehensweise, bei der TP zunächst im Labor erzeugt und bewertet und anschließend in aquatischen Systemen gezielt analysiert werden, kann einen wichtigen Beitrag zur Regulatorik des Einsatzes und der Zulassung von Pestiziden leisten. Die Arbeit liefert wichtige Erkenntnisse und Methodenvorschläge um, im Sinne der Ziele einer nachhaltigen Entwicklung der Vereinten Nationen, einer Verschmutzung der Gewässer in qualitativer und quantitativer Hinsicht vorzubeugen.

Abstract

Pesticides are used as plant protection agents in agricultural areas and as biocides e.g. in industry, households, and communities. They were transformed already at the applied areas (e.g. on facades) or in the connected aquatic environment where they were dissipated in by stormwater events. By these processes, the formation of transformation products (TPs) does occur. The consideration of these TPs is of great importance for the implementation of a comprehensive risk management in environmental risk assessments. However, there is still lack of clarity regarding the transformation processes and the resulting TPs. Moreover, the entry of TPs of pesticides into the aquatic environment especially of biocides was marginally researched until now. As TPs could have various effects on aquatic organisms and there is a great number of TPs that need to be assessed, the ecotoxicological risk of TPs is hitherto still little understood and there is a need for fast and comprehensive toxic screening methods to handle the great variety of TPs deriving from the increasing number of chemicals coming to market.

Hence, the aim of the doctoral thesis was to determine the environmental behavior and fate of selected pesticide-TPs in the aquatic environment. For this purpose direct and indirect phototransformation processes of pesticides were analyzed. Additionally, the entry of biocides and their TPs from facades into the aquatic environment via stormwater infiltration systems of the German city Freiburg was analyzed. Finally, ecotoxicological properties of 45 TPs were examined by a combination of experimental and in silico tools in a tiered approach.

In the first part of the doctoral thesis, the influence on the formation of TP by direct and indirect phototransformation processes of the pesticides Penconazole, Terbutryn, and Mecoprop was analyzed. Moreover, the formation of TPs was examined by the use of different irradiation sources. Results show that different transformation processes could lead to different types of TPs. As for Mecoprop indirect photolysis, two TPs were formed that differ from the TPs formed by direct photolysis. In contrast, the formation of TPs of Penconazole and Terbutryn was similar in both processes. The comparison of three different irradiation sources showed that it is of great importance to define and specify irradiation sources as precisely as possible regarding the spectral range emitted and the absolute photon flux used to avoid the determination of false rate constants of direct photolysis. This is especially important for weakly sunlight-absorbing pesticides.

In the second part of the doctoral thesis, the entry of biocides that were applied on facades and their TPs via urban stormwater infiltration systems into groundwater was examined. By means of a combination of different screening methods to detect and quantify TPs of the biocides Diuron, Terbutryn, and OIT, it could be shown that this methodological approach contributes to the identification of important entry paths into aquatic environment and to the clarification about the behaviour of known and unknown TPs in the respective media. Thus, stormwater infiltration systems were identified as pathway of biocides and their TPs into groundwater for the first time. The origin from facades was verified for Diuron and its TP-219 by a sprinkling experiment of a 14-year old facade.

The combination of experimental and computer based in silico prediction analysis of ecotoxicological properties of 45 pesticide TPs was analyzed in a tiered approach in the third part of the doctoral thesis. At tier I, literature review and in silico methods were used to determine the environmental bacterial toxicity and the genotoxicity of TPs. In case of indications to be toxic, photolytic mixtures containing parent compound and TPs were used for the consecutive toxicity test. Therefore, Microtox assay for the parent compounds and the photolytic mixture was conducted to determine the acute and chronic toxicity and the growth inhibition of luminescent bacteria at tier II. Genotoxicity tests were conducted to determine primary DNA damage. At tier III, single substance standards were used to conduct toxicity tests in case of toxic indication by previous tiers and availability of analytical standard. It turned out that the tiered approach was a suitable tool for an initial assessment of the ecotoxicological properties of TPs. The combination of computer based methods and experiments can be advantageous for the huge amount of chemical and especially for substances with difficulties regarding synthesis and analysis. By this approach, 96 % of TPs could be assessed.

Overall, this thesis shows that the consideration of TPs led to an increased diversity of substances that need to be assessed within water monitoring and risk assessment. The application of different methods to generate, assess, and detect TPs in aquatic systems was provided and thus makes an important contribution to the monitoring and regulation of pesticides. This thesis offers important knowledge and methods proposal to prevent the pollution of the aquatic environment in terms of the goals of a sustainable development of the United Nations.

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Abkürzungsverzeichnis

LBT	Leuchtbakterientest (engl. *Luminescent Bacteria Test*)
LC-HR-MS	Flüssigkeitschromatographie mit gekoppeltem hochauflösenden Massenspektrometer
LC-MS/MS	Flüssigkeitschromatographie mit gekoppeltem Tandemmassenspektrometer
OECD	Organisation für wirtschaftliche Zusammenarbeit und Entwicklung (engl. *Organisation for Economic Co-operation and Development*)
OIT	Octhilinon
PSM	Pflanzenschutzmittel
QSAR	Quantitative Struktur-Wirkungs-Beziehung (engl. *Quantitative Structure-Acitivity Relationship*)
RVA	Regenwasserversickerungsanlage
SPE	Festphasenextraktion (engl. *Solid Phase Extraction*)
TEG	Teileinzugsgebiet
ThOD	Theoretischer Sauerstoffbedarf (engl. *Theoretical Oxygen Demand*)
TP	Transformationsprodukt(e)

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1 Einleitung

Als eines der 17 Ziele der Vereinten Nationen stellt die Gewährleistung von sauberem Wasser eine der Grundvoraussetzungen für eine nachhaltige Entwicklung dar. Dabei soll u. a. durch die Minimierung der Chemikalienfreisetzung die Wasserqualität bis zum Jahre 2030 verbessert werden (Vereinte Nationen 2020). Um diese Ziele umzusetzen, bedarf es einer ganzheitlichen Analyse und Beurteilung der Gewässerbelastung, sowohl bezüglich der in die Gewässer gelangenden Ausgangssubstanzen als auch der durch abiotische und biotische Abbauprozesse entstehenden TP (Schwarzenbach et al. 2006; Somasundaram und Coats 1991). Letztere stellen eine besondere Herausforderung dar, da die physikochemischen und toxischen Eigenschaften in vielen Fällen stark von der Ausgangssubstanz abweichen und oftmals nur unzureichend untersucht sind (Fenner et al. 2013).

Nicht zuletzt durch hochauflösende Massenspektrometrie gelingt es zunehmend, eine größere Anzahl an TP aufzuklären und diese teilweise im niedrigen Konzentrationsbereich von einigen ng L^{-1} in Gewässern nachzuweisen (Kümmerer 2010). Dennoch gibt es nach wie vor große Wissenslücken bezüglich des Abbaus von organischen Spurenstoffen sowie des Umweltverhaltens und Verbleibs von TP in aquatischen Systemen. Dies lässt sich vor allem mit der Vielzahl der auf dem Markt verfügbaren Stoffe und der sich daraus ergebenden Vielzahl zu berücksichtigender TP begründen, die eine Erfassung und Bewertung aller Substanzen zu einer großen, kaum zu bewältigenden Aufgabe der Umweltchemie und Ökotoxikologie machen.

Zu der Substanzgruppe der Pestizide zählen im europäischen Recht sowohl Pflanzenschutzmittel (PSM) als auch Biozide (Europäische Union 2009a). Der maßgebliche Unterschied liegt in den jeweiligen Schutzgütern[1], die verwendeten Substanzen können jedoch identisch sein (Europäische Union 2009b, 2012). Es gibt Wirkstoffe, die aufgrund ihrer toxischen Wirkung in einigen Ländern der Europäischen Union verboten wurden, z. B. die Herbizide Diuron und Terbutryn (Europäische Kommission 2020). Aufgrund von Übergangsregelungen für Altwirkstoffe können diese Substanzen weiterhin als biozide Wirkstoffe in Biozidprodukten eingesetzt werden (Europäische Union 2014). In Kombination mit der Betrachtung von TP stellen Pestizide ein relevantes Forschungsfeld dar.

Ein TP gilt dann als ökotoxisch relevant, wenn umfassende Nachweise für das Risiko vorliegen, wobei es ein höheres oder vergleichbares Risiko gegenüber Organismen aufweisen oder

[1] PSM werden zum Schutz von Pflanzen vor Schadorganismen, wie Unkräuter, Pilze und Insekten hauptsächlich im landwirtschaftlichen Bereich eingesetzt. Biozide hingegen werden zum Schutz des Menschen und seiner Produkten vor Schadorganismen vorwiegend im urbanen (Haushalte, öffentliche Einrichtungen, etc.) und im industriellen Bereich eingesetzt.

über bestimmte toxische Eigenschaften verfügen muss (Europäische Union 2009b; Europäische Chemikalienagentur 2018). Da oftmals weder das Risiko bekannt ist, noch Grenzwerte vorliegen, ist das übergreifende Ziel der vorliegenden Dissertation die Untersuchung der Entstehung und des Verbleibs von TP von ausgewählten Pestiziden sowie eine initiale Bewertung ihrer Stoffeigenschaften. Dazu wurden sowohl massenspektrometrische als auch ökotoxikologische Analysen in Kombination verwendet. Damit wurden relevante Transformationsprozesse und -produkte identifiziert sowie Kenntnislücken über bisher unbekannte Eintragspfade in die aquatische Umwelt geschlossen. Auf diese Weise leistet die Arbeit einen wissenschaftlichen Beitrag zur Erhaltung der Wasserqualität im Sinne des sechsten Ziels für eine nachhaltige Entwicklung der Vereinten Nationen (Vereinte Nationen 2020).

1.1 Strahlungsquellen und Abbauwege

Die Aufklärung der Struktur von TP hängt vom Wissensstand über mögliche Abbauprozesse sowie von den vorhandenen experimentellen bzw. analytischen Möglichkeiten ab, wie z. B. eine realitätsgetreue Nachstellung im Labormaßstab bzw. eine hochauflösende Analytik. In Zulassungsbescheiden von Pestiziden finden häufig jene TP Berücksichtigung, die durch metabolische Prozesse entstehen. Dass TP abiotischer Prozesse, wie die photochemische Umwandlung, weniger Achtung erfahren, liegt u. a. an der Tatsache, dass die direkte Photolyse für Pestizide in vielen Zulassungsbescheiden als nicht relevanter Abbaupfad deklariert wird, da die Absorptionsspektren vieler Pestizide außerhalb des Emissionsspektrums terrestrischer Sonnenstrahlung liegen (Fenner et al. 2013). Daher ist es gesetzlich nicht erforderlich, diesen Abbaupfad zu analysieren. Dennoch können auch indirekte photolytische Prozesse eine wesentliche Rolle beim Abbau und der Transformation von Pestiziden spielen, die durch gelöste organische Substanzen oder Hydroxylradikale initiiert werden (Remucal 2014). Letztere werden beispielsweise durch die photochemische Anregung von Nitrat, Karbonat oder Huminsäuren gebildet, welche auf natürliche Weise in Gewässern vorkommen. Die Kinetik dieser Abbauprozesse ist für viele bekannte TP ausreichend untersucht (Bachman und Patterson 1999; Frimmel 1994; Kamiya und Katsura 1998; Sakkas et al. 2002; Zepp et al. 1985; Brezonik und Fulkerson-Brekken 1998; Fulkerson Brekken und Brezonik 1998; Haag und Hoigné 1985; Zepp 1987), die Entstehung von TP durch indirekte Photolyse im Vergleich zur direkten hingegen eher weniger (Khan und Gamble 1983; Torrents et al. 1997; Meunier und Boule 2000). Aus diesem Grund liegt der Fokus in diesem Teil der Arbeit auf der indirekten Photolyse und den dabei entstehenden TP.

Weiterhin können die Bedingungen der photolytischen Experimente starke Auswirkungen darauf haben, welche TP identifiziert werden. Die zur Simulation von Photolyse oftmals verwendete Strahlung der Xenonbogenlampe ist dem Sonnenspektrum sehr ähnlich, da sie ein kontinuierliches Emissionsspektrum im Wellenlängenbereich zwischen 200 und 1000 nm erzeugt (Yager und Yue 1988). Eine größere Ähnlichkeit mit dem auf der Erdoberfläche auftreffenden Emissionsspektrum der Sonne kann jedoch durch Filter mit geeigneter Absorptionskante erreicht werden (Winer et al. 1979). Die Absorptionskante wird bei der Simulation des photolytischen Abbaus von Pestiziden oftmals nicht genau definiert (Dimou et al. 2005; Sakkas et al. 2005). Dies ist jedoch vor allem für Substanzen relevant, die ein sehr schwaches Absorptionsspektrum im Bereich der terrestrischen Solarstrahlung aufweisen.

Neben dem Einfluss der indirekten Photolyse, am Beispiel von Nitrat auf drei ausgewählte Pestizide, war daher eine weitere Fragestellung dieser Arbeit die Untersuchung des Einflusses unterschiedlicher Xenonlampen mit unterschiedlichen optischen Filtern auf den Abbau von Pestiziden und die entstehenden TP.

1.2 Eintrag von Bioziden und Transformationsprodukten in die aquatische Umwelt

Da, wie bereits beschrieben, die Schutzgüter von Bioziden und PSM unterschiedlich sind, unterscheiden sich entsprechend ihre Anwendungsgebiete und die daraus folgenden Eintragspfade der Wirkstoffe und TP in Gewässer. Sowohl an dem behandelten Schutzgut (z. B. auf Pflanzen und Fassaden) (Fenner et al. 2013; Bollmann et al. 2017b; Minelgaite et al. 2015) als auch in angrenzenden Gewässern (Minelgaite et al. 2015) können Pestizide den beschriebenen Abbauprozessen unterliegen. Dabei können TP entstehen, die in Abhängigkeit ihrer physikochemischen Eigenschaften in die aquatische Umwelt eingetragen und verteilt werden (Abbildung 1).

Der bereits gut untersuchte Umwelteintrag von PSM über die Auswaschung von landwirtschaftlichen Flächen erfolgt entweder diffus durch Regenereignisse in Oberflächengewässer oder punktuell über das Abwasser des Landwirtschaftsbetriebes mit zwischengeschalteter Klärung in der Kläranlage und anschließendem Eintrag in Oberflächengewässer (Gavrilescu 2005). Die Eintragsquellen von Bioziden sind diverser, da die Anwendungsgebiete weitaus vielfältiger sind. So können Biozide beispielsweise über das häusliche Abwasser (Wieck et al. 2018) in die Kläranlage und von dort in Oberflächengewässer gelangen (Chen et al. 2012).

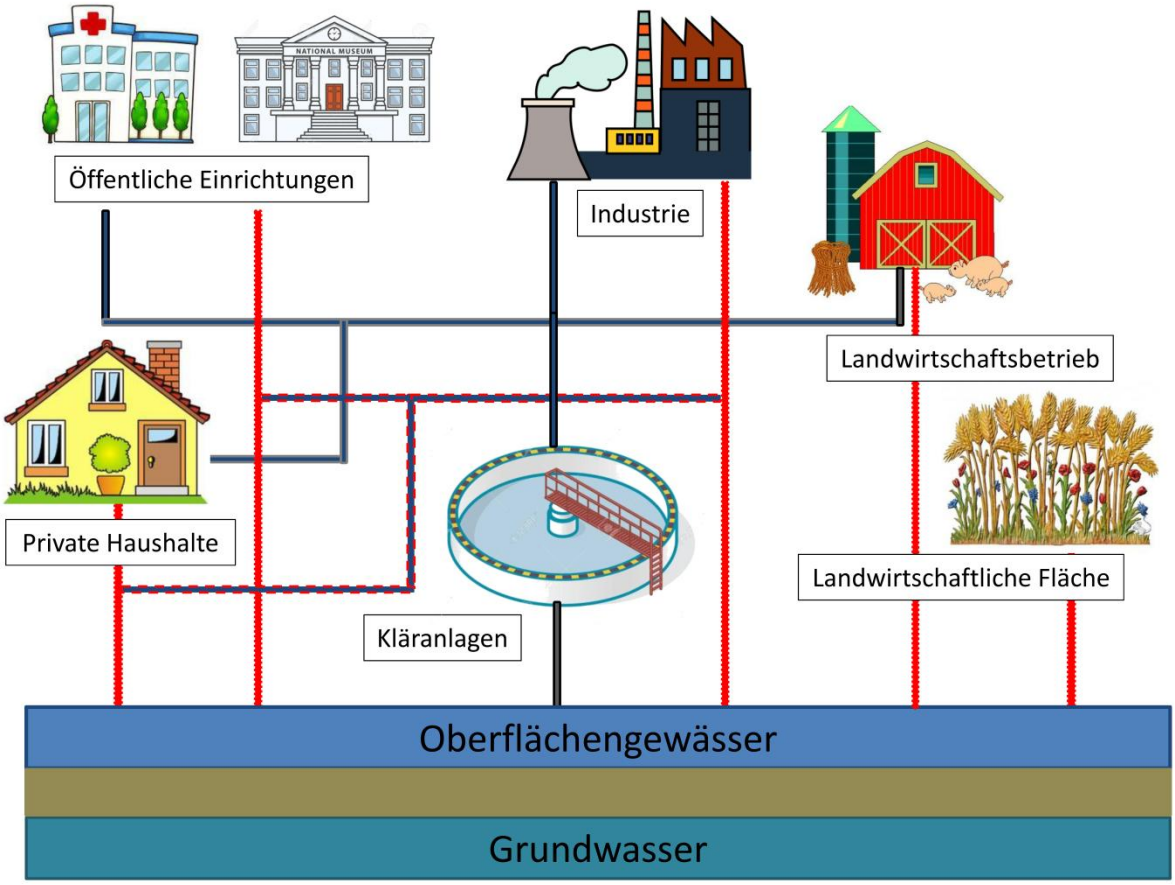


Abbildung 1: Schematische Darstellung des Eintrags von PSM und Bioziden in Gewässer in Anlehnung an (Umweltbundesamt 2020). Die blauen Linien zeigen den Eintrag in Oberflächengewässer über vorherige Klärung in der Kläranlage. Die roten Linien zeigen den diffusen Eintrag, der durch Regenwasser in die angrenzenden Oberflächengewässer eingebracht wird. Die blau-roten Linien zeigen Mischwassersysteme an.

Ein weiterer Eintragspfad in Gewässer, welcher in der vorliegenden Arbeit betrachtet wird, ist die Auswaschung aus mit Bioziden behandelten Materialien durch Regenereignisse. Hierbei spielen dementsprechend die Biozide eine Rolle, die im Außenbereich Anwendung finden, wie z. B. Biozide, die in speziellen Farben und Lacken für Fassadenanstriche eingesetzt werden. Nach der Auswaschung aus Fassaden werden die im Regenwasser enthaltenen Biozide (Burkhardt et al. 2011; Gasperi et al. 2014) entweder diffus an der Hauswand versickert (Bollmann et al. 2017a) oder über gepflasterte Oberflächen in Trenn- oder Mischkanalisationen geleitet. In Mischkanalisationen wird das belastete Regenwasser in die Kläranlage und anschließend ins Oberflächengewässer geleitet, wohingegen in Trennkanalisationen das Regenwasser direkt in Oberflächengewässer oder in Regenwasserversickerungsanlagen (RVA) abgeleitet wird (Bollmann et al. 2014a; Bollmann et al. 2014b; Launay et al. 2016). In beiden Varianten der Trennkanalisation können Biozide in den Wasserkreislauf gelangen ohne zuvor eine Abwasserbehandlung durchlaufen zu haben.

Inwiefern Biozide und TP über die RVA in das angrenzende Grundwasser eingetragen werden, wurde bisher wenig untersucht. Aus diesem Grund anaylsiert die vorliegende Arbeit den

Eintrag der drei ausgewählten Biozide Diuron, Terbutryn und OIT und deren TP über RVA in das angrenzende Grundwasser exemplarisch anhand eines Studiengebiets in der Stadt Freiburg.

1.3 Relevanz von Transformationsprodukten

Im Vergleich zu ihren Ausgangssubstanzen verfügen TP über veränderte Molekülstrukturen und deshalb auch über andere physikochemische und umweltrelevante Eigenschaften. Dies führt dazu, dass TP im Rahmen der Umweltrisikobewertung nicht außer Acht gelassen werden dürfen. So wurde in der Vergangenheit oftmals festgestellt, dass TP zum einen langlebiger als ihre Ausgangssubstanz sein können und damit über längere Zeiträume in den jeweiligen Umweltkompartimenten persistieren (Fenner et al. 2013). Zum anderen sind viele der TP mobiler als die Ausgangssubstanzen und reichern sich entsprechend überwiegend in der aquatischen Umwelt an (Hu et al. 2009). Letztlich wurde zudem durch vorangegangene Studien gezeigt, dass TP eine erhöhte toxische Relevanz gegenüber Umweltorganismen aufweisen können (Fenner et al. 2002; Neuwoehner et al. 2010; Sinclair und Boxall 2009; Belfroid et al. 1998; Bustos et al. 2019; Gutowski et al. 2015b).

Da TP oftmals unbekannte chemische Stoffe sind, für die experimentelle Untersuchungen aus verschiedenen Gründen nicht zur Verfügung stehen, ist es folglich eine große Herausforderung, die Vielzahl an Substanzen zu bewerten (Kümmerer et al., 2019). Unter Zuhilfenahme von computerbasierten Methoden, wie z. B. Quantitative Struktur-Wirkungs-Beziehung[2] (QSAR), kann eine erste, orientierende Bewertung vorgenommen werden, was erheblich zeit- und ressourceneffizienter ist als ein rein experimenteller Ansatz.

Ein weiterer Teil der vorliegenden Arbeit war daher die kombinierte Anwendung von experimentellen und computerbasierten Methoden zur Vorhersage der umweltrelevanten Eigenschaften von 45 bekannten Pestizid-TP in einem mehrstufigen Screening-Ansatz.

[2] Oft auch im weiteren Sinne von Struktureigenschaftsbeziehungen verwendet, also auch physikochemische Eigenschaften umfassend.

2 Ziele und Übersicht des Rahmenpapiers

Das sich aus den Wissenslücken ableitende, übergeordnete Ziel der vorliegenden Dissertationsschrift ist die Analyse des Umweltverhaltens und des Verbleibs von TP ausgewählter PSM und Biozide in aquatischen Systemen. Die sich daraus ableitenden Unterziele der Arbeit lauten:

i) Erfassung möglicher Effekte von indirekter Photolyse auf die Bildung von TP in aquatischen Systemen unter Berücksichtigung geeigneter Strahlungsquellen zur Simulation im Labormaßstab (Transformationsprozesse).
ii) Generierung neuester Erkenntnisse zum Umwelteintrag von Bioziden und TP aus Fassadenanstrichen in das urbane Oberflächengewässer und das Grundwasser über RVA durch Starkregenereignisse (Umwelteintrag).
iii) Entwicklung eines Untersuchungsschemas zur Toxizitätsbewertung von Pestizid-TP durch kombinierte Anwendung von computerbasierten und (bio-) analytischen Methoden (Eigenschaften).

Die sich daraus ableitenden Arbeitsschwerpunkte sind in Klammern genannt. Die Arbeit liefert dementsprechend wichtige Erkenntnisse bezüglich der ablaufenden Transformationsprozesse, dem Eintrag von TP in die Umwelt und ihrer (ökotoxikologischen) Eigenschaften.

Die Ergebnisse der Analysen aus den drei Arbeitsschwerpunkten wurden in drei Artikeln in internationalen wissenschaftlichen Zeitschriften mit Peer-Review-Verfahren veröffentlicht (Tabelle 1).

Tabelle 1: Übersicht über die Arbeitsschwerpunkte (AP) und die daraus entstandenen veröffentlichten Artikel.

	AP	**Titel der Veröffentlichung**
Artikel 1	Transformations-prozesse	Hensen, B.; Olsson, O.; Kümmerer, K. (2019): The role of irradiation source setups and indirect phototransformation: Kinetic aspects and the formation of transformation products of weakly sunlight-absorbing pesticides. Science of the total Environment, 695: 133808.
Artikel 2	Umwelteintrag	Hensen, B.; Lange, J.; Jackisch, N.; Zieger, F.; Olsson, O.; Kümmerer, K. (2018): Entry of biocides and their transformation products into groundwater via urban storm-water infiltration systems. Water Research 144: 413-423.
Artikel 3	Eigenschaften	Hensen, B.; Olsson, O.; Kümmerer, K. (2020): A strategy for an initial assessment of the ecotoxicological effects of transformation products of pesticides in aquatic systems following a tiered approach. Environment International, 137: 105533.

Weitere Ergebnisse der Promotion sind in die Ergebnisse des vom Bundesministerium für Bildung und Forschung geförderte Projekt *MUTReWa* (Maßnahmen für einen nachhaltigeren Umgang mit Pestiziden und deren Transformationsprodukten im Regionalen Wassermanagement) und weitere projektbezogene Publikationen eingeflossen (siehe Publikationsverzeichnis).

3 Methoden

Das methodische Vorgehen ist in Abbildung 2 skizziert und umfasste im Wesentlichen:

i) Spezifizierung dreier Strahlungsquellen im Hinblick auf deren Eignung für die Simulation der direkten Photolyse durch terrestrische Solarstrahlung sowie die Untersuchung der photochemischen Transformation von drei Pestiziden durch direkte und indirekte Photolyse und Aufklärung der TP mittels LC-HR-MS (Artikel 1).

ii) Untersuchung des photochemischen Abbaus von Bioziden und Identifizierung der dabei entstehenden TP mittels LC-HR-MS. Entwicklung einer quantitativen Methode zur Bestimmung von bekannten TP und einer qualitativen Methode zum Nachweis von unbekannten TP, für die kein analytischer Standard zur Verfügung steht. Messung von 110 Regen- und Grundwasserproben aus dem Stadtteil Vauban in Freiburg mit Hilfe dieser zwei Methoden (Artikel 2).

iii) Ermittlung von umweltrelevanten Eigenschaften mittels experimentell-analytischer Methoden in Kombination mit computerbasierten Methoden zur Vorhersage von quantitativen Struktur-Eigenschafts-Beziehungen (QSAR) von 45 ausgewählten TP sechs verschiedener PSM und Bioziden (Artikel 3).

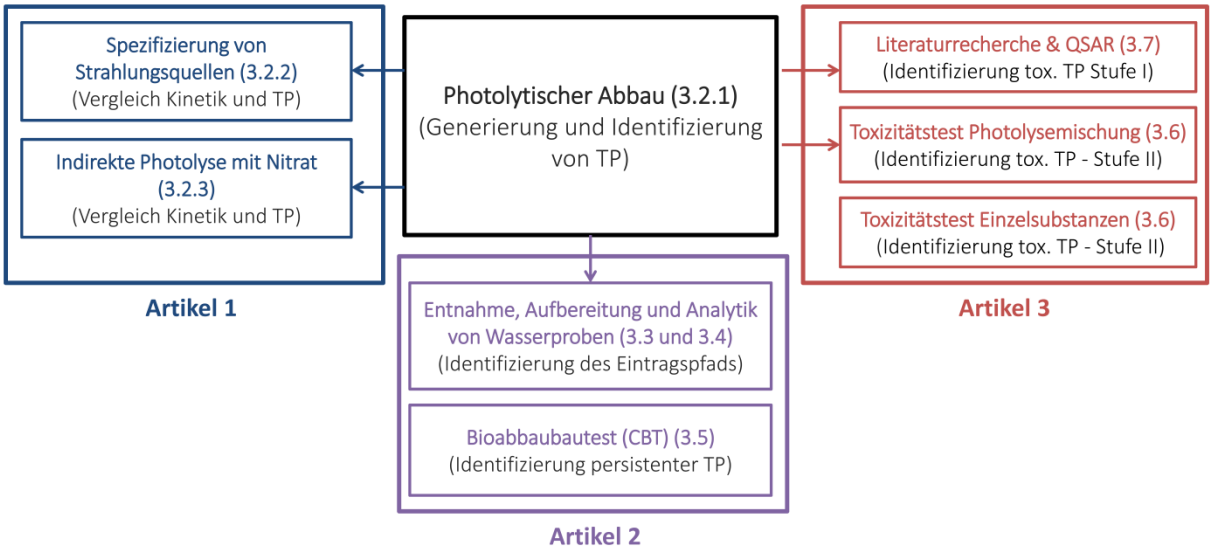


Abbildung 2: Übersicht über das methodische Vorgehen der Arbeit. Die Photolyse der Pestizide und die anschließende Identifikation der dabei entstehenden TP ist der methodische Grundbaustein. Dieser war Bestandteil aller davon abzweigenden experimentellen Schritte, welche sich wiederum in Artikel 1-3 kategorisieren lassen. Das genaue Vorgehen ist in den jeweils in Klammern genannten Unterkapiteln zu finden.

3.1 Stoffauswahl

Insgesamt wurden acht verschiedene Pestizide ausgewählt. Die Auswahl der Substanzen erfolgte aufgrund der unterschiedlichen Grundstruktur und demnach unterschiedlichen photochemischen (spektralen) sowie ökotoxikologischen Eigenschaften. Weiterhin unterscheiden sich die Substanzen bezüglich ihrer Anwendungsbereiche und damit in ihren Eintragswegen in die Umwelt. Vier der ausgewählten Substanzen werden in Deutschland als PSM und drei als Biozide eingesetzt. Die Substanz Mecoprop wird sowohl als PSM als auch als Durchwurzelungshemmer in Bitumenbahnen[3] eingesetzt. Tabelle 2 zeigt alle analysierten Substanzen mit ihren Eigenschaften und Anwendungsgebieten. Die letzten vier Spalten zeigen, in welcher Veröffentlichung die jeweiligen Ergebnisse Eingang gefunden haben.

Tabelle 2: Übersicht über das untersuchte Substanzspektrum der vorliegenden Arbeit. Aufgelistet ist die Verwendungsart (als Pflanzenschutzmittel (PSM) oder Biozid), deren Wirkungsweise sowie das jeweilige Anwendungsgebiet. Außerdem ist angegeben, in welchem Artikel (1-3) die Substanz in die Untersuchung Eingang gefunden hat. P steht für weitere projektbezogene Veröffentlichungen (siehe Publikationsverzeichnis).

Substanz	Verwendung	Wirkung	Anwendungsgebiet	Artikel			P
				1	2	3	
Boscalid	PSM	Fungizid	Weinanbau			X	
Penconazol	PSM	Fungizid	Weinanbau	X		X	
Metazachlor	PSM	Herbizid	Raps und Mais				X
Flufenacet	PSM	Herbizid	Raps und Mais				X
Diuron	Biozid	Herbizid	Fassadenanstriche		X	X	
Terbutryn	Biozid	Herbizid	Fassadenanstriche	X	X	X	
OIT	Biozid	Fungizid	Fassadenanstriche		X	X	
Mecoprop	PSM/ Durchwurzelungshemmer	Herbizid	Sommergetreide; Bitumenbahnen	X		X	

3.2 Photolytischer Abbau von Pestiziden

3.2.1 Photolyse zur Identifizierung von Transformationsprodukten und Herstellung von Photolysemischungen zur weiteren Untersuchung

Die Photolyse wurde mittels Xenonlampe (TXE 150, UV consulting Peschl, Germany) in Reinstwasser über einen Zeitraum von 8 Stunden für alle Testsubstanzen durchgeführt. Dabei wurde stündlich eine Probe genommen und zur Analyse eingefroren. Zusätzlich wurden Proben einer Lösung unter Ausschluss von Licht (Dunkelproben) für alle Substanzen genommen, um einen möglichen Reaktionsweg über Hydrolyse auszuschließen.

[3] Auch wenn Mecoprop aufgrund seiner Auslobung als Materialschutzmittel in vielen Veröffentlichungen als Biozid benannt wird, handelt es sich hier um keinen Wirkstoff gemäß Biozid-Verordnung (Europäische Union 2012, sondern wurde im Rahmen der Europäischen Chemikalienverordnung zur Registrierung, Bewertung, Zulassung und Beschränkung chemischer Stoffe als Industriechemikalie registriert.

3.2.2 Spezifizierung von Strahlungsquellen zur Simulation von Photolyse

In einem weiteren Schritt wurden zwei weitere Strahlungsquellen verwendet und bezüglich ihres Einflusses auf den Abbau der Substanzen Penconazol, Terbutryn und Mecoprop (siehe Tabelle 2) sowie der Bildung von TP untersucht. Die Spezifizierung und der Vergleich der Strahlungsquellen wurden demensprechend anhand dreier verschiedener Xenonbogenlampen in unterschiedlichen Testsets durchgeführt. Die Testsets variierten hinsichtlich der verwendeten optischen Filter und der Leistung der Lampe. Als Erstes wurde ein Batch-Reaktor mit unfiltriertem Xenonlicht verwendet (siehe 3.2.1). Als zweiter Reaktor wurde der Suntest® CPS+ benutzt, welcher mit einem Tageslicht-Filter ausgestattet ist. Das Xenonlicht in der als Drittes verwendeten optischen Bank wurde durch einen Borosilikatfilter geleitet. Die genaue Beschreibung der Lampen und deren Eigenschaften sind in Artikel 2 zu finden.

3.2.3 Indirekte Photolyse mit Nitrat

Die indirekte Photolyse wurde unter Zugabe von Nitrat (c = 100 mg L^{-1}) als Quelle von Hydroxylradikalen durchgeführt (Palm et al. 2003). In allen weiteren Testparametern erfolgte die Durchführung identisch zu der Untersuchung der direkten Photolyse. Weitere Details sind in Artikel 2 zu finden.

3.3 Entnahme und Aufbereitung der Umweltproben

Die untersuchte RVA befindet sich im Süden der Stadt Freiburg und weist als zentrales Element Kaskaden aus begrünten Mulden-Rigolen-Systemen auf, die das abfließende Regenwasser zuerst oberirdisch in den Mulden sammeln und anschließend über eine Boden- oder Kiesschicht in eine unterliegende Rigole leiten. Aufgrund der Speicherräume kann eine große Menge Regenwasser auf kleiner Fläche gesammelt und abgeleitet werden. Es wurden zwei Teileinzugsgebiete (TEG) beprobt, mit jeweils einer separat angeschlossenen Mulde. An drei Niederschlagsereignissen wurde der Niederschlagsabfluss aus den TEG am Muldenzulauf und das versickerte Wasser im Rigolenkörper beprobt. Weiterhin wurden Proben aus insgesamt sieben Grundwassermessstellen entnommen, drei im Anstrom und vier im Abstrom der Versickerungsanlage. Diese wurden an Niederschlagsereignissen genommen, weshalb der Grundwasserabstrom durch das versickerte Wasser aus dem Mulden-Rigolen-System gespeist wird. Außerdem wurde beispielhaft ein Gebäude des TEG 1 künstlich mit Wasser benetzt und das abfließende Wasser analysiert. Weitere Details zur Probenahme finden sich in Artikel 1.

Das untersuchte Substanzspektrum umfasste die bioziden Wirkstoffe Diuron, Terbutryn und OIT sowie die korrespondierenden TP Diuron-Desmethyl (TP-219), Hydroxy-Terbutryn (TP-

212), Terbutryn-Desethyl (TP-214) und Terbumeton (TP-226). Für die genannten Substanzen lagen analytische Standards vor, weshalb die Konzentration über ein quantitatives Target-Screening ermittelt werden konnte. Neben diesen TP wurden die Proben auf weitere TP, die durch die Photolysetests ermittelt wurden, untersucht (siehe Tabelle 3). Da für diese Substanzen jedoch kein analytischer Standard vorhanden war, erfolgte für diese TP lediglich ein qualitativer Nachweis (siehe 3.4.2). Die Aufbereitung der Umweltproben erfolgte mittels Festphasenextraktionsverfahren (SPE). Das genaue Vorgehen ist in Artikel 1 zu finden.

3.4 Analytik

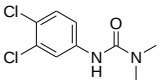
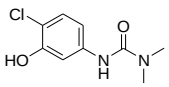
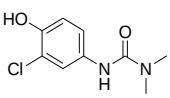
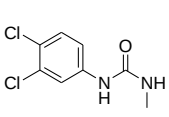
3.4.1 Strukturaufklärung

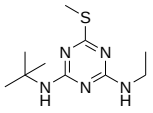
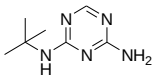
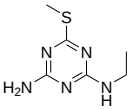
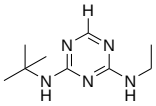
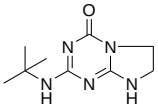
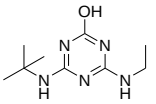
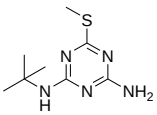
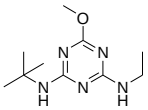
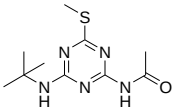
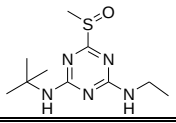
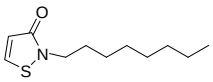
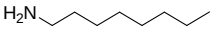
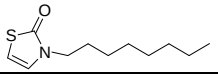
Die Struktur der gebildeten TP wurde mit hochauflösender Massenspektrometrie identifiziert und mit der Literatur abgeglichen. Es kamen LC-Systeme mit gekoppelter Iontrap (Bruker Daltonic Esquire 6000+, Karlsruhe, Germany, niedrig auflösende MS) und Oribtrap (Q Exactive™ HF Hybrid Quadrupol-Orbitrap, Thermo Fisher Scientific, Darmstadt, Germany, hoch auflösende MS) zum Einsatz. Die Details sind in Tabelle 3 dargestellt.

3.4.2 Konzentrationsbestimmung

Die Quantifizierung der Zielsubstanzen erfolgte mit der für diesen Zweck besser geeigneten Triple Quadrupol LC-MS/MS (Agilent Technologies, 1200 Infinity LC-System and 6430 Triple Quad, Waldbronn, Germany).

Tabelle 3: Untersuchtes Substanzspektrum der Beprobung der RVA und des Grundwassers in der Stadt Freiburg. Summen- und Strukturformel der aufgeklärten TP sowie Fragmentorspannung (FV) und Kollisionsenergie (CE) des ersten und zweiten Massenübergangs. AS (Analytischer Standard) gibt an, ob für die Substanz ein Standard vorhanden ist. Die Strukturen der Terbutryn-TP TP-186, TP-210 und TP-156 sind lediglich Vorschläge, da sie bisher nicht durch einen analytischen Standard oder durch Literatur verifiziert wurden.

Substanz	Bekannte Synonyme	Summenformel	Strukturformel	Übergang 1 (FV/CE)	Übergang 2 (FV/CE)	AS
Diuron		$C_9H_{12}ClN_2O$		233.0→72.1 (96/17)	233.0→ 56.0 (96/57)	Ja
TP-215a	3-(3-chloro-4-hydroxyphenyl)-l, 1-dimethylurea	$C_9H_{10}ClN_2O_2$		215.1→72.1 (98/25)	215.1→ 46.1 (98/13)	Nein
TP-215b	3-(4-chloro-3-hydroxyphenyl)-l, 1-dimethylurea	$C_9H_{12}ClN_2O_2$		215.1→ 72.1 (98/25)	215.1→ 46.1 (98/13)	Nein
TP-219	DCPMU; Diuron-Desmethyl; 1-(3,4-dichlorophenyl)-3-methylurea	$C_8H_8Cl_2N_2O$		219.0→ 127.0 (98/33)	219.0→ 162.0 (98/13)	Ja

Terbutryn		$C_{10}H_{19}N_5S$		242.2→ 186.1 (91/13)	242.15 → 68.0 (91/45)	Ja
TP-168	Desthiomethyl-Desethyl-Terbutryn	$C_7H_{13}N_5$		168.1→ 112.0 (98/13)	168.1→ 70.0 (98/29)	Nein
TP-186	-	$C_6H_{11}N_5S$		186.1 → 68.0 (195/37)	186.1 → 91.0 (195/17)	Nein
TP-196	Desthiomethyl-Terbutryn	$C_9H1_7N_5$		196.2→ 140.1 (88/13)	196.2→ 45 (88/25)	Nein
TP-210	-	$C_9H_{15}N_5O$		210.1→ 112.1 (112/25)	210.1→ 154.0 (112/9)	Nein
TP-212	2-Hydroxy-Terbutryn; 2-Hydroxy-Terbutylazin	$C_9H_{17}N_5O$		212.2→ 56.0 (107/13)	212.2→ 69.0 (107/45)	Ja
TP-214	Terbutryn-Desethyl; M1; Descyclopropyl-irgarol	$C_8H_{15}N_5S$		214.1→ 158 (93/13)	214.1→ 68.0 (93/45)	Ja
TP-226	Terbumeton	$C_9H_{19}N_5O$		226.2→170.1 (102/13)	226.2→ 128.1 (102/17)	Ja
TP-256	-	$C_{10}H_{18}N_5SO$		256.2→ 158.0 (122/25)	256.2→ 200.0 (122/13)	Nein
TP-258	Terbutryn sulfoxid	$C_{10}H_{19}N_5OS$		258.1→ 186.0 (107/17)	258.1→ 202.0 (107/13)	Nein
OIT		$C_{11}H_{19}NOS$		214.1→ 102.0 (86/9)	214.1→ 57.1 (86/13)	Ja
TP-130	Octylamine	$C_8H_{19}N$		130.2→ 57 .0 (73/8)	130.2→ 43.1 (73/16)	Ja
TP-214	3-Octylthiazol-2(3 H)-one	$C_{11}H_{19}NOS$		214.1→102.0 (86/9)	214.1→ 57.1 (86/13)	Nein

3.5 Bioabbau der Ausgangssubstanzen und photolytischen Mischungen

Zur Bestimmung der leichten biologischen Abbaubarkeit wurde der Closed Bottle Test (CBT) nach OECD 301D durchgeführt. Der Test wurde sowohl mit der Muttersubstanz als auch mit der Photolysemischung (siehe 3.2.1) aus achtstündiger Bestrahlung mittels Xenonlampe (Batch-Reaktor) durchgeführt.

Voraussetzung für die Durchführung des biologischen Abbautests nach OECD-Richtlinie ist eine gewisse Löslichkeit der Testsubstanzen in Wasser, um die geforderte minimale Anfangskonzentration im jeweiligen Test zu erreichen. In der Regel sollte im CBT pro Substanz ein theoretischer Sauerstoffbedarf (ThOD) von 5 mg L^{-1} vorliegen (OECD). Weitere Informationen sind in Artikel 1 zu finden.

3.6 Toxizität der Muttersubstanzen und photolytischen Mischungen

Um erste Kenntnisse über die Toxizität der TP im Verhältnis zu den Muttersubstanzen zu gewinnen, wurden Proben in einem modifizierten Leuchtbakterientest (LBT) zur Untersuchung der Wachstums- und Leuchthemmung (akut und chronisch) sowie der Zellvermehrungshemmung mit dem Testorganismus *Vibrio fischeri* (NRRL-B-11177; Hach-Lange GmbH, Düsseldorf) untersucht. Zur Untersuchung der genotoxischen Wirkung auf die DNA von *Salmonella typhimurium* (TA1535 psk 1002; Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Deutschland) wurde der umu-Test durchgeführt. Weitere Informationen zum Testaufbau sind in Artikel 3 zu finden.

3.7 Quantitative Struktur-Wirkungs-Beziehungen

Die ökotoxikologischen Eigenschaften der einzelnen TP wurden mit Hilfe von Methoden der Chemieinformatik (Quantitative Struktur-Wirkungs-Beziehungen, QSAR) mittels der Software *MultiCASE* (v. 1.7.0.5, MultiCASE Inc.) berechnet. Es wurde das statistische Modell AUA verwendet, um die akute Toxizität im Leuchtbakterientest (Microtox) zu berechnen. Weiterhin wurde die bakterielle Mutagenität mittels statistischen Modells GT_A7B und mittels regelbasierten Expertensystems GT_EXPERT in Kombination verwendet.

4 Ergebnisse und Diskussion

4.1 Spezifizierung von Strahlungsquellen und Bildung von Transformationsprodukten unter verschiedenen Bedingungen

Die Spezifizierung der Strahlungsquellen bestätigte, dass ein genaues Wissen über den emittierten Wellenlängenbereich sowie den absoluten Photonenfluss von großer Bedeutung für die Ermittlung von Geschwindigkeitskonstanten des direkten Abbaus ist. Abbildung 3 zeigt die Überlappung der Absorptionsspektren der drei untersuchten Substanzen Penconazol, Terbutryn und Mecoprop mit dem jeweiligen Emissionsspektrum der drei untersuchten Strahlungsquellen über einen Wellenlängenbereich von 200 bis 400 nm. Die Filterwirkung des optischen Filters der optischen Bank zeigt die größte Absorptions- bzw. Filterwirkung in einem Wellenlängenbereich unterhalb von 290 nm. Die Übereinstimmung mit dem Emissionsspektrum der terrestrischen Solarstrahlung ist für diese Strahlungsquelle am größten, da die Emission dieser Strahlungsquelle unterhalb von 290 nm näherungsweise null beträgt. Dies bedeutet auch, dass die Substanz Penconazol unter natürlichen Bedingungen photochemisch inert ist und dass das Absorptionsspektrum von Terbutryn und Mecoprop lediglich geringe Überlappungen mit dem Emissionsspektrum der Sonne aufweisen.

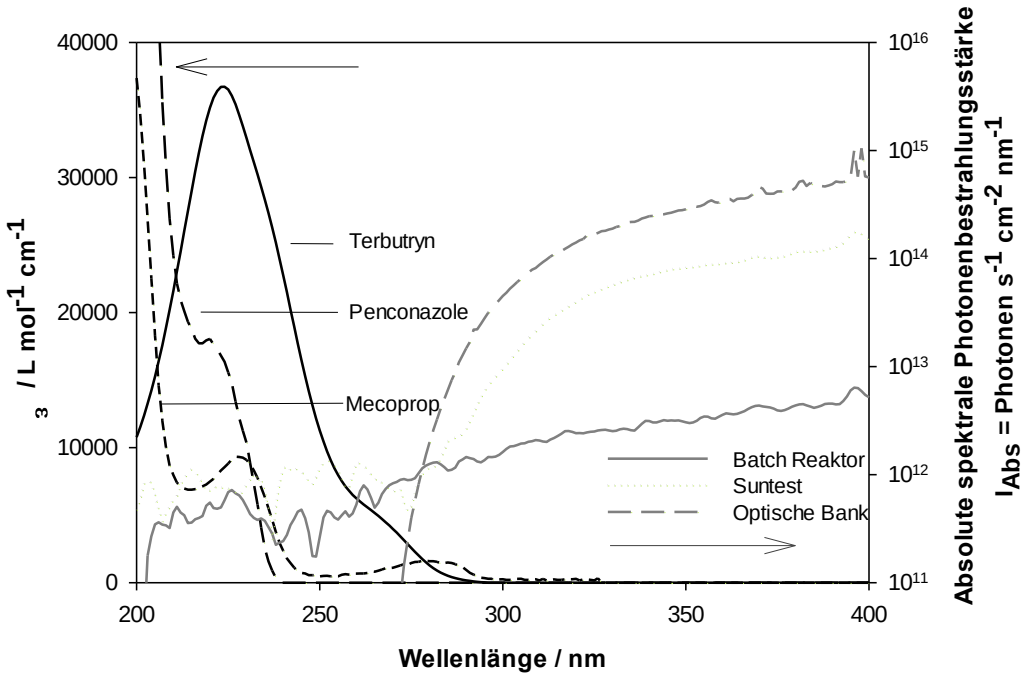


Abbildung 3: Emissionsspektren der verwendeten Strahlungsquellen (rechte Achse) und Absorptionspektren der untersuchten Substanzen (linke Achse), aufgetragen über einen Wellenlängenbereich von 200-400 nm.

Die Kinetik der direkten Photolyse mittels unterschiedlicher Strahlungsquellen zeigt naturgemäß eine starke Abhängigkeit der Überlappung des Absorptionsspektrums der Substanzen

und des absoluten emittierten Photonenflusses der Lampen. Wie zu erwarten, variierten die für die einzelnen Substanzen aus dem photolytischen Abbau ermittelten Geschwindigkeitskonstanten der direkten Photolyse stark. Um eine geringere Streuung der in der Literatur oftmals verwendeten Geschwindigkeitskonstanten zu erreichen, wäre die Verwendung von einheitlichen Strahlungsquellen für die Ermittlung von substanzspezifischen Lebensdauern empfehlenswert. Ein weiterer Lösungsansatz besteht in der Berechnung von Quantenausbeuten der direkten Photolyse, die den jeweiligen absoluten Photonenfluss berücksichtigen und dementsprechend unabhängig von der verwendeten Strahlungsquelle sind.

Bei Betrachtung der in Abhängigkeit der unterschiedlichen Strahlungsquellen gebildeten TP bei der direkten Photolyse zeigte sich, dass die Wahl der Quelle und somit das tatsächliche Emissionsspektrum bei gegebenem absoluten Photonenfluss keinen Einfluss auf die Bildungskinetik sowie die TP-Typen hat (Tabelle 4), was für eine wellenlängenunabhängige Bildung von TP spricht. Die indirekte Photolyse in Anwesenheit von Nitrat ergab für die Substanzen Penconazol und Terbutryn ein identisches Spektrum an TP mit leicht unterschiedlicher Bildungskinetik. Durch die indirekte Photolyse von Mecoprop entstanden hingegen gänzlich andere TP im Vergleich zur direkten Photolyse. Die durch direkte Photolyse entstehenden TP wurden unter indirekter Photolyse nicht gebildet. Stattdessen wurden zwei TP mit gleicher Masse gebildet, was auf die Entstehung von Isomeren hinweist. Die Struktur der TP konnte jedoch nicht eindeutig identifiziert werden.

Tabelle 4: Entstandene TP in den drei untersuchten Strahlungsquellen Batch Reaktor, Suntest CPS+ und Optische Bank sowie unter Zugabe von Nitrat. Untersucht wurden die Substanzen Penconazol, Terbutryn und Mecoprop.

		Batch Reaktor	**Suntest CPS+**	**Optische Bank**	**Optische Bank + NO_3^-**
Penconazol	**TP-184**	+	+	+	+
	TP-248(a)	+	+	+	+
	TP-248(b)	+	+	+	+
	TP-264(a)	+	+	+	+
	TP-264(b)	+	+	+	+
	TP-266(a)	+	+	+	+
	TP-266(b)	+	+	+	+
Terbutryn	**TP-168**	+	+	+	+
	TP-186	+	+	+	+
	TP-196	+	+	+	+
	TP-210	+	+	+	+
	TP-212	+	+	+	+
	TP-214	+	+	+	+
	TP-226	+	+	+	+
	TP-256	+	+	+	+
	TP-258	+	+	+	+
Mecoprop	**TP-137(a)**	-	-	-	+
	TP-137(b)	-	-	-	+
	TP-141	+	+	+	-
	TP-195	+	+	+	-
	TP-213	+	+	+	-

Die Ergebnisse belegen folglich, dass bei der Simulation von photolytischen Prozessen von Pestiziden stets eine genaue Spezifizierung der Strahlungsquellen vorgenommen sowie weitere indirekte Transformationspfade untersucht werden sollten. Dadurch wird eine umfassende Einschätzung von Transformationsprozessen von Pestiziden, vor allem im Rahmen der Zulassung von Pestiziden, ermöglicht. Auch wenn im Rahmen der Arbeit lediglich einer der möglichen indirekten Transformationspfade untersucht wurde, konnte belegt werden, dass indirekte photolytische Prozesse hinsichtlich der Abbaukinetik und Bildung von TP große Auswirkungen haben können. Weitere indirekte Prozesse, die durch gelöste organische Substanzen und weitere Vorläuferverbindungen wie Carbonat initiiert werden (Remucal 2014), sollten daher zukünftig im Rahmen der Risikobewertung von Pestiziden vermehrt Berücksichtigung finden. Die Untersuchung dieser möglichen Transformationspfade gilt insbesondere für Substanzen, die aufgrund geringer bzw. fehlender Überlappung mit dem Emissionsspektrum terrestrischer Strahlung generell als photochemisch inert deklariert werden (Fenner et al. 2013).

4.2 Eintrag von Bioziden und Transformationsprodukten aus Fassaden ins Grundwasser

Die Beprobungen der RVA, das Mulden-Rigolen-System, im Studiengebiet der Stadt Freiburg ergaben Biozidkonzentrationen von bis zu 160 ng L^{-1} für Terbutryn, 5 ng L^{-1} für Diuron und 67 ng L^{-1} für OIT (Abbildung 4). Die Werte liegen im Bereich der gemessenen Konzentrationen von Bioziden in anderen städtischen Gewässern (Bollmann et al. 2014a; Burkhardt et al. 2011). Darüber hinaus ließen sich sieben TP von Terbutryn und ein TP von Diuron, jedoch keines von OIT im Muldenwasser nachweisen. Der fehlende Nachweis von OIT-TP lässt sich anhand des geringen Austrags aus Fassaden (Bollmann et al. 2017b) und des raschen Abbaus im Boden (Bollmann et al. 2017a) erklären. Der Vergleich beider TEG zeigte, dass die mittleren Konzentrationen aller Substanzen des TEG 1 um einen Faktor 10 höher waren als in TEG 2. Dabei dominiert die Konzentration von Terbutryn und dessen TP (TP-212 und TP-214; 20-40 ng L^{-1}) in den Niederschlagsabflussproben. Die höhere Konzentration in TEG 1 deutet auf eine, im Vergleich zu TEG 2, größere Anzahl an mit Bioziden behandelten Fassaden hin, die in die RVA ableiten. Weiterhin können Alter und Typ der Fassade die Höhe der Konzentration beeinflussen.

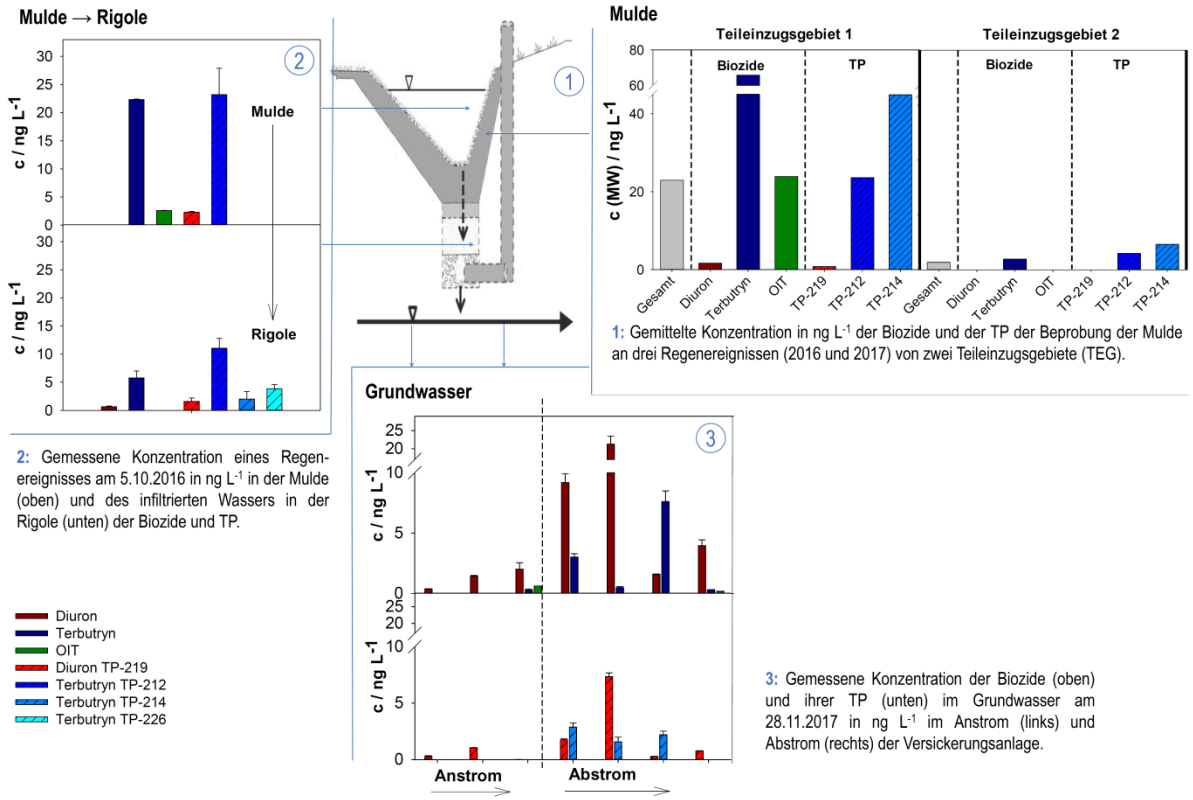


Abbildung 4: Ergebnisse der Beprobung der RVA und des Grundwassers der Stadt Freiburg. Der gesammelte Oberflächenabfluss in der Mulde (1), der Übergang von Mulde zur Rigole (2) sowie das Grundwasser im An- und Abstrom der Versickerungsanlage (3).

Die Wasserproben aus dem Rigolenkörper wiesen geringere Konzentrationen der Substanzen im Vergleich zum Niederschlagsabfluss auf, was auf eine Retentionswirkung durch die Bodenpassage hinweist. Dennoch wurde deutlich, dass eine Vielzahl der Substanzen und insbesondere der TP auch in den Rigolen nachweisbar waren, was bedeutet, dass die Boden-Sand-Passage die untersuchten Substanzen nicht vollständig aus der Wasserphase eliminierte. Außerdem wurden zusätzliche TP im Rigolenwasser nachgewiesen, die im Muldenwasser nicht detektiert wurden. Dies deutet auf eine Remobilisierung der Substanzen von Bodenpartikeln hin, die mit vorherigen Regenereignissen in das Mulden-Rigolen-System eingetragen wurden.

Die vier Beprobungen des Grundwassers im An- und Abstrom der RVA zeigten, dass im Grundwasser zeitweise alle drei untersuchten Biozide mit maximalen Konzentrationen von 22 ng L^{-1} (Diuron), 8 ng L^{-1} (Terbutryn) und 2 ng L^{-1} (OIT) nachweisbar waren. Es konnte zudem jeweils ein TP von Terbutryn und Diuron quantifiziert werden. Für Diuron-Desmethyl (TP-219) konnte eine maximale Konzentration von 7 ng L^{-1} im Grundwasserabstrom der untersuchten RVA ermittelt werden. Während Diuron und Terbutryn in allen Proben nachgewiesen wurden, konnte OIT lediglich bei einer Stichtagsbeprobung detektiert werden. Dies kann damit begründet werden, dass OIT eine im Vergleich zu Diuron und Terbutryn schnellere Bodenabbaubarkeit aufweist (Bollmann et al. 2017a).

Durch Verdünnung im Aquifer lagen die Konzentrationen zwar deutlich unterhalb der europaweit festgelegten Trinkwassergrenzwerten für Pestizide und deren Abbauprodukte (Europäische Union 2017), dabei ist jedoch zu bedenken, dass die Toxizität der TP oft nicht hinreichend bekannt ist und zum Teil größer ist als die der Ausgangssubstanz (Bustos et al. 2019; Gutowski et al. 2015a; Gutowski et al. 2015b). Die Konzentrationen waren insbesondere für Diuron im Grundwasserabstrom höher als im Grundwasseranstrom. Generell zeigen die Ergebnisse, dass die Anzahl der Biozide und TP sowie die gemessenen Konzentrationen im Grundwasserabstrom der RVA höher waren als im Grundwasseranstrom. Der Eintrag von Bioziden und TP in das Grundwasser über die untersuchte RVA konnte damit zweifelsfrei belegt werden.

Um die Herkunft der Biozide und vor allem der TP genauer zu untersuchen, wurde eine nordexponierte 14 Jahre alte Fassade über zwei Stunden intensiv bewässert und das abtropfende Wasser auf seinem Weg in die Versickerungsmulde zu verschiedenen Zeitpunkten beprobt (Abbildung 5). Es gab Positivbefunde in vereinzelten Proben für Terbutryn (Muldenzulauf und Mulde) und OIT (Fassade). Insbesondere Diuron und dessen TP Diuron-Desmethyl waren in allen Proben in hohen Konzentrationen nachweisbar, wobei direkt an der Fassade mit ca. 700 ng L^{-1} eine 30-fach höhere Konzentration für Diuron-Desmethyl als für die Muttersubstanz Diuron (max. 18 ng L^{-1}) ermittelt wurde. Einerseits wurde ersichtlich, dass auch aus älteren Fassaden noch Stoffe ausgetragen werden und andererseits, dass TP bereits direkt auf der Fassade gebildet werden und daher vermehrt Aufmerksamkeit verdienen.

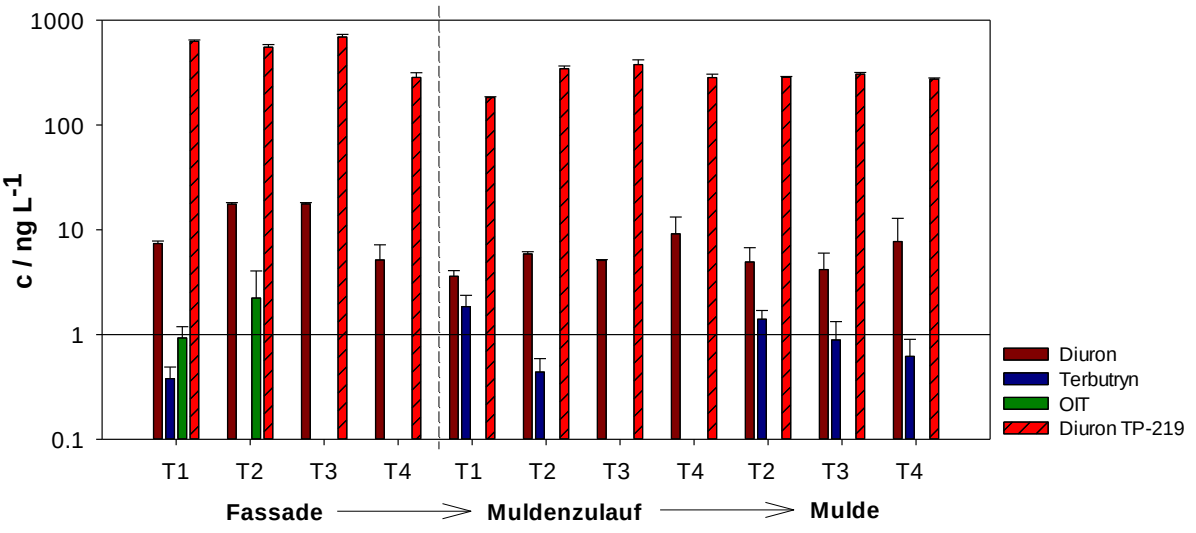


Abbildung 5: Gemessene Konzentrationen der Biozide und ihrer TP im abfließenden Wasser des Beregnungsexperiments vom 27.02.2017 in ng L^{-1}.

Zusammenfassend zeigen die Ergebnisse, dass Fassaden eine Quelle für Schadstoffe im aquatischen, urbanen Raum sein können. Sie legen den Schluss nahe, dass die Barrierewirkung und Schutzfunktion des Mulden-Rigolen-Systems bezüglich einer Schadstoffkontamination des Grundwassers unzureichend ist. Es gilt künftig zu untersuchen, inwieweit auch andere Eintragspfade, z. B. diffuse Bodenversickerung, eine Rolle spielen und inwieweit diese Befunde verallgemeinerbar sind. Zieht man die vorhandenen Grenzwerte heran, ist für das Grundwasser von keiner akuten Gefährdung durch die Biozide auszugehen. Da die Toxizität der TP jedoch weitestgehend unbekannt ist, kann ein Risiko nicht ausgeschlossen werden. Darüber hinaus können Langzeiteffekte durch eine zunehmende Anreicherung der Substanzen in Gewässern das Risiko erhöhen. Zieht man in Betracht, dass sich die aktuellen Messprogramme vor allem auf die Analyse von bekannten (und relevanten) Metaboliten stützen (Bundesministerium für Ernährung und Landwirtschaft 2013), wird deutlich, dass hier Bedarf an Methoden besteht, die auch weniger bekannte TP einbeziehen. Dadurch kann u. a. das Eintragsverhalten in die aquatische Umwelt aufgezeigt werden, wie der hier erstmalig belegte Eintrag von Bioziden und deren TP über RVA in das angrenzende Grundwasser.

Es wurde deutlich, dass die untersuchten Mulden-Rigolen-Systeme im Sinne einer Regenwasserbewirtschaftung nur hinsichtlich der Wassermenge und nicht hinsichtlich des Stoffrückhalts geplant und ausgelegt wurden. Dies könnte beispielsweise durch den Einbau von Passagen mit erhöhter Filterwirkung erreicht werden. Da der Rückhalt von Spurenstoffen im Regenwasserabfluss durch derartige End-of-pipe-Maßnahmen nicht umfassend belegt ist, sollten zusätzlich stets Maßnahmen zur Reduktion an der Emissionsquelle erfolgen. Dies könnten beispielsweise bauliche Maßnahmen hinsichtlich Dach- oder Fassadengestaltung sein, die den Einsatz von Bioziden an Fassaden reduzieren können.

4.3 Ermittlung ökotoxikologischer Eigenschaften von Transformationsprodukten durch die kombinierte Anwendung experimenteller und computerbasierter Methoden

Die Auswertung der Literatur der zwei PSM Boscalid und Penconazol sowie der Substanzen Diuron, Terbutryn, OIT und Mecoprop ergab eine Gesamtanzahl von 45 bekannten, umweltrelevanten TP (Abbildung 6). Daraus wird deutlich, dass die Anzahl der zu bewertenden Substanzen durch den unvollständigen Abbau versiebenfacht wird, im Vergleich zur alleinigen Betrachtung der Ausgangssubstanzen. Die Literaturanalyse ergab, dass 33 % der TP bereits in der aquatischen Umwelt nachgewiesen wurden, wobei bisher kein Nachweis der TP von Boscalid und Penconazol erfolgte. Der Großteil der Konzentrationen lag im Bereich von einigen

100 ng L^{-1}. Die höchste Konzentration (7.9 µg L^{-1}) in Oberflächenwasser wurde für Diuron-TP-219 gemessen (Field et al. 1997). Im Grundwasser wurde hingegen die höchste Konzentration für das Mecoprop-TP-141 mit einer Konzentration von 1.4 µg L^{-1} gemessen (McManus et al. 2014).

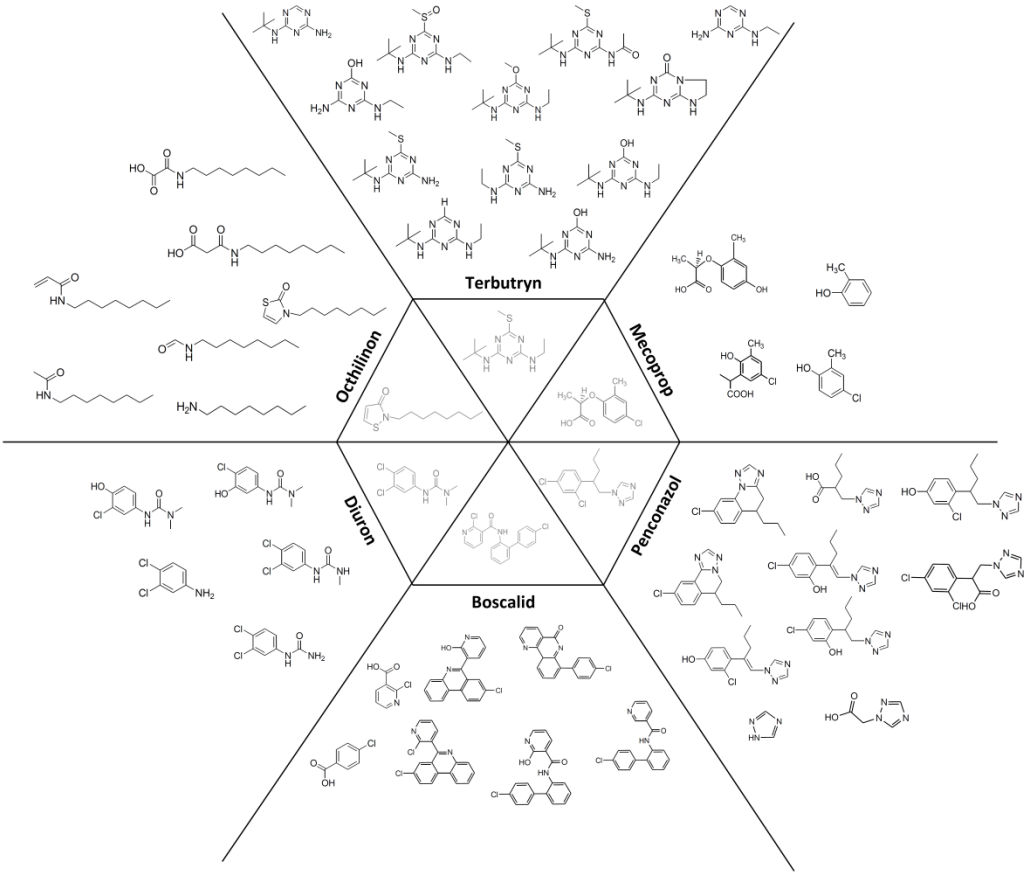


Abbildung 6: Verdeutlichung der Zunahme der Substanzanzahl durch die Berücksichtigung von TP. Die Anzahl der zu bewertenden Substanzen hat sich, im Vergleich zur alleinigen Betrachtung der Ausgangssubstanzen, mehr als versiebenfacht.

Durch die kombinierte Anwendung von computerbasierten und experimentellen Ansätzen konnte die Ökotoxizität von 43 der 45 TP (96 %) bewertet werden.

Das Wissen über die ökotoxikologischen Eigenschaften der TP konnte anhand dieses Ansatzes verdreifacht werden, da zuvor die ökotoxikologischen Eigenschaften von lediglich 13 TP bekannt waren. Für die Bewertung wurde eine Wahrscheinlichkeitseinstufung durchgeführt, bei der die Anzahl der Methoden, die angewendet werden konnten, ausschlaggebend für die Kategorisierung ist. Konnte die Toxizität eines TP auf nur einer der drei Stufen erfasst werden, so erfolgte daraus eine Kategorisierung in „wahrscheinlich ökotoxisch“ im Falle eines positiven Befunds oder „wahrscheinlich nicht ökotoxisch“ im Falle eines negativen Befunds. Konnte die Toxizität eines TP auf mindestens zwei Stufen bewertet werden, erfolgte daraus

eine Kategorisierung in „sehr wahrscheinlich ökotoxisch“ bzw. „sehr wahrscheinlich nicht ökotoxisch“ (Tabelle 5). Daraus wurde ersichtlich, dass 44 % der TP wahrscheinlich ökotoxisch und 20 % der TP sogar sehr wahrscheinlich ökotoxisch sind. 13 % der TP sind wahrscheinlich nicht ökotoxisch und weitere 18 % sind sehr wahrscheinlich nicht ökotoxisch. Penconazol-TP-130 und OIT-TP-158 konnten nicht bewertet werden, da die Ökotoxizität durch keines der drei Verfahren ermittelt werden konnte.

Die Anzahl der Substanzen, die wahrscheinlich bzw. sogar sehr wahrscheinlich ökotoxisch sind, hat sich im Vergleich zur alleinigen Berücksichtigung der Ausgangssubstanzen vervierfacht. Von diesen TP sind die Penconazol-TP-70 und TP-286 sowie Diuron-TP-205 und TP-219 bereits im Rahmen von Zulassungsberichten bekannt und als ökotoxisch relevant eingestuft worden. OIT-TP-214, Mecoprop-TP-141, TP-195 und TP-213 sowie Diuron-TP-162 sind zwar bekannt, jedoch nicht als relevant eingestuft worden. Keines der TP, das als wahrscheinlich ökotoxisch eingestuft wurde, wurde bisher in den Zulassungsverfahren berücksichtigt.

Tabelle 5: Einteilung der 45 bewerteten TP anhand von Hinweisen darauf, toxisch bzw. nicht-toxisch gegenüber aquatischen Organismen zu sein. Die Wahrscheinlichkeitseinstufung erfolgte aus der Anzahl der Methoden, die angewendet werden konnten.

***Sehr wahrscheinlich* ökotoxisch**	***Wahrscheinlich* ökotoxisch**	**Nicht bewertbar**	***Wahrscheinlich* nicht ökotoxisch**	***Sehr wahrscheinlich* nicht ökotoxisch**
Penconazol-TP-70	Boscalid- TP-307(a)	Penconazol-TP-130	Boscalid- TP-157	Terbutryn-TP-140
Penconazol-TP-286	Boscalid-TP-307(b)	OIT-TP-158	Boscalid- TP-158	Terbutryn-TP-168
Diuron-TP-162	Bosclaid-TP-325(a)		Boscalid- TP-309	Terbutryn-TP-196
Diuron-TP-205	Boscalid-TP-325(b)		Terbutryn-TP-156	Terbutryn-TP-212
Diuron-TP-219	Penconazol-TP-184		OIT-TP-202	Terbutryn-TP-226
OIT-TP-214	Penconazol-TP-248(a)		OIT-TP-216	OIT-TP-172
Mecoprop-TP-141	Penconazol-TP-248(b)			OIT-TP-130
Mecoprop-TP-195	Penconazol-TP-264(a)			Mecoprop-TP-107
Mecoprop-TP-213	Penconazol-TP-264(b)			
	Penconazol-TP-266(a)			
	Penconazol-TP-266(b)			
	Diuron-TP-215(a)			
	Diuron-TP-215(a)			
	Terbutryn-TP-184			
	Terbutryn-TP-186			
	Terbutryn-TP-210			
	Terbutryn-TP-214			
	Terbutryn-TP-256			
	Terbutryn-TP-258			
	OIT-TP-184			

Die Ergebnisse zeigen, dass eine kombinierte Anwendung aus Literaturrecherche sowie computerbasierten und experimentellen Methoden das Wissen über die ökotoxikologischen Eigenschaften vervielfachen kann. Wohingegen sich der angewandte mehrstufige Ansatz für die Ermittlung der Genotoxizität als nicht geeignet herausstellte, da diese sowohl in den computerbasierten als auch in den experimentellen Verfahren in den meisten Fällen nicht ermittelt werden konnte.

Die hier gezeigte große Anzahl an zu bewertenden TP ist umso bedeutsamer, wenn man in Betracht zieht, dass selbst aktuelle Empfehlungen zur künftigen Risikobewertung von Pestiziden die Berücksichtigung von TP vernachlässigen (Schäfer et al. 2019). Die Vielzahl der verwendeten Stoffe und der sich daraus bildenden TP macht deutlich, dass einer umfassenden ökotoxikologischen Bewertung Grenzen gesetzt sind. Einerseits ist aufgrund analytischer Grenzen, wie z. B. Löslichkeit der Substanzen oder Höhe der Nachweis- und Bestimmungsgrenzen, die Aufklärung der Gesamtheit aller TP nahezu unmöglich. Andererseits erfordert eine umfassende Analyse eine Vielzahl (öko-)toxischer Endpunkte. Dabei liegt die Herausforderung sowohl in der Verwendung geeigneter computerbasierter Methoden als auch in validen experimentellen Testverfahren (Kümmerer et al. 2019). Da die alleinige Anwendung von experimentellen Methoden sehr ressourcen- und zeitintensiv ist, sollten derartige Methoden lediglich für die Entwicklung ebendieser computerbasierten Modelle sowie für die genauere Untersuchung möglicher ökotoxikologisch relevanter Substanzen angewandt werden. Die ökotoxikologische Relevanz der Substanzen kann im Rahmen eines wie hier vorgestellten Screenings identifiziert werden.

4.4 Zusammenfassung

Im Rahmen der vorliegenden Dissertationsschrift wurden bisher wenig berücksichtigte Transformationsprozesse unter Laborbedingungen sowie ein bislang unbekannter Eintragspfad von TP in die aquatische Umwelt analysiert. Außerdem wurde mittels kombinierter Anwendung von experimentellen und computerbasierten Methoden das Wissen über die ökotoxikologischen Eigenschaften bereits bekannter Pestizid-TP erweitert. Im Einzelnen lassen sich folgende Erkenntnisse ableiten:

i. Beim Vergleich von drei verschiedenen Strahlungsquellen zur Simulation von Photolyse im Labormaßstab zeigte sich, dass eine genaue Spezifizierung der Lampen notwendig ist, um Fehler bezüglich der Geschwindigkeit des direkten Abbaus vor allem von schwach absorbierenden Pestiziden zu vermeiden.

ii. Die indirekte Photolyse in Gegenwart von Nitrat wurde als wichtiger Transformationsprozess für Pestizide im Hinblick auf die Entstehung von TP identifiziert. So konnte für Mecoprop ein im Vergleich zur direkten Photolyse abweichender Transformationspfad zu zwei differenten TP ermittelt werden. Die TP von Penconazol und Terbutryn waren hingegen bei beiden Prozessen identisch.

iii. Dezentrale Versickerungssysteme wurden erstmalig als Eintragspfad für biozide Wirkstoffe und insbesondere deren TP ins Grundwasser identifiziert. Dabei wurden

vor allem die Ausgangssubstanzen Diuron und Terbutryn sowie jeweils ein zugehöriges TP im Grundwasserabstrom einer Versickerungsanlage detektiert.

iv. Weiterhin wurden Fassaden als Quelle von Biozid-TP identifiziert. Dies konnte insbesondere anhand der Ausgangssubstanz Diuron und des TP-219 am Beispiel einer 14 Jahre alten Fassade verifiziert werden. Dieses TP ist bekannt dafür, stark toxisch gegenüber aquatischen Organismen zu wirken.

v. Die Analyse von 45 Pestizid-TP zeigte, dass eine kombinierte Analyse mittels experimenteller und computerbasierter Methoden ein gutes und zeiteffektives Werkzeug zur Ermittlung von ökotoxikologischen Eigenschaften ist.

vi. Hierbei zeigte sich, dass vor allem OIT-TP-214, Mecoprop-TP-141, TP-195 und TP-213 vermehrt Aufmerksamkeit bezüglich weiterer sowie umfassenderer ökotoxikologischer Tests verdienen, da diese TP bisher in Zulassungsbescheiden nicht berücksichtigt wurden und aufgrund der hier durchgeführten Tests als sehr wahrscheinlich toxisch eingestuft wurden.

5 Fazit und Ausblick

Das Ziel der Arbeit war es, das Umweltverhalten und den Verbleib ausgewählter Pestizid-TP in aquatischen Systemen zu analysieren. Zu diesem Zweck wurden der Abbau von Pestiziden, die umweltrelevanten Eigenschaften der dabei entstehenden TP sowie der Eintrag in Gewässer untersucht. Alles in allem konnten durch die hier stattgefundenen Untersuchungen sowohl neue Erkenntnisse bezüglich des methodischen Vorgehens bei der Analyse von TP als auch bezüglich der Eigenschaften von Ausgangssubstanzen und TP gewonnen werden. Durch die angewandten Methoden wurde gezeigt, dass eine Kombination aus bereits existierenden Messverfahren und einer Erweiterung durch neue, dem aktuellen Wissensstand angepasste Methoden nützlich ist, um das Vorkommen in der Umwelt und die Eigenschaften der TP umfassend zu analysieren.

Die Analyse unterschiedlicher Transformationsprozesse ergab, dass die indirekte Photolyse ein wichtiger Transformationspfad vor allem für diejenigen Substanzen ist, deren photolytische Reaktionen unter Umweltbedingungen bis dato als irrelevant galten. Am Beispiel des Eintrags von Bioziden und deren TP aus Fassaden ins Grundwasser konnte belegt werden, dass die TP auch unter Umweltbedingungen gebildet und in das aquatische System eingetragen werden. Durch die kombinierte experimentelle und computerbasierte Analyse der ökotoxikologischen Eigenschaften von Pestizid-TP konnte schließlich die Toxizität von 96 % der untersuchten TP bewertet werden. Es stellt sich demnach als ein geeignetes Vorgehen zur Ersteinschätzung von Pestizid-TP dar.

Die Ergebnisse machen deutlich, dass ein Eintrag der Substanzen, insbesondere der TP, aufgrund der ökotoxikologischen Relevanz vermieden werden sollte. Der Rückhalt sollte dabei entweder direkt an der Quelle oder aber zumindest durch einen verbesserten, nachgeschalteten Stoffrückhalt (z. B. an den Versickerungssystemen) gewährleistet werden.

Um einer Belastung der Gewässer durch Chemikalien, wie z. B. durch Pestizide, vorzubeugen, müsste die Umweltrisikobewertung in der Hinsicht angepasst werden, dass für die Risikoabschätzung weitere qualitative Screening-Methoden einbezogen werden. Dadurch können Hinweise darauf ermittelt werden, inwieweit auch bis dato als nicht relevant geltende TP in die Umwelt eingetragen werden und sich dort verhalten. Im Hinblick auf die Wirkung von Chemikalien empfiehlt es sich, das hier vorgestellte mehrstufige Verfahren für eine Erstabschätzung einzubeziehen, um einerseits die Vielzahl an Substanzen aber andererseits auch schlecht analysierbare Substanzen untersuchen zu können. Im Falle eines möglichen Risikos

sollten eingehendere Tests mit Einzelsubstanzen und Substanzmischungen erfolgen. Hierbei sollte vor allem die kombinierte Wirkung von verschiedenen Substanzen inklusive ihrer TP in vorherigen computerbasierten und experimentellen Screenings Gegenstand zukünftiger Forschung sein.

Die Arbeit liefert wichtige Erkenntnisse und Methodenvorschläge zum Umgang mit der zunehmenden Anzahl von Substanzen und dazugehöriger TP. Die Erkenntnisse und Methoden sind unter der Berücksichtigung von physikochemischen Eigenschaften und Anwendungsspektren auch auf andere Pestizide und ferner auf andere organische Substanzgruppen (u. a. Arzneimittel) übertragbar. Damit können die Ergebnisse dieser Arbeit in Zukunft verwendet werden, um im Sinne der Ziele einer nachhaltigen Entwicklung einer Verschmutzung der Gewässer in qualitativer und quantitativer Hinsicht vorzubeugen.

6 Literaturverzeichnis

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7 Artikel zur kumulativen Dissertation

Artikel 1 Birte Hensen, Oliver Olsson, Klaus Kümmerer (2019): The role of irradiation source setups and indirect phototransformation: Kinetic and mechanistic aspects of weakly sunlight-absorbing pesticides. Science of the total Environment: 695: 133808. DOI: https://doi.org/10.1016/j.scitotenv.2019.133808.

Artikel 2 Birte Hensen, Jens Lange, Nicole Jackisch, Franziska Zieger, Oliver Olsson, Klaus Kümmerer (2018): Entry of biocides and their transformation products into groundwater via urban stormwater infiltration systems. *Water research*. 144: 413-423. DOI: https://doi.org/10.1016/j.watres.2018.07.046.

Artikel 3 Birte Hensen, Oliver Olsson, Klaus Kümmerer (2020): A strategy for an initial assessment of the ecotoxicological effects of transformation products of pesticides in aquatic systems following a tiered approach. Environment International, 137: 105533. DOI: https://doi.org/10.1016/j.envint.2020.105533.

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The role of irradiation source setups and indirect phototransformation: Kinetic aspects and the formation of transformation products of weakly sunlight-absorbing pesticides

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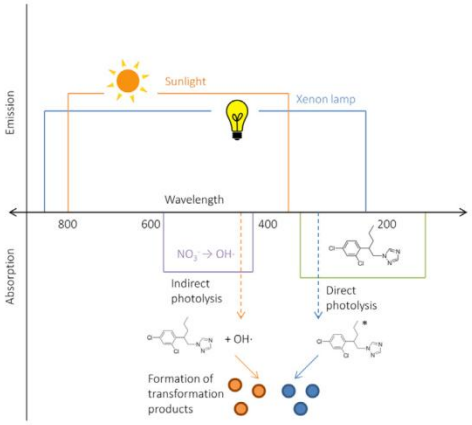
HIGHLIGHTS

- Importance of characterization of irradiation source setups for laboratory photolysis
- Consideration of indirect photolysis regarding degradation rates and TP formation
- Indirect photolysis led to different TPs for Mecoprop compared to direct photolysis.
- Photolysis led to TPs for Penconazole that were hitherto unknown.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, emission spectra of three different commonly used xenon irradiation sources were analyzed and compared for the first time to ascertain the most suitable setup to simulate natural solar radiation. In order to demonstrate setup differences, absolute photon fluxes of irradiation sources were received by actinometry. Verification was done by measuring quantum yields of the model compounds Penconazole, Terbutryn, and Mecoprop in every setup. Differences regarding kinetic aspects and the formation of transformation products (TPs) was evaluated by analyzing direct phototransformation and additionally photolysis in presence of Nitrate as a photosensitizer in one irradiation setup (optical bench). Results showed that a precise setup characterization is needed to estimate whether irradiation sources are suitable to simulate terrestrial sunlight. This was found to be especially important for weakly sunlight-absorbing substances. In comparison with direct photolysis, indirect photolysis led to an enhancement of degradation rate constants for all substances and in case of Mecoprop to different types of TPs that were formed during irradiation. This study underlined that there are big knowledge gaps regarding irradiation sources setups and conditions. It is therefore absolutely necessary to consider those factors while simulating substance degradation and the TP formation under environmental conditions.

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1. Introduction

The release of pesticides from agricultural and urban areas affects water quality of aquatic systems in large parts of the world (Schwarzenbach et al., 2006). In water, they are subject to various biotic and abiotic transformation processes resulting in transformation products (TPs). Photolysis of pesticides in aqueous solutions were investigated extensively (Burrows et al., 2002). It is certain that direct photodegradation of many pesticides occurs rarely in the aquatic environment under environmental conditions (Konstantinou et al., 2001), as an overlap of the pesticide absorption spectrum with the emission spectrum of sunlight is often small (Fenner et al., 2013).

However, the precise characterization of the irradiation source setup is often neglected while simulating pesticide degradation. Xenon arc lamps are a commonly used irradiation source to simulate environmental photolysis of chemicals due to their continuous spectrum and close match to sunlight (OECD, 2008; Yager and Yue, 1988). Different photolytic devices equipped with a xenon arc lamp e.g. merry-go-round (Palm and Zetzsch, 1996; Werner et al., 2005), optical bench (Palm et al., 2003; Palm, 2017; Schindelin and Frimmel, 2000), batch immersion tube (Gutowski et al., 2015; Wilde et al., 2016), and Suntest® apparatuses (Dimou et al., 2005; Durand et al., 1990; Hustert et al., 1999; Sakkas et al., 2002; Sakkas et al., 2011) were used in a wide range of studies. As for solar radiation, the lower cut-off wavelength is at about $\lambda = 290$ nm (Burrows et al., 2002) due to atmospheric scattering and absorption of wavelengths below. Though, without convenient long-pass filter (e.g. borosilicate filter), xenon arc lamps do not emit solar like spectra due to their emission below 290 nm (Winer et al., 1979). A comparison of different light sources was hitherto done for low versus high pressure UV-light, UV-light versus xenon-light or fluorescent light, polychromatic versus monochromatic light, and artificial versus natural sunlight in a variety of studies (Crosby, 1969; Hirahara et al., 2001; Lemaire et al., 1985; Pirisi et al., 1996; Willach et al., 2018; Wong and Chu, 2003). Different irradiation source setups equipped with xenon arc lamps were not evaluated regarding their suitability to simulate environmental conditions (wavelengths < 290 nm) so far.

Besides direct photolysis, the indirect one is at least equally important for the assessment of pesticides fate in the aquatic environment (Remucal, 2014). Via the indirect photolysis pathway in water pesticides may react with a reactive species such as hydroxyl radicals or singlet oxygen. Reactive species can be formed previously due to photochemical excitation of e.g. Nitrate, Carbonate, or Humic and Fulvic acids representing natural photosensitizer (Burrows et al., 2002; Klöpffer, 2013; Remucal, 2014). Most studies relating to indirect photolysis of pesticides focused on kinetic aspects by analyzing the influence of Humic and Fulvic acids (Bachman and Patterson, 1999; Frimmel, 1994; Gonçalves et al., 2006; Kamiya and Katsura, 1998; Sakkas et al., 2002) or Nitrate (Dimou et al., 2005; Fulkerson Brekken and Brezonik, 1998). They compared the received degradation rates to rate constants of direct photolysis. Only a few studies investigated discrepancies of mechanistic aspects and thereby formed types of TPs of pesticides by direct and indirect photolysis. For instance, it was found that the presence of Humic and Fulvic acids led to another dealkylated TP of Prometryn that was not found by direct photolysis (Khan and Gamble, 1983). Likewise, one additional TP due to nitrate-induced photolysis of Atrazine compared to direct photolysis was formed (Torrents et al., 1997). For Mecoprop, different formation patterns by nitrate-induced photolysis compared to direct photolysis but no different types of TPs were analyzed (Meunier and Boule, 2000). However, studies on TP formation by indirect photolytic processes of pesticides are rare and there is a lack of information on decisive aspects that influence the formation of TPs.

Summarizing, this study should emphasize the necessity on following information and investigations to simulate photolysis under environmental conditions. The following protocol allows for a more precise view on fate and transformation of pesticides:

a) Irradiation source setup
- Application of optical filters to restrict wavelength (material, thickness, and transmission)
- Determination of exact wavelength ranges and absolute photon fluxes of irradiation sources

b) Parent compounds
- Comparison of absorption spectrum of test substance and its overlap with both the emission spectrum of sunlight and the irradiation source
- Determination of quantum yields of direct photolysis in water
- Investigation of kinetic differences between direct and indirect photodegradation rates

c) Transformation products
- Identification of differences of TP formation between direct and indirect photolysis
- Investigation of kinetic differences between direct and indirect TP formation

To analyze these aspects, we selected three model compounds Penconazole, Terbutryn, and Mecoprop as they are represent a variety of pesticides that have weak spectral overlap with sunlight. Moreover, the substances cover different basic structures and therefore a broad range of different photolytic characteristics (e.g. photon absorption, degradation rates, number and pattern of TP formation). The formation of TPs of Penconazole was analyzed in different solvents (Schwack and Hartmann, 1994) and in water by sunlight and UV-light (Rodríguez-Cabo et al., 2018). Thereby formed products occurred by cyclization of triazolic and the phenyl ring. Direct Phototransformation of Terbutryn was analyzed by e.g. Bollmann et al. (2016) and Hensen et al. (2018) and eleven TPs were elucidated in total. Mecoprop photolysis was analyzed under various conditions, e.g. influence of Nitrate (Meunier and Boule, 2000) and Fulvic acids (Halladja et al., 2009; Lányi and Dinya, 2005) as well as Photo-Fenton (Flox et al., 2007) and TiO_2 (Abramović et al., 2019; Topalov, 2000). In these studies, Mecoprop was photolysed by low and medium pressure mercury light in different devices and setups and by sunlight. Generally, four different types of TPs were found that were formed independent from irradiation source and conditions.

To reduce the knowledge gaps associated with the above shortcomings the present study focused on lamp characterization and influence of indirect photolysis on kinetic aspects and the formation of TPs. In order to identify an irradiation sources with an appropriate emission spectrum to simulate environmental conditions absolute photon fluxes of three irradiation sources equipped with a xenon arc lamp were determined by actinometry. Quantum yields of direct photolysis of the three model substances in all devices were calculated to verify these results. To examine the influence of indirect photolysis, this study analyzed the use of nitrate as photosensitizer on degradation kinetics of three pesticides in optical bench. Nitrate was used due to the fact that it is a well studied and defined source of hydroxyl radicals (see S4). As another scope was to give an overview of the formation of TPs that were formed by these different photolytic processes, this study rather targeted on the elucidation of finally contained TPs by LC-MS than to provide exact degradation mechanisms.

2. Materials and methods

2.1. Chemicals

Analytical standards of Penconazole (99.1%), Terbutryn (99.1%), Mecoprop (99.6%), and Metamitron as an actinometer (Palm, 2017) were purchased from Sigma Aldrich. Sodium Nitrate and Sodium Benzoate were obtained from Roth and Fluka, respectively. Acetonitrile (LC-

MS grade; VWR International GmbH, Darmstadt, Germany) was used as organic mobile phase in chromatography and for the preparation of stock solutions. Aqueous mobile phase and solutions for the implementation of phototransformation experiments were prepared with ultrapure water (Membra Pure, Germany; Q1:16.6 MΩ and Q2: 18.2 MΩ). Chromatographic settings can be received from S1.

2.2. Analytical procedure

LC analysis was done using a RP-column (Nucleodur 100-3, 125/2, c18 ec; Macherey Nagel, Düren, Germany). Reaction kinetics were analyzed with an Agilent LC-Triple Quad (1200 Infinity LC-System coupled with 6430 Triple Quad, Agilent Technologies, Waldbronn, Germany). The structure of TPs of Penconazole, Terbutryn, and Mecoprop were elucidated using a LC-Iontrap (Dionex Ulitmate 3000 UHPLC system, Dionex, Idstein, Germany) and a LTQ Orbitrap-XL high-resolution mass spectrometer with ESI source (Thermo Scientific, Dreieich, Germany) in a full scan. Applied setting can be received from S1. Intra- and inter-day precision are listed in S2.

2.3. Photochemical setups

Photolytic experiments were performed in ultrapure water with an initial concentration of 100 ng L^{-1} and 5 mg L^{-1} of each substance. Optically dilute solutions ($E < 0.02$; (OECD, 2008)) of 100 ng L^{-1} were used to determine quantitative aspects of photolytic reactions. This is also a concentration that is environmentally relevant (Singer et al., 2010). Higher concentrated solutions of 5 mg L^{-1} were used to enable the detection of TPs occurring with weak peak-intensity. Following irradiation sources were used for the photolysis of pesticides:

(1) **Batch immersion tube photo-reactor** (**"batch reactor"**) with an immersion xenon lamp (TXE 150, 150 W, UV consulting Peschl, Germany; $\lambda = 200$ to 850 nm). The flask (1 L) was filled with about 0.8 L of test substance solution. Experiments were conducted under constant temperature ($T = 293 \pm 2$ K) by cooling the lamp setup with tap water (WKL230, LAUDA, Berlin) and constant stirring. No optical filter was applied to restrict wavelength.

(2) **SUNTEST® apparatus** ("**suntest**") (Atlas Material Testing Technology GmbH, Linsengericht-Altenhaßlau, Germany) equipped with a xenon lamp and a set power of 500 W m^{-2}. The lamp was attached above the sample compartment. The integrated air-cooling device was set to ambient temperature ($T = 295 \pm 2$ K). To restrict evaporation, the flask (20 mL) was covered with a quartz plate that do not absorb at wavelengths that were important for our study. Reflection in the compartment was controlled by placing the glass flask in a concrete shell. Constant stirring was enabled by installing a magnetic stirrer below the sample compartment. An optical filter (daylight-filter) was installed that restricts wavelengths < 290 nm. It was used by many studies before (Gonçalves et al., 2006; Kah et al., 2018; Solís et al., 2019). The measured transmission of the optical filter showed an absorption edge at $I0_{10\%} = 320$ nm (Fig. 1).

(3) **Optical bench** (**"optical bench"**) (AMKO-LTI, Tornesch, Germany) with a xenon lamp (1000 W, Osram, Germany – set to 500 W) was used. Information about the exact setup can be found in Palm (2017). The solution of the pesticide was applied in a cuvette (3 mL) and constantly stirred. Cooling was done by an open water bath ($T = 293 \pm 3$ K). Transmission of optical glass-filter showed an absorption edge at $I0_{10\%} = 289$ nm (Fig. 1).

2.4. Photochemical analysis

All photolytic experiments were conducted for eight hours. Aliquots were hourly drawn from the photo-reactors in order to monitor the kinetics and the pathway of degradation and were stored until analysis at −20 °C. Instead of ultrapure water as it was used for direct photolysis experiments, aqueous solutions for indirect photolysis in optical bench

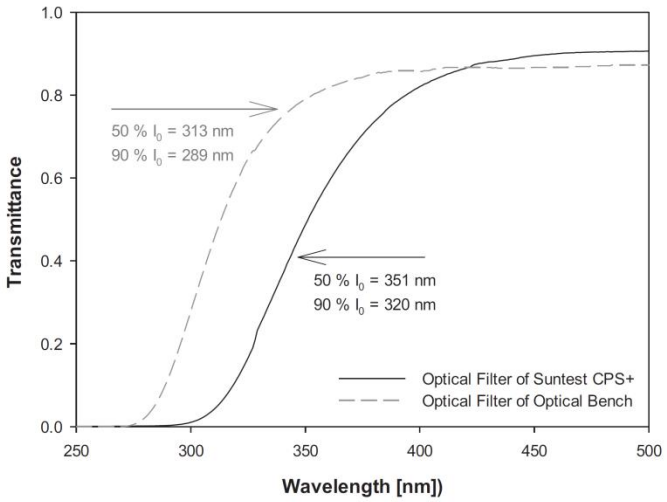

Fig. 1. Transmittance of the optical filter of the explored irradiation sources "Suntest" (solid line) and optical bench (dashed line). Calculated absorption edges at 50% and 90% transmittance.

were added with Sodium Nitrate (Palm et al., 2003) as a source of hydroxyl radicals (see S4). It was used in a concentration of 1.6 mM to enable a surplus and therefore a constant concentration of hydroxyl radicals. Determination of the hydroxyl rate constant of the analyzed pesticides $K_{OH,Pes}$ was done by (Eq. (1)) assuming a constant OH-concentration and therefore a first-order-reaction (Palm et al., 2003) with rate constants of pesticides (k_{Pes}) and Benzoate (k_B).

$$k_{OH.Pes} = K_{OH.B} \frac{k_{Pes}}{k_B} \quad (1)$$

Chemical actinometry was performed to calculate absolute photon fluxes of all three devices by using Metamitron as an actinometer. Quantum yields Φ of each substance were determined by the following equation:

$$\Phi = \frac{k}{2.303 \cdot J_{\lambda,abs} \cdot \varepsilon_\lambda} \quad (2)$$

Formation rate constants of TPs at every measured time point (k_t) were evaluated by setting the peak area of TPs at a certain time point ($A_{TP,t}$) in relation to the area of parent compound at test start ($A_{Sub,t0}$)

$$k_t = \ln\left(\frac{A_{TP.t}}{A_{Sub.t_0}}\right) \quad (3)$$

More details on the calculations carried can be found in S4.
Sigma Plot (version 12.5) was used for the creation of graphs.

3. Results and discussion

3.1. Characterization of irradiation sources

Absolute photon fluxes of the three irradiation sources calculated by actinometry are depicted in Fig. 2. Spectra of all irradiation sources matched that of sunlight in different extents. Optical bench showed <0.001% transmission at wavelengths <270 nm and 3.3% transmission between 270 and 290 nm (Fig. 1). Batch reactor and "suntest" showed summed photon fluxes of about 10^{13} photons s^{-1} cm^{-2} nm^{-1} in the wavelength range of 200 to 290 nm. This fact was somewhat expected for the batch reactor since no optical filter was applied in this setup. As for "suntest", there seemed to be an effect of the optical filter by reducing the radiation by a factor of 100 in this range compared to

wavelengths >290 nm. But there was still high emission obvious that could also be emphasized by the transmission data of the filter. Contrary to the manufacturer's specification (see S3), 0.14 ± 0.05% transmission on average could be observed at <290 nm. Other studies, where suntest® apparatuses with optical filters to restrict radiation <290 nm were used, underline this fact as high degradation rates were measured although analyzed pesticides showed absorption spectra < 290 nm (Dimou et al., 2005; Sakkas et al., 2005). It appeared that both optical filters applied in optical bench and "suntest" varied regarding their amount of extinction of radiation in wavelength ranges < 290 nm as well as the steepness of filter edges, which seemed to be higher in the case of the optical bench. This could be explained by different thicknesses of optical filters that have been already reported as a relevant factor (Winer et al., 1979).

While dealing with different irradiation source setups, quantum yields of direct photolysis become important due to the fact that they are consistent values that are independent from irradiation sources, i.e. absolute photon fluxes (Lemaire et al., 1985; Zepp, 1976). To this reason they were calculated for the three pesticides to verify the absolute photon fluxes of all three devices. This has also been done in the past to evaluate seven types of irradiation apparatuses (Lemaire et al., 1985). Calculated quantum yields (Fig. 3A) in the batch reactor, optical bench, and "suntest" showed variation coefficients found to be 1.2 % (Penconazole), 12.4 % (Terbutryn), and 38.3 % (Mecoprop). The high accuracy of Penconazole and Terbutryn quantum yields underlines that calculated absolute photon fluxes for all devices were properly calculated. The lower precision of Mecoprop quantum yields must be caused by a slightly differing quantum yield in the optical bench in comparison to the batch reactor and "suntest" as well as from literature. However, this result is perfectly in line with variation coefficients of quantum yields obtained in another study exploring different irradiation sources (Lemaire et al., 1985).

Considering these outcomes and the abovementioned wavelength cut-off in the environment at 290 nm, emission spectra of the batch reactor and the "suntest" apparatus could be characterized as different to that of sunlight (Palm et al., 2003). Within our study, the optical bench seemed to be the most suitable device to simulate solar radiation in laboratory scale experiments. Thus, it confirmed the current knowledge on the importance of optical filters and highlighted the urgency to consider these aspects while simulating degradation rates of weakly sunlight-absorbing substances.

3.2. Monitoring of the photolysis of parent compounds

Absorption spectra of all three pesticides showed strong absorption bands in the wavelength range of 220 to 250 nm and weak extensions up to λ=290 nm (Fig. 2). This implies that there is generally a weak probability that the substances undergo direct photolysis under environmental conditions. Calculated quantum yields of direct photolysis of Terbutryn ($\Phi = 5.61 \cdot 10^{-3} \pm 0.001$) were not studied before but were comparable to those of Atraton which has also a triazine-based substance and showed similar absorption spectrum (Palm and Zetzsch, 1996). Determined quantum yields of Mecoprop ($\Phi = 1.97 \cdot 10^{-1} \pm 0.08$) are in accordance with literature data (Meunier and Boule, 2000). No quantum yield of Penconazole was found in literature and could only be calculated for batch reactor and "suntest" in this work due to the lacking overlap of absorption and emission spectrum of optical bench ($\Phi = 8.28 \cdot 10^{-3} \pm 0.001$). However, except for Mecoprop, quantum yields imply long lifetimes of pesticides by direct photolysis in the environment.

Mecoprop rate constant of dark experiments was below 10^{-8} s^{-1}. Kinetic differences of direct and indirect photolysis were analyzed in optical bench due to its close match to sunlight. Terbutryn and Penconazole showed low rate constants of direct photolysis (S6). They were rather more comparable with those found under exclusion of light (Penconazole = $8.70 \cdot 10^{-2}$ M^{-1} s^{-1}, Terbutryn = $5.61 \cdot 10^{-2}$ M^{-1} s^{-1}. In fact, direct photolysis rate constants of Penconazole were even slightly lower than those in the dark. However, the deviation was within the analytical error (Fig. 3B). It emphasizes once more that the optical bench do not emit at wavelengths in the range of penconazole absorption (Fig. 2). Conversely, Mecoprop showed higher rate constants (2.16 M^{-1} s^{-1}) by direct photolysis in the optical bench setup. These observations can be explained by the amount of overlap of absorption spectra with the emission spectrum of devices (Fig. 2) that were found to be three times higher for Mecoprop than for Terbutryn (S5).

Rate constants of indirect photolysis were in all cases higher compared to direct photolysis (Fig. 3A). These results were somewhat expected since many studies found that Nitrate enhances rate constants of various pesticides (Dimou et al., 2005; Sakkas et al., 2005; Stangroom et al., 1998). Direct photolysis could be excluded due to the optical filter and the significant inner filter effect of Nitrate through the use of concentrations of about 100 mg L^{-1}

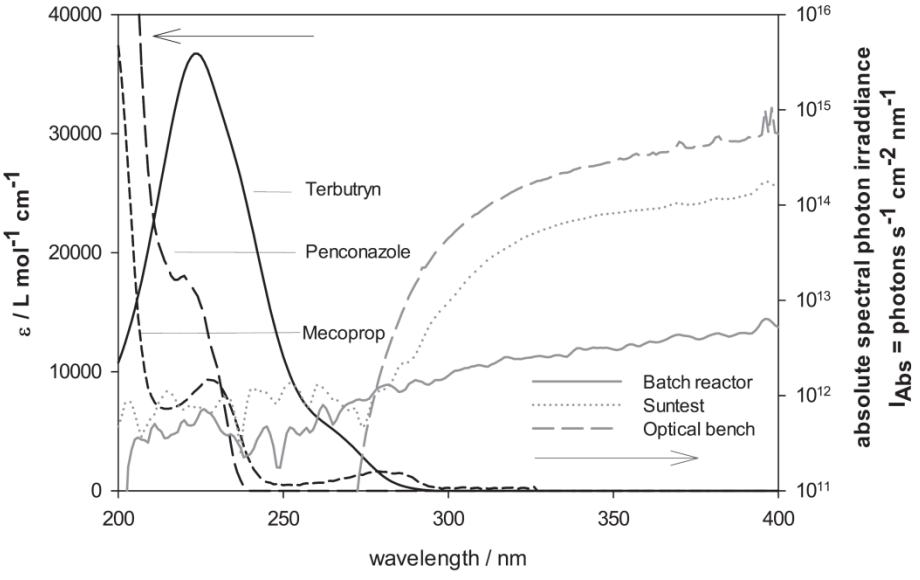

Fig. 2. Absorption spectra (ε in L mol^{-1} cm^{-1}) of the analyzed substances Terbutryn, Penconazole, and Mecoprop (black dashed and solid lines; left ordinate) as well as the calculated absolute spectral photon fluxes (I_{abs} in photons s^{-1} cm^{-2} nm^{-1}) of the three irradiation sources setups (grey dashed and solid lines, right ordinate) over a wavelength range from 200 to 400 nm.

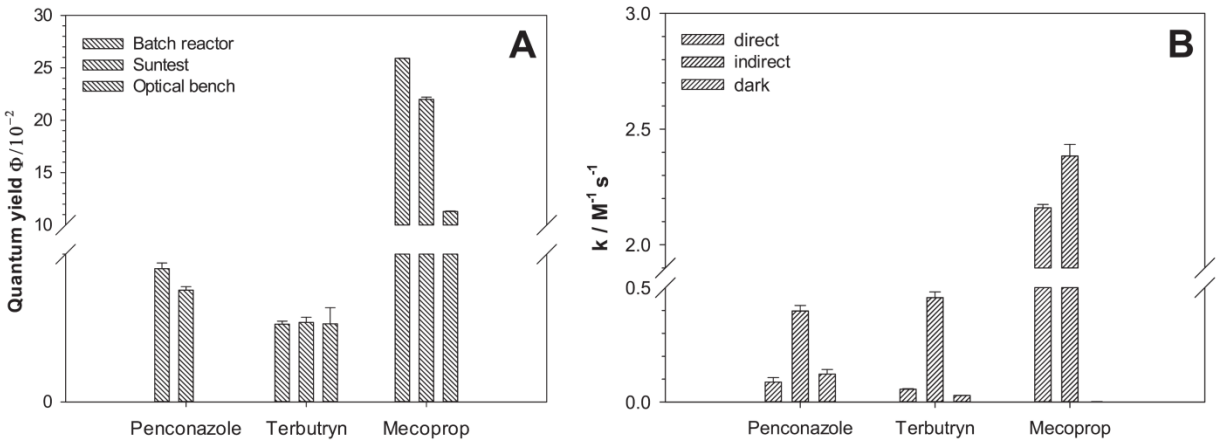

Fig. 3. A: Calculated quantum yields (Φ) by irradiation by three different irradiation sources (batch reactor, suntest, and optical bench). No quantum yield for Penconazole in optical bench could be calculated due to the lacking spectral overlap. B: Rate constants (k / s^{-1}) of direct and indirect photolysis (with Nitrate) using xenon light (optical bench) and under exclusion of light (dark).

(Mack and Bolton, 1999). The determined indirect rate constants were eight times higher for Terbutryn and five times for Penconazole compared to direct rate constants. Rate constants of indirect photolysis in the presence of nitrate of Mecoprop are slightly higher than direct ones (Fig. 3A). This can be explained by the hydroxyl rate constant, which was examined to ascertain the reactivity of substances with hydroxyl radicals (S7). That of Mecoprop closely matches the diffusion limit of aromatic compounds of $k_{OH} = 10^{10}$ M s^{-1} in water (Haag and Yao, 1992). A higher rate constant in water was therefore not possible. Hydroxyl radical rate constant of Mecoprop received here ($k_{OH} = 1.12 \cdot 10^{10}$ M^{-1} s^{-1}) was about 4.5 times higher than stated in the literature (Armbrust, 2000). The use of different competitors (e.g. Acetophenone) in that study could be an insufficient but reasonable explanation. Terbutryn hydroxyl rate constant ($k_{OH} = 1.86 \cdot 10^{9}$ M^{-1} s^{-1}) was comparable with other triazines (e.g. Atrazine, Simiazine) (Haag and Yao, 1992). Penconazole hydroxyl radical rate constant ($k_{OH} = 1.88 \cdot 10^{9}$ M^{-1} s^{-1}) has never been reported before.

It turned out that direct photolysis under environmental conditions was not relevant for Penconazole and Terbutryn but indirect photolysis might be a relevant pathway for such substances. To this reason the formation of TPs in such processes become important and should be analyzed in the following.

3.3. Formation of TPs by direct and indirect photolysis

The monitoring of the formation of TPs formed by direct photolysis in different irradiation sources showed that formation was similar in each device (Table 1). More details can be received from S6. The similarity of TPs formed by different irradiation sources, i.e. different wavelengths and power, confirmed the results of other studies (Pirisi et al., 1996; Willach et al., 2018). A formation of TPs in dark samples was not observed. Thus, hydrolysis could be excluded. There is generally no indication whether the formation of TPs was dependent on irradiation source or on indirect photolysis, except for Mecoprop. For this substance not the irradiation source but the use of Nitrate influences the formation of TPs, as two completely different TPs were found in indirect photolytic processes (Table 1). This effect was seen to some extent by previous studies (Khan and Gamble, 1983; Torrents et al., 1997), where a slightly differing spectrum of TP was found.

Table 1
Elucidated TPs that were formed by irradiation with different irradiation sources and in addition with Nitrate in optical bench of Penconazole, Terbutryn, and Mecoprop with Retention times (RT) and exact masses ($[M + H]^+$/$[M\text{-}H]^-$). Molecular formula of TP-137(a) and TP-137(b) are proposed since they are not verified by an analytical standard.

	TP	RT[min]	Exact mass [Da]	Molecular formula	Ionization mode	Batch reactor	Suntest	Optical bench	Optical bench + NO_3^-
Penconazole	TP-184	1.8	184.108	$C_8H_{13}N_3O_2$	+	+	+	+	+
	TP-248(a)	10.5	248.095	$C_{13}H_{14}ClN_3$	+	+	+	+	+
	TP-248(b)	1.5	248.139	$C_{13}H_{18}N_3O_2$	+	+	+	+	+
	TP-264(a)	2.9	264.089	$C_{13}H_{14}ClN_3O$	+	+	+	+	+
	TP-264(b)	4.2	264.090	$C_{13}H_{14}ClN_3O$	+	+	+	+	+
	TP-266(a)	3.6	266.105	$C_{13}H_{16}ClN_3O$	+	+	+	+	+
	TP-266(b)	5.1	266.105	$C_{13}H_{16}ClN_3O$	+	+	+	+	+
Terbutryn	TP-168	1.0	168.127	$C_7H_{13}N_5$	+	+	+	+	+
	TP-186	2.4	186.081	$C_6H_{11}N_5S$	+	+	+	+	+
	TP-196	1.5	196.158	$C_9H_{17}N_5$	+	+	+	+	+
	TP-210	1.3	210.138	$C_9H_{15}N_5O$	+	+	+	+	+
	TP-212	1.2	212.153	$C_9H_{17}N_5O$	+	+	+	+	+
	TP-214	3.8	214.115	$C_8H_{15}N_5S$	+	+	+	+	+
	TP-226	3.6	226.132	$C_9H_{19}N_5O$	+	+	+	+	+
	TP-256	8.0	256.126	$C_{10}H1_8N_5SO$	+	+	+	+	+
	TP-258	2.7	258.142	$C_{10}H_{19}N_5OS$	+	+	+	+	+
Mecoprop	TP-137(a)	3.2	137.068	$C_8H_{10}O_2^*$	−	−	−	−	+
	TP-137(b)	3.9	137.068	$C_8H_{10}O_2^*$	−	−	−	−	+
	TP-141	5.9	141.011	C_7H_7ClO	−	+	+	+	−
	TP-195	2.1	195.066	$C_{10}H_{12}O_4$	−	+	+	+	−
	TP-213	5.3	213.032	$C_{10}H_{11}ClO_3$	−	+	+	+	−

3.3.1. Penconazole

By photolysis of Penconazole eight TPs could be identified (Fig. 4A). With the exception of TP-248(a) and TP-248(b) none of them are currently described in literature (Rodríguez-Cabo et al., 2018; Schwack and Hartmann, 1994). According to these studies, they emerged by loss of chlorine in ortho position and following ring closure with second nitrogen (TP-248(a)) and fifth carbon (TP-248(b)) of the heterocyclic ring. TP-184 might be formed due to break down of the dichlorophenyl group and subsequent oxidation to carboxyl group. TP-266(a) and TP-266(b) with $\Delta\, m/z = 18$ compared to Penconazole might have occurred by substitution of one chlorine by hydroxyl group. TP-264(a) and TP-264(b) with $\Delta\, m/z = 16$ might have formed by additional dehydrogenation and sp^2-hybridisation of two carbon atoms of the pentyl group. For TP-248(b) with Δ m/z = 36 substitution of both chlorine atoms by hydroxyl groups can explain the found mass differences. These possible transformation processes could be confirmed, to some extent, by exact

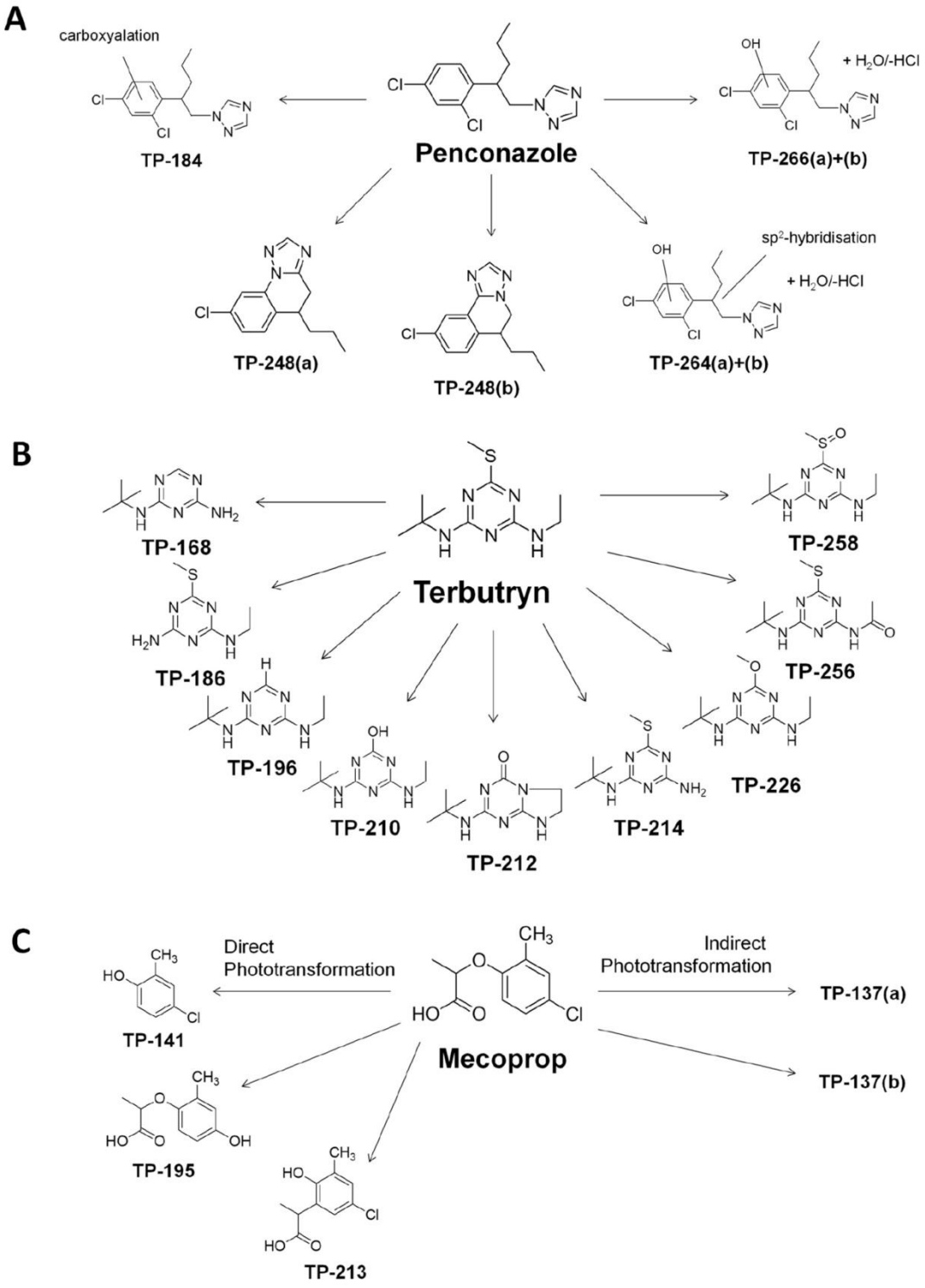

Fig. 4. Possible transformation mechanism of Penconazole (A), Terbutryn (B), and Mecoprop (C) by direct and indirect photolysis in optical bench.

masses and fragmentation pattern in high resolution mass spectrometry (S8). Although confirmation of exact isomeric structures are missing due to the non availability of analytical standards, analysis of the m/z values at a certain retention time allowed for a kinetic analysis as relative changes are sufficient for this. A unique enhancement of formation rates by indirect photolysis could be found for neither TP, although degradation of penconazole was enhanced (Fig. 5). It is seen that formation rates of indirect phototransformation showed both acceleration and deceleration compared to rates of direct phototransformation.

3.3.2. Terbutryn

In the direct and indirect phototransformation experiments for Terbutryn the formation of eleven TPs was found (Fig. 4B). As Bollmann et al. (2016) stated phototransformation of Terbutryn resulted in the substitution of the methylthio group by hydrogen (TP-168, TP-196), oxygen (TP-212), and ether (TP-226) or methyl (TP-214) groups. TP-168 and TP-214 were formed through the loss of the ethyl group. TP-258 was formed by oxidation of the methylthio group resulting in methylsulfoxide group. Those TPs were already verified by an analytical standard in that study. Three new TPs with *m/z* 186, 210, and 256 were found in a study (Hensen et al., 2018) that were also found here but could not be confirmed due to the lack of analytical standards. Similarity of TP formation by both processes could be explained by the fact that most TPs resulted from dealkylation and hydroxylation, as it is the most abundant formation pathway with hydroxyl radicals, e.g. by previous hydrogen abstraction (Gligorovski et al., 2015). Another study analyzed photolysis of Atrazine, which is such as Terbutryn a triazine-based pesticide. They showed that nitrate-induced photolysis led to an additional dealkylated TP that were not found in direct photolysis processes (Torrents et al., 1997). They stated that an hydroxylated TP - similar to TP-212 of Terbutryn here - was not a product of hydroxyl radical process as another study suggested (Kolpin and Kalkhoff, 1993). Results of latter one could be somewhat verified by our results since types of TPs of both processes were identical. Kinetic analysis showed that there is a slight acceleration of the formation rate of TPs evident by a factor of 1.6 on average by indirect phototransformation (Fig. 5).

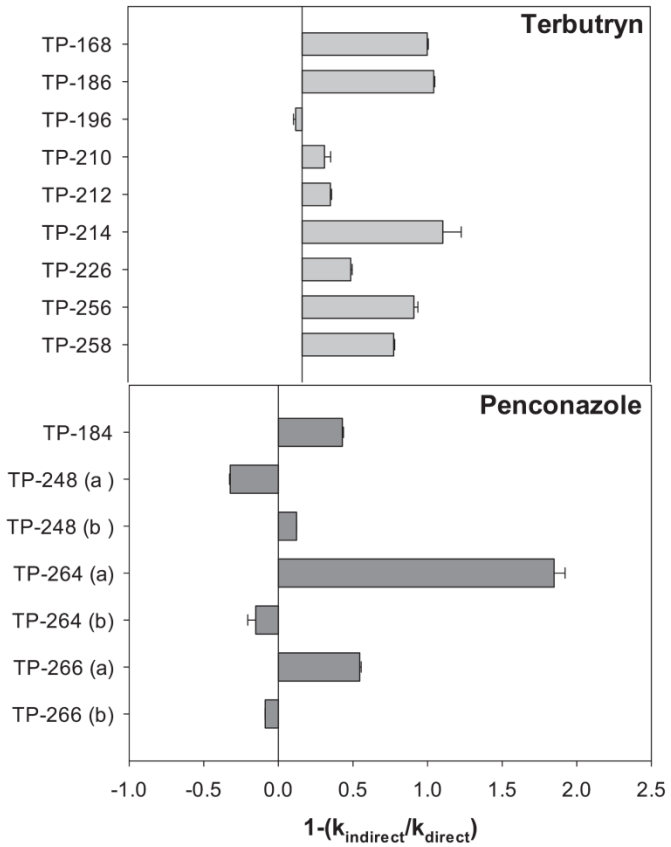

Fig. 5. Indirect and direct rate constants (k / h^{-1}) of TP-formation of Penconazole (A) and Terbutryn (B). Values > 0 imply faster formation of a certain TPs by indirect photolysis and values < 0 faster formation by direct photolysis.

3.3.3. Mecoprop

Direct photolysis of Mecoprop led to the formation of three TPs with different masses. Amongst a few others already known TPs, TP-141, TP-195, and TP-213 were found in other studies, e.g. (Boule et al., 2002; Meunier and Boule, 2000). According to these studies, TPs were formed by dechlorination and subsequent hydroxylation (TP-195) and by "Claisen"-rearrangement (TP-213). For TP-141 oxidation of the acetic moiety and formation 4-chloro-2-methylphenol was proposed. As shown in Fig. 4C, indirect photolysis led to the formation of two completely different TPs with *m/z* 137 at different retention times. An explanation could be the formation of constitutional isomers which did not occur by direct photolysis. TPs with those masses were not found for Mecoprop before. This might be probably due to different elucidation setups and parameters. Other studies also analyzed TP formation by hydroxyl radical induced photolysis of Mecoprop (Abramović et al., 2019; Flox et al., 2007; Meunier and Boule, 2000). According to Meunier and Boule (2000), nitrate-induced photolysis led not to different TPs compared to direct one but to a different formation pattern. We assume that TPs formed by indirect photolysis occurred by decarboxylation of acetic acid moiety and loss of chlorine and methyl substituents followed by hydroxylation at one part of the aromatic ring (para- or ortho-position). Thereby probably formed Ethoxyphenol derivatives were compared by available standards of para- and ortho-Ethoxyphenol in a short screening test but turned out to be wrong. According to analyzed fragments (*m/z* 65.1) other aromatic derivatives were assumed but could not clearly be identified.

4. Conclusion

In general, the state of knowledge regarding mechanistic aspects of the TP formation of direct and indirect photolysis seems to be vague and nowhere near understood. While there was no difference in the type of TPs formed by indirect photolysis of Penconazole and Terbutryn, indirect photolysis of Mecoprop led to the formation of different types of TPs compared to direct photolysis. This effect was not stated before.

It could be shown that the choice of an applicable irradiation source setup for laboratory photolysis is of great importance in order to analyze substances with weak overlap of their absorption spectrum with the emission spectrum of terrestrial sunlight (< 290 nm). It was shown that minimal spectral differences can have severe impact on degradation rates, which is why either well characterized and appropriate source setups quantum yields that are independent from the irradiation source should be used to analyze and estimate substances lifetime.

Although the analyzed substances are only a small group of compounds that do not represent the entire class of pesticides, this study showed that indirect phototransformation could be a relevant pathway of weakly sunlight-absorbing pesticides. This applies in particular for the formation of TPs since they reveal often unknown substances with even more unknown properties. Since the role of indirect processes of pesticides are generally not considered within the approval procedure so far, it should be taken into account when dealing with regulatory aspects, especially for substances with weak photon absorption in wavelength > 290 nm.

Acknowledgement

The authors thank Dr. Wolf-Ulrich Palm and Theo Reymann for their support. This research was funded by the Federal Ministry of Education and Research (BMBF) (02WRM1366A) support measure "Regional

water resource management (ReWaM)" in the project MUTReWa (Measures for a sustainable approach to pesticides and their transformation products in the regional water management).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.133808.

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The role of irradiation source setups and indirect phototransformation: Kinetic aspects and the formation of transformation products of weakly sunlight-absorbing pesticides

- Supplementary data -

Hensen. B.; Olsson. O.; Kümmerer. K.

S1: Chromatographic and mass spectrometric setting of analyzed substances

Parameter	**Penconazole**	**Terbutryn**	**Mecoprop**	**Benzoate**	**Metamitron**
Temperature	40°C	30!C	30°C	30°C	25°C
Flow	0.2 mL/min	0.4 mL/min	0.4 mL/min	0.3 mL/min	0.2 mL/min
Gradient	isocratic mode (45 % B)	0-1 min (10 % B), 1-11 min (50 % B), 11-18 min (85 %), 18-21 min (90 % B), 21-24 min (90 % B), 24-26 min (10 % B), 26-29 min (10 % B)	0-1 min (10 % B), 1-11 min (50 % B), 11-18 min (85 %), 18-21 min (90 % B), 21-24 min (90 % B), 24-26 min (10 % B), 26-29 min (10 % B)	isocratic mode (50 % B)	0-3 min (15 % B), 8-10 min (65 % B), 11-18 min (15 % B).
Ionization mode	+	+	-	-	/
Ionization source	ESI	ESI	ESI	ESI	/
Scan method	MS2 scan	MS2 scan	MS2 scan	/	/
Cut-off mass	100 Da	100 Da	100 Da	/	/

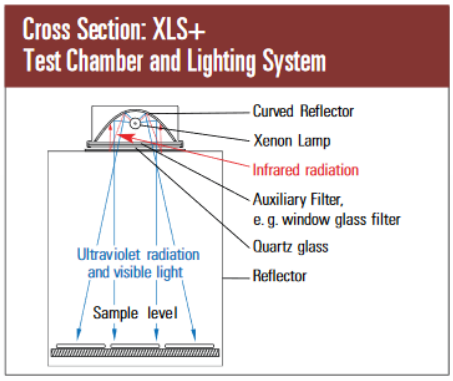
S2: Evaluation of the intraday and interday precision (in %).

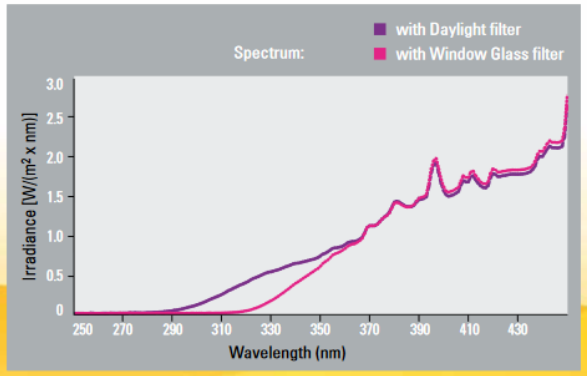
Substance	Intraday precision (n=3)	Interday precision (n=3)
Penconazole	1.7	2.0
Terbutryn	1.3	2.1
Mecoprop	2.1	2.2

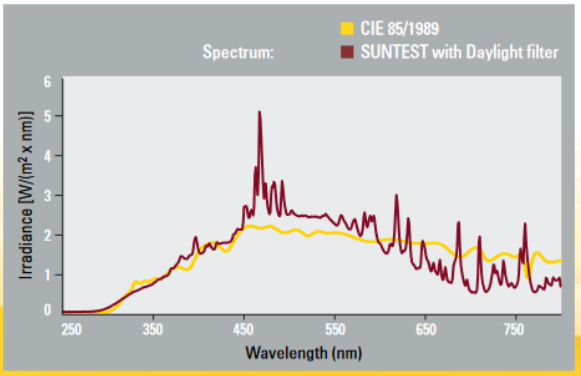
S3: Manufacturer's specification of the daylight filter (Suntest Xenon Test Instruments by ATLAS Material Testing Solutions; Source: https://www.atestor.hu/editor_up/up/egyeb/2017_02/26/14881371677176433 7/Atlas_SunTest_XLS__fenyallosag_vizsgalo_keszulek.pdf)

Solar Simulation

Atlas xenon lamps deliver consistent, even irradiance and a stable spectral power distribution. The spectral output closely matches solar radiation. The distinct advantage of the simulation of the total solar spectrum lies in the realistic reproduction of the comparable natural sample heating due to VIS and IR radiation correlated to sample color. Atlas offers a range of filters to meet industry standards such as ISO 4892-2 and ASTM G155 including both daylight and daylight behind window glass filters. Special filters tailored to specific applications are also available (please see "Optional Accessories" section).







S4: Calculation of hydroxyl radical rate constants and quantum yields

Theoretical background of the photolysis of nitrate:

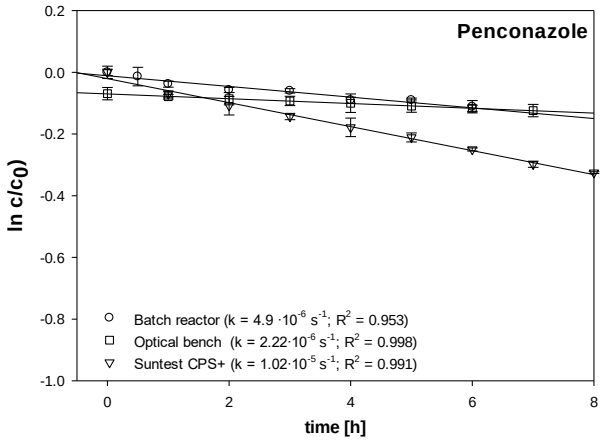
$NO^{3-} + H_2O + h\nu \rightarrow NO_2 + OH^- + OH$

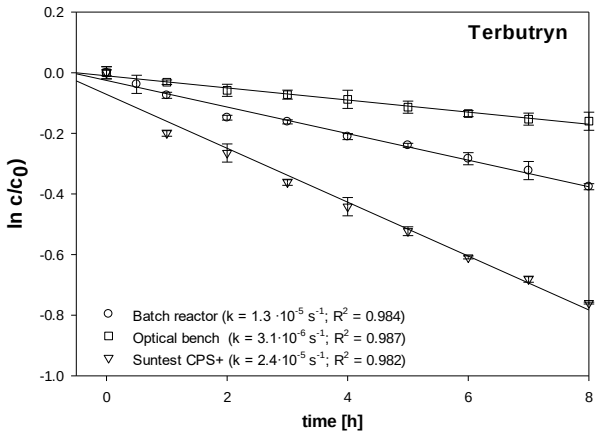
Parameter	**Description**	**Formula**
Hydroxyl rate constants	Hydroxyl radical rate constants were calculated relative to that of Benzoate as a competitor with a very well known hydroxyl radical rate constant of $k_{OH,B} = 5{,}9 \cdot 10^9$ M^{-1} s^{-1}. Benzoate was used in higher concentrations of 34 µM to eliminate the possibility of OH reacting with itself (Buxton et al. 1988). Determination of the hydroxyl rate constant of the analyzed pesticides $K_{OH,Pes}$ was done by (Eq. 2) assuming a constant OH-concentration and therefore a first-order-reaction (Palm et al. 2003) with rate constants of pesticides (k_{Pes}) and Benzoate (k_B).	$k_{OH,Pes} = K_{OH,B} \frac{k_{Pes}}{k_B}$
Actinmetry and quantum yield	Actinometric measurements were implemented with Metamitron as an actinometer (Palm 2017) to determine the absolute photon flux $J_{\lambda,abs}$ of the used photolytic devices. Absolute photon fluxes were determined by measuring the relative emission spectra ($J_{\lambda,rel}$ in counts) by an UV-Vis Spectrometer (BLACK-Comet, StellarNET, Tempa, USA) within a range of λ = 190 to 850 nm (integration time = 10 ms) and calculating the factor between relative and absolute photon flux (Eq. 3). Eq. 4 was calculated by the degradation of the actinometer Metamitron at t_0 (dc/dt) in mol L^{-1} s^{-1}, the well known quantum yield ϕ of the actinometer (Palm 2017), the molar absorption coefficient ε in L mol^{-1} cm^{-1}, the concentration of the substance in mol L^{-1} and the penetration depth of the light through the solution in cm.	$J_{\lambda,abs} = J_{\lambda,rel} \cdot F$ Factor $F = \frac{\frac{dc}{dt}}{\Phi_{Act} \cdot (\sum_{200nm}^{400nm} (J_{\lambda,rel} \cdot 2{,}303 \cdot \varepsilon_\lambda \cdot c \cdot l)}$ $\Phi = \frac{k}{2.303 \cdot J_{\lambda,abs} \cdot \varepsilon_\lambda}$
Formation rate constants	Formation rate constants of TPs at every measured time point (k_t) were evaluated by setting the peak area of TPs at a certain time point ($A_{TP,t}$) in relation to the area of parent compound at test start ($A_{Sub,t0}$)	$k_t = \ln\left(\frac{A_{TP,t}}{A_{Sub,t_0}}\right)$

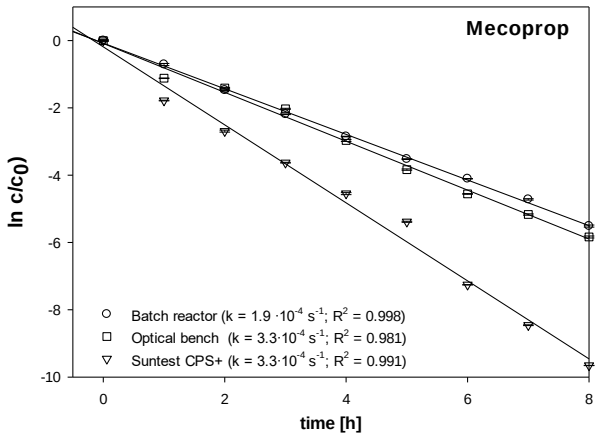
S5: Action spectra [s^{-1}] of substances of each irradiation source

Substance	Batch reactor	Suntest ® apparatus	Optical bench
Penconazole	$2.38 \cdot 10^{-4}$	$5.84 \cdot 10^{-4}$	-
Terbutryn	$1.05 \cdot 10^{-3}$	$1.95 \cdot 10^{-3}$	$2.52 \cdot 10^{-4}$
Mecoprop	$3.15 \cdot 10^{-4}$	$6.42 \cdot 10^{-4}$	$7.75 \cdot 10^{-4}$

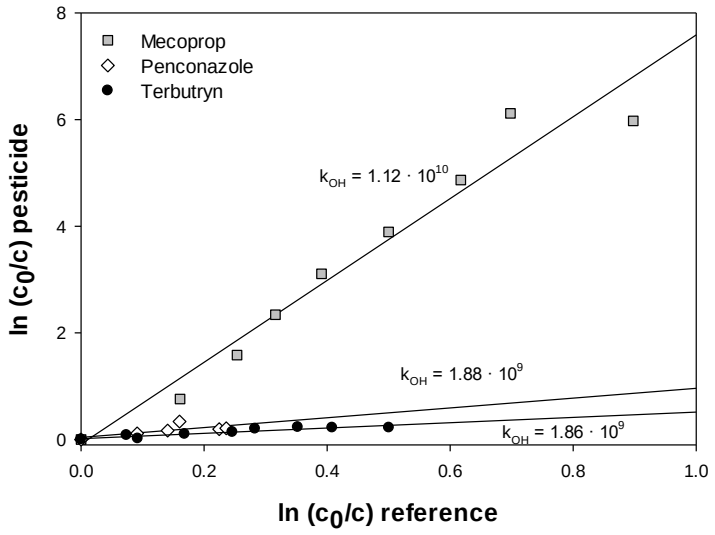
S6: Plots of Degradation rates of direct photolysis in different irradiation source (n = 2).



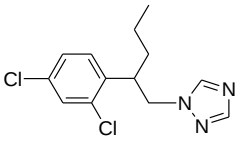
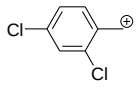
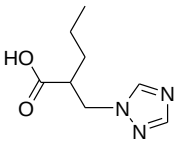
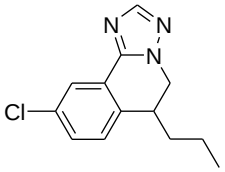
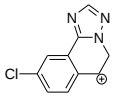
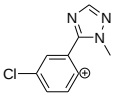
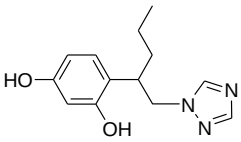
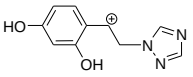
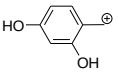
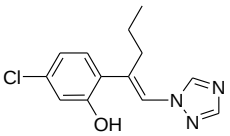
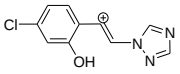
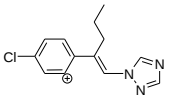
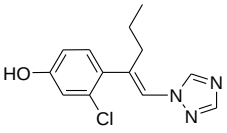
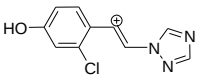
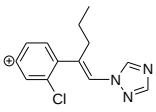
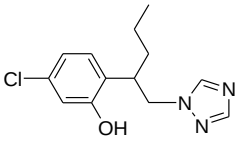
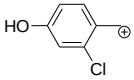
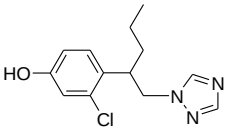
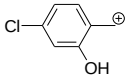


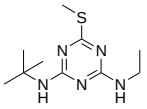
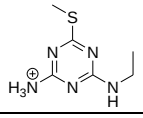
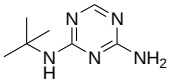
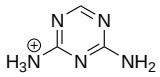
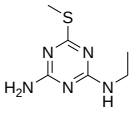
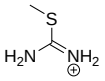
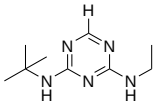
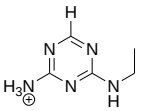
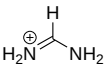
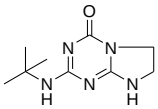
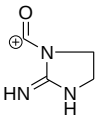
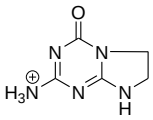
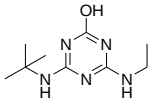
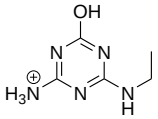
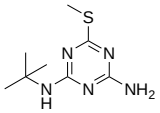
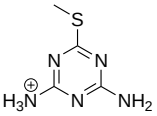
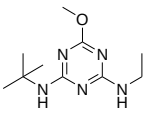
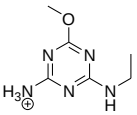
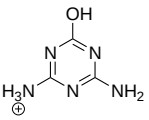
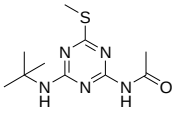
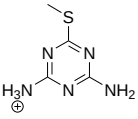
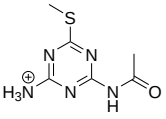
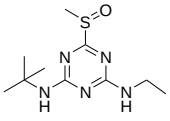
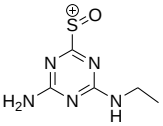
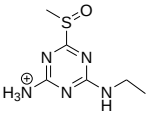


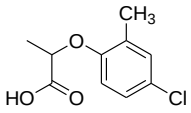
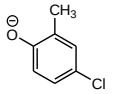
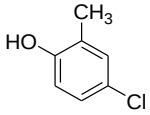
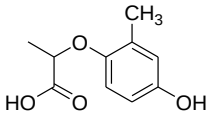
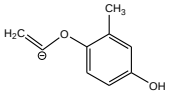
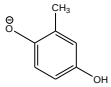
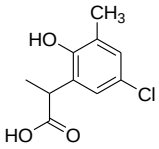
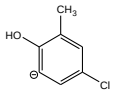
S7: Hydroxyl rate constants of Penconazole, Terbutryn, and Mecoprop with Benzoate as competitor. Degradation rates of pesticides were plotted against degradation rates of Benzoate.



S8: Fragmentation pattern of transformation products

Substance	Structural formula (proposed)	Transition 1	Transition 2
Penconazol		284.1 → 70.1  $C_2H_4N_3^+$ Exact Mass: 70.04	284.1 → 159  $C_7H_5Cl_2^+$ Exact Mass: 158.976
TP-184*		No fragments	
TP-248(a)		248. → 204  $C_{10}H_7ClN_3^+$ Exact Mass: 204.032	248.1 → 192  $C_9H_7ClN_3^+$ Exact Mass: 192.032
TP-248(b)*		248.1 → 204  $C_{10}H_{10}N_3O_2^+$ Exact Mass: 204.077	248.1 → 123  $C_7H_7O_2^+$ Exact Mass: 123.044
TP-264(a)*		264.1 → 220.1  $C_{10}H_7ClN_3O^+$ Exact Mass: 220.027	264.1 → 246.1  $C_{13}H_{13}ClN_3^+$ Exact Mass: 246.079
TP-264(b)*		264.1 → 220.1  $C_{10}H_7ClN_3O^+$ Exact Mass: 220.027	264.1 → 246.1  $C_{13}H_{13}ClN_3^+$ Exact Mass: 246.079
TP-266(a)*		266.1 → 141  $C_7H_6ClO^+$ Exact Mass: 141.01	266.1 → 69.9  $C_2H_4N_3^+$ Exact Mass: 70.04
TP-266(b)*		266.1 → 141  $C_7H_6ClO^+$ Exact Mass: 141.01	266.1 → 69.9  $C_2H_4N_3^+$ Exact Mass: 70.04

Terbutryn		242.15 → 186.1 	242.15 → 68 
TP-168		168.13 → 112 	168.13 → 70 
TP-186*		186.1 → 68.0  $C_2H_2N_3^+$ Exact Mass: 68.024	186.1 → 91.0  $C_2H_7N_2S^+$ Exact Mass: 91.032
TP-196		196.16 → 140.1 	196.16 → 45 
TP-210*		210.14 → 112.1 	210.14 → 154 
TP-212		212.2 → 156.0 	212.2 → 69.0 
TP-214		214.11 → 158 	214.11 → 68 
TP-226		226.2 → 170.1 	226.2 → 128.1 
TP-256*		256.16 → 158 	256.16 → 200 
TP-258		258.1→ 186.0  $C_5H_8N_5OS^+$ Exact Mass: 186.044	258.1 → 202.0  $C_6H_{12}N_5OS^+$ Exact Mass: 202.076

Mecoprop		213.0 → 141.0  $C_7H_6ClO^-$ Exact Mass: 141.011	213.0 → 70.9  $C_3H_3O_2^-$ Exact Mass: 71.014
TP-137(a)	-	65.1	-
TP-137(b)	-	65.1	-
TP-141		141.1 → 141.1 No fragments	-
TP-195		195.1 → 149.2  $C_9H_9O_2^-$ Exact Mass: 149.061	195.1 → 122.9  $C_7H_7O_2^-$ Exact Mass: 123.045
TP-213		155.0 → 141.0  $C_7H_6ClO^-$ Exact Mass: 141.011	-

Artikel 2

Birte Hensen, Jens Lange, Nicole Jackisch, Franziska Zieger, Oliver Olsson, Klaus Kümmerer (2018).

Entry of biocides and their transformation products into groundwater via urban stormwater infiltration systems.

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Entry of biocides and their transformation products into groundwater via urban stormwater infiltration systems

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Groundwater contamination

ABSTRACT

Biocides are, inter alia, applied as preservatives on facades to prevent the growth of microorganisms. Their incomplete mineralization results in new compounds, so-called transformation products (TPs). Rain causes that both applied biocides and their TPs leach from facades with stormwater into the urban aquatic environment. This study is the first to investigate the introduction of the biocides Diuron, Terbutryn, and Octylisothiazolinone (OIT) and their TPs into the groundwater via urban stormwater infiltration systems. In this study, the TPs of these biocides were created by laboratory photolysis and elucidated using LC-HRMS. The results were then used to analyze TPs by LC-MS/MS in stormwater and groundwater samples, which were taken from an urban swale-trench system and from groundwater wells upgradient and downgradient of the infiltration system. A sprinkling experiment was conducted to evaluate facades as a contamination source. Biodegradation tests were conducted to determine biopersistence of biocides and their TPs.

Fourteen TPs were identified under laboratory photolysis. TP-186, TP-210, and TP-256 of Terbutryn were hitherto unknown. Nine TPs were qualitatively detected in environmental water samples. Parent compounds, TP-219 of Diuron and TP-212, TP-214, and TP-226 of Terbutryn were detected at a maximum concentration of 140 ng L^{-1} during stormwater events. Concentrations in groundwater were considerably below German drinking water limits, but were higher in groundwater samples downgradient from the investigated swale-trench system than in those collected upgradient. Neither the biocides nor most of their TPs were readily biodegradable under simulated surface water conditions. The results show that entry of biocides and their TPs into groundwater is caused by infiltration of urban stormwater.

1. Introduction

Biocides are commonly applied to external thermal insulation composite systems of buildings to prevent the growth of microorganisms such as algae, fungi, and bacteria. They are, inter alia, added to water-based polymer resin paints and renders. Stormwater events cause leaching of biocides from facades and entry into urban aquatic environment, where they pose a risk to human and environment due to their toxic potential (Burkhardt et al., 2009).

The leaching processes of biocides from facade coatings by stormwater (SW) have been analyzed in a variety of studies. For example, the influence of external factors (e.g. architecture, weather exposure, and climate conditions) or physicochemical properties of substances (e.g. water solubility and octanol-water partition coefficient) were examined (Breuer et al., 2012; Burkhardt et al., 2012; Jungnickel et al., 2008; Schoknecht et al., 2009). For most substances, leaching is non-continuous and takes place at the beginning of a rain event (first flush effect). The amount of biocides on facades declines with the age of facades and is particularly high early in the lifetimes of coatings (Burkhardt et al., 2012). Various biocides have been detected in SW of urban areas (Burkhardt et al., 2011; Gasperi et al., 2014). In these areas, large fractions of SW from facades drain on paved surfaces and reach combined or separated sewer systems. In combined sewer systems,

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contaminants are discharged via wastewater treatment plants, whereas in separated sewer systems, they are dissipated into rivers or infiltration systems (Bollmann et al., 2014a, 2014b).

During the entire lifetime of biocides, transformation processes influence the fate of biocides, e.g. hydrolysis, biodegradation, and direct and indirect photolysis (Fenner et al., 2013). In most cases, incomplete degradation of organic chemicals leads to the formation of TPs, whose molecular structure, physicochemical properties, toxicity, and environmental behavior is often unknown. TPs and their parent compounds differ regarding their persistence and toxic potential (Gutowski et al., 2015; Herrmann et al., 2015; Khaleel et al., 2017; Menz et al., 2017). It is also known that TPs of several biocides are formed directly on facades by photolysis (Bollmann et al. 2016, 2017b). TPs are not only little understood but also difficult to purchase, and targeted synthesis is expensive and time-consuming. Hence, analytical data on the molecular structures and behavior are missing. Alternatively, solutions received from photolysis can be used to provide further information on chromatographic and mass-spectrometric parameters, that allow for an analysis of environmental samples (Ibánez et al., 2004). This qualitative target screening can be ranked between commonly applied suspected target screening and quantitative target screening methods (Hernández et al., 2005; Krauss et al., 2010). Moreover, this procedure has been used to verify already known or to identify hitherto unknown TPs (Herrmann et al., 2016).

Because they are commonly used in facade paints (Burkhardt et al., 2009; Bollmann et al., 2017a), this study focuses on the biocides Diuron (3-(*3,4-Dichlorophenyl*)-*1,1-dimethylurea*), Terbutryn (2-N-*tert-butyl*-4-N-*ethyl-6-methylsulfanyl-1,3,5-triazine-2,4-diamine*), and OIT (*2-octyl-1,2-thiazol-3-one*) and their related TPs.

Main focus of this study was on the pathway identification of these substances into groundwater via infiltration systems. In a first step, photodegradation experiments were performed to establish the molecular structure of TPs by means of LC-HRMS due to its high resolution and mass accuracy. Results of the photodegradation experiments were used to optimize MS-parameters in LC-MS/MS (e.g. mass transitions and quantifier/qualifier ratio) for a sensitive and selective target screening of TPs in stormwater and groundwater samples. Samples were taken from an urban district with a separated sewer system and a connected swale-trench system for SW infiltration. Finally, a sprinkling experiment was conducted on a 14-year-old facade in the contributing catchment to identify potential sources of biocide residues in the investigated swale-trench system. Closed Bottle Test (CBT) was used to assess the ready biodegradability of parent compounds and their TPs.

2. Methods

2.1. Laboratory experiments and analytical methods

2.1.1. Chemicals and reagents

Analytical standards of Diuron (99.6%), Terbutryn (99.1%), OIT (99.1%), Terbumeton (99.2%), and 2-Hydroxy-Terbutylazin (97.3%) were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Diuron-Desmethyl (97.5%), Terbutryn-Desethyl (99.8%) and internal standards Terbutryn-D5 (98.5%) and Diuron-D6 (99.0%) already dissolved in Acetonitrile (100 µg/mL) were received from Neochema (Bodenheim, Germany). Acetonitrile (LC-MS grade; VWR International GmbH, Darmstadt, Germany) was used as organic mobile phase in chromatography and for the preparation of stock solutions. Aqueous mobile phase and solutions for the implementation of photodegradation experiments were prepared with ultrapure water (Membra Pure, Germany; Q1:16.6 MΩ and Q2: 18.2 MΩ).

2.1.2. Photolytic degradation experiments

An initial concentration of 10 mg L^{-1} of test substances Diuron, Terbutryn, and OIT were used to create and subsequently identify TPs. An additional experiment was conducted twice ($c = 100$ ng L^{-1}) to determine the quantum yield (Φ) of parent compounds. Photolysis was conducted with an initial volume of 800 mL using a xenon arc lamp (TXE 150, Peschl Ultraviolet, Mainz, Germany) which closely matches the solar radiation (Yager and Yue, 1988). It was equipped with an ilmasil quartz immersion tube in a cylindrical batch reactor ($T = 20 \pm 2$ °C). Photolysis experiments were performed over a time period of 8.0 h with hourly sampling (1 mL). The absolute photon flux and the quantum yield were determined as described in supplementary material (SM) 1. As mass balances are known (Bollmann et al. 2016, 2017b), this study focused on the analysis of hitherto unknown TPs and production of solutions with TPs to receive analytical data for MS/MS to analyze the pathway of the substances into groundwater.

2.1.3. Biodegradation testing, Closed Bottle Test

A Closed Bottle Test (CBT) was performed in accordance with the OECD guideline 301D using low concentration of minerals and inoculums to evaluate whether a substance is readily biodegradable in infiltrated water and aquatic environments. A substance is classified as readily biodegradable if the measured biochemical oxygen demand (BOD) reaches at least 60% of the theoretical oxygen demand (ThOD) (OECD, 1992) within a 10-day window. 1 L of mineral medium was inoculated with two drops of sewage treatment plant effluent (approx. 500 CFU mL^{-1}). Inoculum was received from the municipal sewage treatment plant of Lüneburg (144,000 population equivalents). The test was conducted in the dark for 28 days at $T = 20 \pm 1$ °C. The initial concentration of Diuron, Terbutryn, and OIT was 4 mg L^{-1}, 3 mg L^{-1}, and 2.2 mg L^{-1}, respectively, corresponding to a ThOD of 5 mg L^{-1}, which is most suitable for the CBT (SM 8). That was done for the parent compounds and the photolytic mixture in a same manner. Samples taken at the beginning and the end of the CBT underwent LC-MS analysis. The basic experimental setups and validity criteria are described in detail elsewhere (Herrmann et al., 2015; Khaleel et al., 2017).

2.1.4. Chromatographic analysis

The elimination of Diuron, Terbutryn, and OIT and the relative abundance of newly formed compounds in the photolysis mixture were monitored by LC-ITMS [Agilent 1100; (Agilent Technologies, Waldbronn, Germany) connected to a Bruker Daltonic Esquire 6000+ (Bruker Daltonics, Bremen, Germany)] and LC-HRMS [Dionex Ultimate 3000 UHPLC system (Dionex, Idstein, Germany) coupled to a LTQ Orbitrap-XL (Thermo Fisher Scientific, Darmstadt, Germany)] in full scan and positive ionization mode. A NUCLEODUR® RP-C18 (125/2100–3 µm C18 ec) column (Macherey Nagel, Düren, Germany) was used as stationary phase, whereas 0.01% Formic acid (A) and Acetonitrile (B) were used as mobile phases with a flow of 0.4 mL/min and following gradient: 0–1 min (10% B), 1–11 min (10–50% B), 11–18 min (50–85% B, 18–21 min (85–90% B), 21–24 min (90% B), 24–26 min (90-10% B), 26–30 (10% B). Oven temperature was $T = 30$ °C and injection volume 5 µL.

2.2. Study site

The study site, situated in the southern part of the City of Freiburg (Germany) (SM 3), is located in a residential district that was developed on a former military base. The residential area was established between 1997 and 2004. In total, 153,500 m^2 of urban area are drained by a separate infiltration system, where excess SW from roofs, roads, and public places is collected via open gutters and pipes in swales. 25 swales are allocated in two linear cascades

which extend over the full length of the study site (Fig. 1). Each swale has an underground percolation trench and consists of 0.5 m organic-rich topsoil with grass vegetation located on top of a 0.2 m layer of fine sand to avoid groundwater contamination by infiltrating SW in accordance with the German technical guideline A-138 (German Association for Water, Wastewater and Waste (DWA), 2005). Trenches consist of a 0.7 m gravel layer, partly substituted by plastic blocks (Rigofill inspect ©) to increase porosity. During rain events, SW pools in swales, infiltrates via the soil-sand layer, and is temporarily stored in the trenches before it percolates towards groundwater. Two swale-trench systems were chosen for sampling (Fig. 1). The aquifer underneath the study site consists of alluvial fan deposits of up to 11 m depth underlain by an impermeable bottom layer. The groundwater is shallow with mean water tables close to the bottom level of the trenches. Seven groundwater wells are spatially distributed over the entire study site, whereby three of them are located in the upgradient (UG 1–3) and four in the downgradient (DG 1–4) of the swale-trench systems.

In total, an urban catchment of 2.95 ha (Subcatchment 1) is connected to swale S1 and trench T1. A part consists of a public/commercial area, where runoff from facades can reach the storm drainage through arcade-like walls overhanging the pervious pavement of sidewalks and open areas but also pavements touching the basement of buildings. Facades of the residential part can be considered as primarily disconnected from the SW drainage due to gravel-filled rims along the basement often combined with adjacent grassy or horticultural areas that do not generate runoff (SM 4). Subcatchment 2 (8047 m^2) is an entirely private residential area with facade runoff mostly disconnected from SW drainage analogous to the residential part of Subcatchment 1. The corresponding swale, S2, and trench, T2, receive SW from Subcatchment 2 but also excess SW from S1. For more information see Jackisch and Weiler (2015).

2.3. Sampling

Pooled runoff swale water and infiltrated trench water were collected during three SW events (Table 1). In addition, four groundwater samplings were conducted that were independent from stormwater events. A sprinkling experiment was implemented to evaluate facades as a contamination source of biocides from facade runoff into S1 during a heavy rain event. The resulting facades runoff and the runoff draining and pooling in the swale were sampled. Further information on the characteristics and conditions of the sampling site is listed in SM 4.

2.3.1. Event sampling

During each SW event, one sample per event was collected via grab sampling from water pooling in the swale. Trench water was extracted right below the SW sampling points using a peristaltic pump and a Teflon tube. To include quick preferential infiltration, one trench sample was taken with a minimum delay of 15 min to swale sampling. A probe continuously measuring water levels and electrical conductivity was installed inside T1 to verify that freshly infiltrated SW was captured (SM 5). For each sample a water volume of three liters was collected and transferred into two 1 L brown glass bottles with Teflon sealing. Bottles were stored under cool (T = 4 °C) and dark conditions and sent in a cooled package for analysis within 24 h. T1 was usually empty at the onset of rain events, while T2 was often partly filled (possibly due to a high groundwater level). Hence, data on T1 allowed for a better interpretation of biocide retention in the swale-trench system.

2.3.2. Groundwater sampling

To ensure that samples represent the same groundwater layer, wells were chosen from a large pool of monitoring wells based on similarity in electrical conductivity, oxygen, an-/cations, and stable

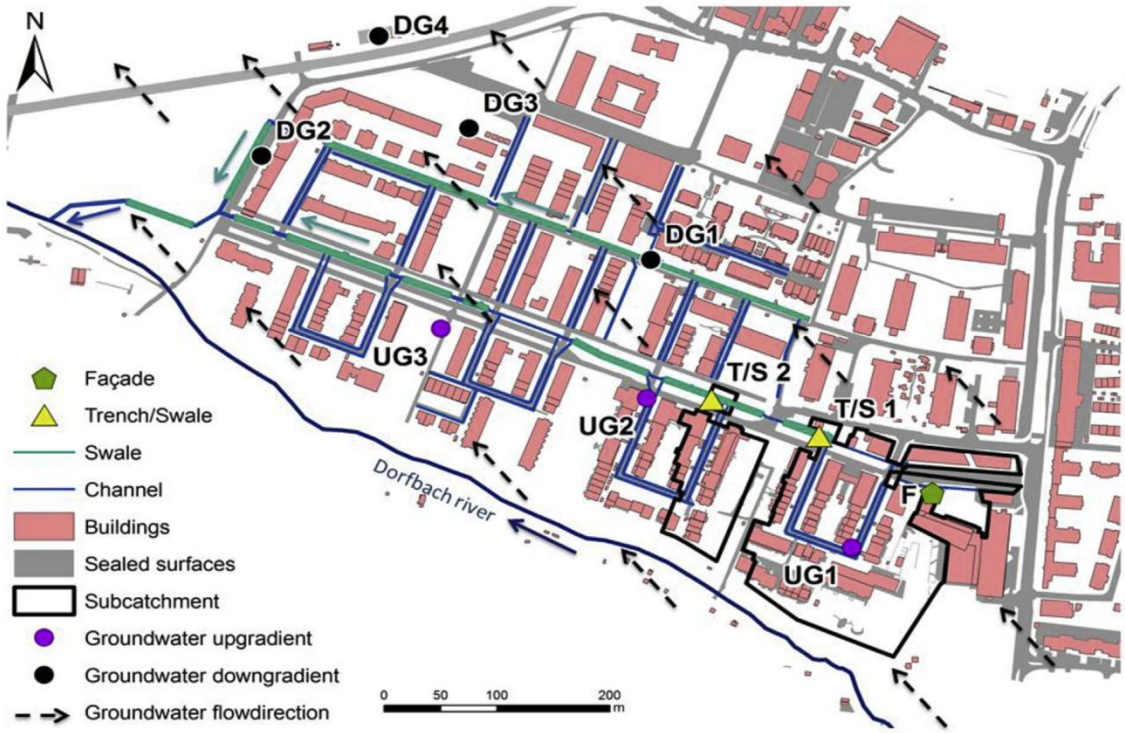


Fig. 1. Map of the study site. Swale and trench (yellow triangles, T/S 1 and T/S 2) of Subcatchment 1 and 2 (bold line) and the groundwater sampling points upgradient (purple dots, UG1-3) and downgradient (black dots, DG1-4) of the infiltration system are shown. Pentagonal, green marking (F) shows the location of the facade sprinkling experiment. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Overview of the sampling procedure implemented in two subcatchments in the City of Freiburg (Germany) during different stormwater (SW) events and four groundwater sampling campaigns. In addition, one sprinkling experiment was conducted in Subcatchment 1.

Sampling type	Sampling date	Labeling	Description	Location
Swale (S)	09.2.2016 06.11.2016 19.5.2017	Event 1 Event 2 Event 3	Pooling SW water during three events	S1 and S2
Trench (T)	09.02.2016 06.-07.11.2016 19.05.2017		Infiltrated trench water within SW events	T1 and T2
Groundwater (GW)	21.3.2016 (E1) 18.10.2016 (E2) 31.5.2017 (E3) 05.12.2017 (E4)	UG 1-3 DG 4-7	GW upgradient of the infiltration system GW downgradient of the infiltration system	Entire study site
Sprinkling experiment	27.2.2017	W T1-4	Water hose (blank sample) Time points of (a) direct facade runoff, (b) draining runoff and (c) pooling runoff in the swale	Facade of Subcatchment 1

oxygen isotopes concentrations. For each sampled well, two liters were extracted twice using a submersible pump (stainless steel) and Teflon tubes following the general guidelines on representative groundwater sampling (ISO 5667-11). Groundwater sampling was carried out as part of a regular monitoring campaign that takes place twice a year, once in early spring (i.e. after the main groundwater recharge period for the study area) and once in autumn (i.e. after the dry summer period usually accompanied by numerous intense storms).

2.3.3. Facade sprinkling experiment

A sprinkling experiment was carried out on a 14-year-old, red-painted facade within Subcatchment 1 (Fig. 1, location F). The objective of this experiment was to verify the release and transport pathway of biocides and TPs from a facade into the infiltration system and to a lesser extent to reproduce leaching under realistic precipitation conditions. Hence, our experiment should be regarded as a worst case scenario, since pressure, amount and pH of the applied water did not correspond to a natural rainfall. The facade still appeared very bright and clean after 14 years of exposure. Using tap water from a nearby hydrant and a water hose (SM 3, Figs. 2), 18,000 L were applied within two hours at a constant rate ($= 150\,L\,min^{-1}$). Four samples from each sampling point, i.e., direct facade runoff, draining runoff, and pooling runoff in the S1 were taken at different time points over a course of two hours (Table 1). In addition, a blank sample was taken directly from the water hose. T1 stayed dry during the experiment, which prevented sampling of the trench water.

2.4. Preparation and measurement of environmental samples

Diuron, Terbutryn, OIT, and TPs were analyzed in a total number of 55 samples in duplicates using Triple Quadrupole (Agilent Technologies, 1200 Infinity LC-System and 6430 Triple Quad, Waldbronn, Germany). Further information on sample preparation and instrumental analysis can be found in the SM 2. After qualitative detection of TP-219 of Diuron and TP-212, TP-214, and TP-226 of Terbutryn in environmental samples, analytical reference standards of Diuron-Desmethyl (TP-219), 2-Hydroxy-Terbutylazin (TP-212), Terbutryn-Desethyl (TP-214), and Terbumeton (TP-226) were used for a second analysis of samples that showed quantifiable peak areas of these TPs.

3. Results and discussion

3.1. Photolysis of Diuron, Terbutryn, and OIT

Calculated quantum yields of $\phi = 0.005 \pm 7\%$ (Diuron), $\phi = 0.006 \pm 9\%$ (Terbutryn), and $\phi = 0.012 \pm 16\%$ (OIT) varied slightly compared to previous studies, where quantum yields were higher by a factor 4 (Diuron) and 8 (Terbutryn) (Canle et al., 2005; Jirkovský et al., 1997). Although quantum yields observed here and in other studies are very low and the overlap of absorption spectra of substances (SM 1) with the emission spectrum of the sunlight (>290 nm) is small, these substances might undergo direct photolytic degradation processes in the environment with a low probability. However, formation of TPs on facades by photolysis was demonstrated by some studies. But they also showed that TPs formed by biodegradation and photodegradation are similar (Bollmann et al. 2016, 2017a, 2017b; Tixier et al., 2001). Thereby TPs that might probably be formed by biodegradation in other compartments of this study area (e.g. soil phases) were included in environmental analyses.

It is been found by photolysis dark experiments and in additional laboratory experiments according to OECD 111 that analyzed biocides are hydrolytically stable over a long time period.

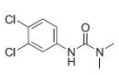
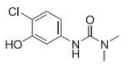
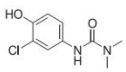
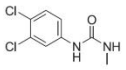
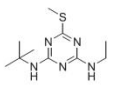
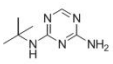
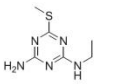
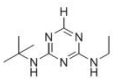
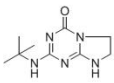
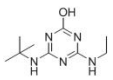
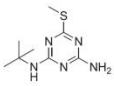
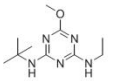
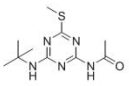
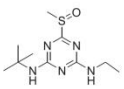
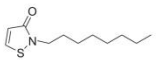
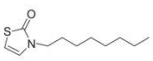
Under given analytical conditions (e.g. ionization and chromatography), LC-MS non-target screening indicated the presence of at least 7 TPs related to Terbutryn, 3 to Diuron, and 2 to OIT. Structures of four out of fourteen TPs were validated by an analytical standard (Table 2).

Photolysis of Diuron led to dehalogenation and subsequent hydroxylation of the phenyl group (TP-215a and TP-215b) and demethylation of dimethylurea group (TP-219). This is a result that is in line with other studies (Jirkovský et al., 1997; Tanaka et al., 1986; Tixier et al., 2001).

As e.g. Bollmann et al. (2016) stated, photolysis of Terbutryn results in the substitution of the methylthio group by hydrogen (TP-168, TP-196), hydroxy (TP-212), and ether (TP-226). TP-168 and TP-214 developed through the loss of the ethyl group. TP-258 was formed by oxidation of the methylthio group resulting in methylsulfoxide group. For Terbutryn, two newly identified TP (with m/z of 210 and 256) were tentatively elucidated, suggesting that TP-210 emerged from replacement of the methylthio group by oxygen and photocyclisation of the ethyl group forming imidazole. TP-256 was probably formed by ketonization of the ethyl group and TP-186 by separation of tert-butyl group. This assumption was confirmed, to some extent, by the observed fragmentation patterns and the exact masses (SM 7). TP-214 is also a metabolite (M1) of the biocide Irgarol and could also be formed by its degradation (Okamura, 2000). TP-212, also known as 2-Hydroxyterbutylazin, was previously analyzed as TP of the plant protection agent Terbutylazin as one of the most frequently detected TPs in surface water and groundwater (Reemtsma et al., 2013).

Photolysis of OIT led to the formation of TP-214 by

Table 2
Molecular and structural formula of elucidated TPs. Fragmentation voltage (FV) and collision energy (CE) and first and second transition to fragmentation mass of biocides and their photo-TPs. Structures of TP-186, TP-210, and TP-256 of Terbutryn are proposed due to missing verification by analytical standard or literature.

Substance	Known synonyms	Molecular formula	Structural formula	Transition 1 (FV/CE)	Transition 2 (FV/CE)	Analytical standard
Diuron		$C_9H_{12}ClN_2O$		233.03 ->72.1 (96/17)	233.03 -> 56 (96/57)	Yes
TP-215a	3-(3-chloro-4-hydroxyphenyl)-l, 1-dimethylurea	$C_9H_{10}ClN_2O_2$		215.06 -> 72.1 (98/25)	215.06 -> 46.1 (98/13)	No
TP-215b	3-(4-chloro-3-hydroxyphenyl)-l, 1-dimethylurea	$C_9H_{12}ClN_2O_2$		215.06 -> 72.1 (98/25)	215.06 -> 46.1 (98/13)	No
TP-219	DCPMU; Diuron-Desmethyl; 1-(3,4dichlorophenyl)-3-methylurea	$C_8H_8Cl_2N_2O$		219.01 -> 127 (98/33)	219.01 -> 162 (98/13)	Yes
Terbutryn		$C_{10}H_{19}N_5S$		242.15 ->186,1 (91/13)	242.15 -> 68 (91/45)	Yes
TP-168	Desthiomethyl-Desethyl-Terbutryn	$C_7H_{13}N_5$		168.13 -> 112 (98/13)	168.13 -> 70 (98/29)	No
TP-186	–	$C_6H_{11}N_5S$		186.1 -> 68.0 (195/37)	186.1 -> 91.0 (195/17)	No
TP-196	Desthiomethyl-Terbutryn	$C_9H1_7N_5$		196.16 -> 140.1 (88/13)	196.16 -> 45 (88/25)	No
TP-210	–	$C_9H_{15}N_5O$		210.14 -> 112.1 (112/25)	210.14 -> 154 (112/9)	No
TP-212	2-Hydroxy-Terbutryn; 2-Hydroxy-Terbutylazin	$C_9H_{17}N_5O$		212.2-> 156.0 (107/13)	212.2 -> 69.0 (107/45)	Yes
TP-214	Terbutryn-Desethyl; M1; Descyclopropyl-irgarol	$C_8H_{15}N_5S$		214.11 -> 158 (93/13)	214.11 -> 68 (93/45)	Yes
TP-226	Terbumeton	$C_9H_{19}N_5O$		226.2-> 170.1 (102/13)	226.2-> 128.1 (102/17)	Yes
TP-256	–	$C_{10}H_{18}N_5SO$		256.16 -> 158 (122/25)	256.16 -> 200 (122/13)	No
TP-258	Terbutryn sulfoxid	$C_{10}H_{19}N_5OS$		258.1-> 186.0 (107/17)	258.1-> 202.0 (107/13)	No
OIT		$C_{11}H_{19}NOS$		214.13 -> 102 (86/9)	214.13 -> 57.1 (86/13)	Yes
TP-130	Octylamine	$C_8H_{19}N$		130.2 -> 57 (73/8)	130.2 -> 43.1 (73/16)	No
TP-214	3-Octylthiazol-2(3 H)-one	$C_{11}H_{19}NOS$		214.13 -> 102 (86/9)	214.13 -> 57.1 (86/13)	No

photoisomerization and TP-130 through breakage of the thiazole ring, as it has been elucidated by Bollmann et al. (2017b). This study observed that those TPs are formed on facades although they found a higher number of TPs than it was here. One possible explanation might be that this study used a different irradiation source (i.e., UV radiation), which implies higher power and a broader wavelength overlap of emission and absorption spectrum.

3.2. Aerobic biodegradation testing

Concerning the biodegradation test, all validity criteria of the OECD guideline were fulfilled. Results of the investigated CBT for the parent compounds showed no biodegradation regarding ThOD and still 0% after 28 days in CBT for Diuron, Terbutryn, and OIT. Therefore, all three substances can be classified as not readily biodegradable substances according to the test guideline, a result that is confirmed by primary elimination rates as found by LC-MS. The recovery of Diuron, Terbutryn, and OIT after 28 days, was $85 \pm 1.7\%$, $102 \pm 2.8\%$, and $90 \pm 0.3\%$, respectively, based on LC-MS analysis. The biodegradation of photolysis mixtures taken after 8 h of irradiation showed a slight increase of elimination of 15% (photolysis mixture of Diuron), 25% (photolysis mixture of Terbutryn), and 30% (photolysis mixture of OIT). This indicates the possibility of biodegradation for some photo-TPs in biodegradation tests. The samples taken at the beginning and end of each CBT were analyzed by LC-MS. Most of the detected TPs showed a recovery of approximately $102 \pm 11\%$ after 28 days, whereas other TPs were further biodegraded: TP-130 of OIT and TP-215(a) and TP-215(b) of Diuron showed a recovery of 0%, $5.8 \pm 0.2\%$ and $16.2 \pm 2\%$, respectively. Results received here are not directly comparable with results from literature due to the usage of differing groups of microorganisms and test conditions. In this study, we analyzed ready biodegradability under conditions which matches those of surface water. High persistence of Terbutryn and Diuron and high degradation rates of isothiazolinones were observed in case of using soil microorganisms over a test period of 120 days by Bollmann et al. (2017a). Tixier et al. (2000, 2001) observed fungal degradation of Diuron. Bollmann et al. (2017a) also stated lower persistence of TPs of OIT than TPs of Terbutryn as it was found here. No further TPs were found to be formed by biodegradation of photo-TPs. Except for the TPs that show high rates of degradation, the biocides and TPs examined here are likely to persist in the aquatic environment. For this reason, it might be possible to detect them in environmental samples.

3.3. Occurrence of biocides and their TPs in stormwater and groundwater

Replicates of all analyzed environmental samples showed errors of 16.2% (Diuron), 13.7% (Terbutryn), and 16.8% (OIT) on average, indicating that the method applied here was very precise. The presence of nine TPs in environmental samples could be shown by qualitative target screening with parameters received from photolysis experiments. Qualitative data was confirmed for TP-212, TP-214, and TP-226 of Terbutryn and TP-219 of Diuron by repeating the measurements with available reference standards. Thereby actual concentrations were determined for four out of 14 TPs. Furthermore, this re-analysis of samples allowed for a confirmation of qualitative results of the previous analyses.

Analyses of the water samples showed that all three biocides could be detected in all sampling types (swale, trench, and groundwater) (Table 3). Apart from TP-215(a) and TP-215(b) of Diuron, TP-130 of OIT, and TP-186 and TP-258 of Terbutryn, all TPs could be detected in at least 5% of analyzed samples. TPs that were persistent in previously described biodegradation tests were found in environmental samples. The general low recovery of TPs of OIT is in line with studies showing that TPs tend to remain on facades (Bollmann et al., 2017b) and degrade rapidly in soil (Bollmann et al., 2017a). TP-226 of Terbutryn is usually linked to the plant protection agent Terbumeton (Bollmann et al., 2016), but since its use was banned in Germany, the present results can be explained by the transformation of Terbutryn. TP-219 of Diuron was the most common one in 69% of samples.

Terbutryn (detected in 81% of all stormwater samples) and its TP-212 (75%) and TP-214 (69%) were predominantly found in stormwater samples and Diuron (100%) and its TP-219 (82%) in groundwater samples. OIT occurred infrequently in all sampling types. This is in accordance with Bollmann et al. (2017a, 2017b), who observed high degradation rates of OIT in soil and high retention on facades. Therefore, higher detection frequency of OIT in swale water and lower one in trench water and groundwater might be explained by high persistence in surface water (chapter 3.2.) but low lifetimes in soil phases (Bollmann et al., 2017a).

3.3.1. Transport of biocides and TPs during SW runoff events

Quantified concentration levels of biocides and their TPs in SW runoff (Fig. 2) were in accordance with findings in urban catchments in Denmark and Switzerland (Bollmann et al., 2014a; Burkhardt et al., 2011). Diuron was only detectable in S1 after SW runoff Event 1 ($c = 4.9\ ng\ L^{-1}$) but in T1 at Event 2, which suggests other sources than the infiltrating water from S1 or rather a continuous release from the soil phase. Its TP-219 was detectable in S1 during Event 2 ($c = 2.3\ ng\ L^{-1}$) and in the underlying trench in similar concentrations (Fig. 3). Most previous studies focused on TP-219 of Diuron as a plant protection agent in agriculture (Field et al., 1997; Gatidou et al., 2004; Reemtsma et al., 2013; Wittmer et al., 2010). Only a few studies have investigated the occurrence of TP-219 of Diuron in urban runoff so far (Reemtsma et al., 2013; Wittmer et al., 2010). If it was detected at all, concentration levels were comparable with those found in this study.

Terbutryn was found in all samples taken from S1 and S2 in a range of $1–160\ ng\ L^{-1}$ (Fig. 2). Concentrations of Terbutryn in S2 ($c_{max,S2} = 6.4\ ng\ L^{-1}$) were much lower than in S1 ($c_{max,S1} = 160\ ng\ L^{-1}$), which indicates that the concentration in the urban SW strongly depends on the amount of facades and especially their type and age connected to the infiltration system. In this case, Subcatchment 2 was smaller than 1.

Seven (S1) and five (S2) Terbutryn-TPs were detected in swale samples. Both TP-256 and TP-214 occurred frequently in runoff water. This result is very much in line with Bollmann et al. (2016), who showed that of the Terbutryn-TPs leaching from facades TP-214 was the most important one. In our study, the maximum concentration was $72.8\ ng\ L^{-1}$. TP-214 was also present at $2–3\ ng\ L^{-1}$ in trench water, a result that might be due to retention by the soil layer during the infiltration process. However, application of its additional parent compound Irgarol in the study site cannot be ruled out. Therefore, measured concentrations might not be a result of Terbutryn alone. TP-212 was present up to $23.2\ ng\ L^{-1}$ in swale samples and up to $11\ ng\ L^{-1}$ in trench samples. It was also detected in urban runoff water in similar concentrations by Reemtsma et al. (2013). However, these authors did not link this TP to Terbutryn as its parent compound but to Terbutylazin instead. TP-226 was not detected in runoff samples. This is in accordance with literature, which suggest that this is one of the Terbutryn-TPs that tends to remain on facades (Bollmann et al., 2016). Here, maximum concentrations of TP-226 were detected in trench water ($15.9\ ng\ L^{-1}$). Because the concentrations in trench water were higher than in swale water, this TP probably occurred by biodegradation of adsorbed Terbutryn on soil (Bollmann et al., 2017a) that might had entered by earlier stormwater events. Therefore, it is

Table 3
Frequency of detection of biocides and their TPs in every analyzed sampling type (stormwater, groundwater, and sprinkling water) of biocides and their TPs. All 55 samples were measured in duplicates (n = 110).

Substance		Frequency of detection > LOQ [%]				
		Stormwater		Groundwater n = 56	Sprinkling experiment n = 22	Total n = 110
		Swale n = 12	Trench n = 20			
Parent compounds	Diuron	17	40	100	100	80
	Terbutryn	100	70	57	55	64
	OIT	50	0	14	18	16
TPs of Diuron	TP-215a	0	0	0	0	0
	TP-215b	0	0	0	0	0
	TP-219	17	30	82	100	69
TPs of Terbutryn	TP-168	33	40	0	0	11
	TP-186	0	0	0	0	0
	TP-196	17	0	11	0	7
	TP-210	50	10	0	0	7
	TP-212	50	90	0	0	20
	TP-214	67	70	50	0	38
	TP-226	0	70	0	0	22
	TP-256	83	40	11	0	22
	TP-258	0	0	0	0	0
TPs of OIT	TP-130	0	0	0	0	0
	TP-214	0	0	14	0	5

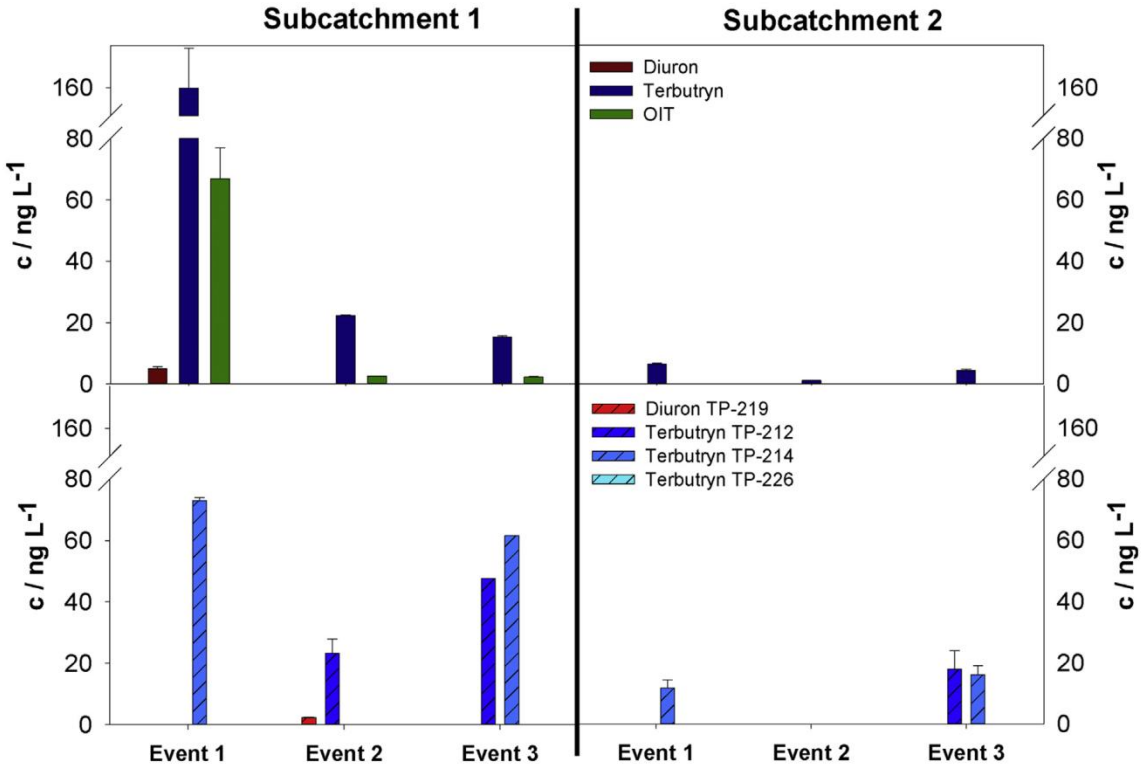


Fig. 2. Concentration of biocides in ng L^{-1} (top) and associated TPs (bottom, dashed bars) in Swale 1 and Swale 2 at three sampling events in 2016 and 2017.

impossible to trace their environmental fate as precisely as the other TPs. In general, number and concentration of the majority of detected TPs decreased in the trench compared to swale water. It may thus be concluded that a part was retained in the soil layer without a complete elimination from the water phase. OIT was only quantifiable in S1 but for all three events in concentrations between 2.5 and 66.8 ng L^{-1}. However, OIT was not detected in the underlying T1, a result that might be due to retention in the soil layer and more likely its rapid degradation in soil as it has been shown by Bollmann et al. (2017a).

The decreasing concentration of biocides during SW events may be caused by the intra-event variability of biocide concentrations since the low temporal resolution of two replicates per event is insufficient to display first flush effects (Olsson et al., 2013).

3.3.2. *Infiltration of biocides and TPs into groundwater*

In this study, biocides and some TPs were detected in infiltrated groundwater of an urban area for the first time. Other studies

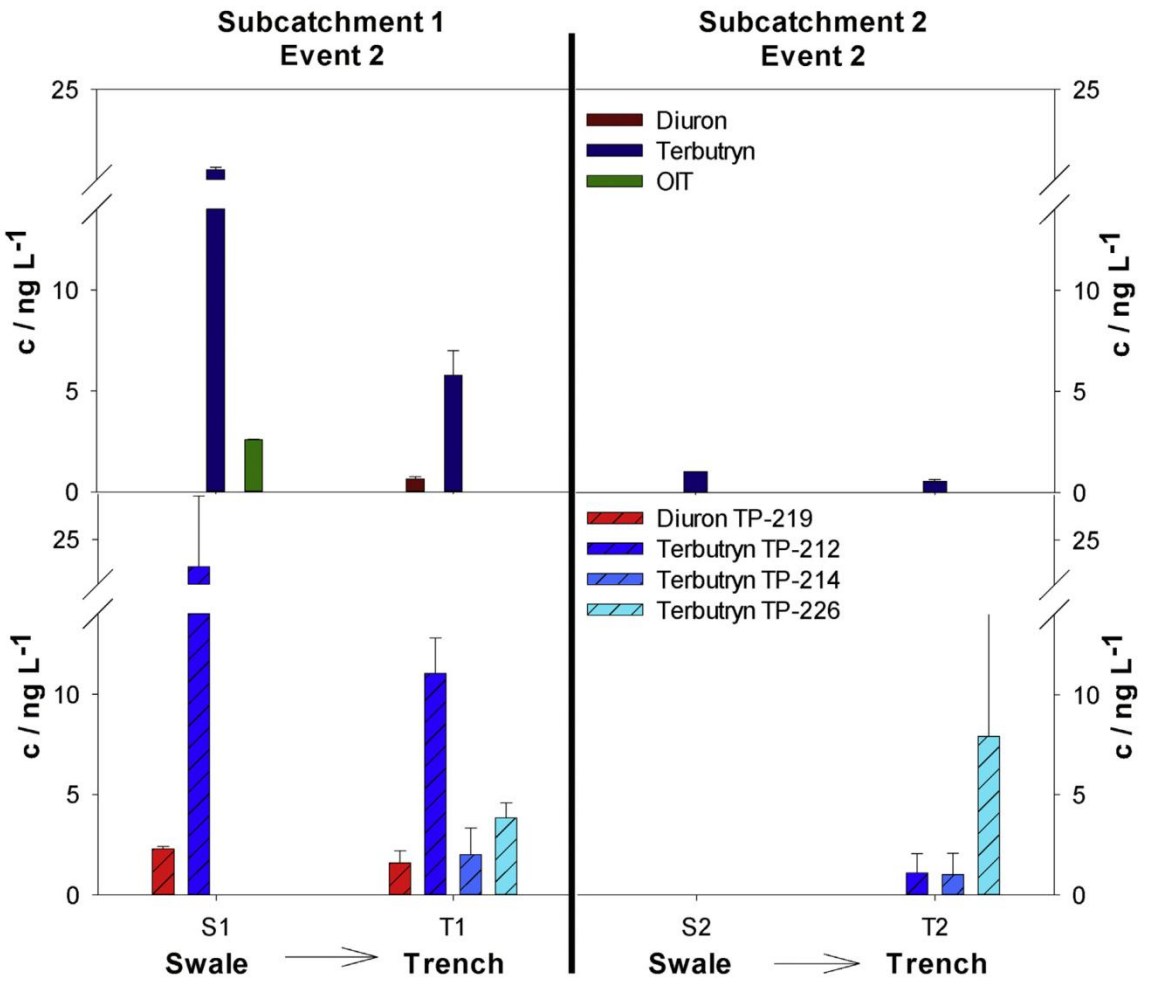


Fig. 3. Concentration of biocides in ng L^{-1} (top) and associated TPs (bottom, dashed bars) in Swale1 and Swale 2 and the infiltrating water in the Trench1 and Trench 2 of the sampling Event 2.

focused on groundwater affected by the use of plant protection agents in agriculture and related TPs (Field et al., 1997; Gooddy et al., 2002; Quednow and Püttmann, 2007; Reemtsma et al., 2013). One study analyzed infiltrated groundwater of a separated sewer system but could not detect biocides (Bollmann et al., 2014b).

The comparison of mean concentrations of downgradient (DG) (8.8 ng L^{-1}) groundwater samples of the swale-trench system to upgradient (UG) ones (c1.1 ng L^{-1}) showed an increase in the mean concentration of Diuron by a factor of 8 (Fig. 4). Concentrations of TP-219 were up to $c_{max} = 7.3$ ng L^{-1}. Increasing concentrations in DG samples (factor of 3.7) were found for this compound as well. Due to its presence in S1 and T1, it might have entered groundwater via the swale-trench system. Observed concentrations in groundwater were higher compared to those in stormwater. There seemed to be continuous release from the soil phase due to the relatively high adsorption affinity of Diuron compared to Terbutryn which could be indicated by the retention times (Terbutryn < Diuron) as a rough approximation. Additionally for TP-219, it is possible that it was formed by microorganisms in the soil layer.

Terbutryn was only detected in DG samples at maximum concentrations (c_{max}) of 7.6 ng L^{-1} except the three UG samples taken during Event 1 and 4. TP-214 of Terbutryn was also only detected in DG at $c_{max} = 3.3$ ng L^{-1}, except two samples in UG. This indicates that Terbutryn and its TP-214 most likely entered the groundwater via the swale-trench system.

In contrast to the trends observed for Terbutryn and Diuron, concentrations of OIT remained unchanged. It was detected rather infrequently at the whole study site and only during the second and fourth sampling event ($c_{max} = 1.4$ ng L^{-1}). The low presence of OIT and its TPs in groundwater samples and the fact that they were not found in trench samples may be an indication for high soil degradation rates as it was stated before (Bollmann et al., 2017a).

In summary, the number of biocides and their TPs were higher in the groundwater downgradient (49%) of the swale-trench-system than upgradient (27%). The increase of concentrations of Diuron, Terbutryn, and their TPs in downgradient samples strongly suggests groundwater contamination by the swale-trench system. However, since some of these substances were also detectable in upgradient samples, other potential pathways into groundwater need to be considered, such as rapid infiltration through gravel-filled rims at the basement of facades and diffuse infiltration through grassed and permeable pavements.

3.3.3. Assessment of biocide transport from facade to SW infiltration by sprinkling experiments

During the artificial sprinkling experiment (Fig. 5), Diuron leached from the facade with maximum concentrations of 17.7 ng L^{-1} and was also found in draining and pooling runoffs in S1 ($c_{max} = 7.7$ ng L^{-1}). TP-219 leached from the facade in thirtyfold higher concentrations ($c_{max} = 691.8$ ng L^{-1}) and was also detected

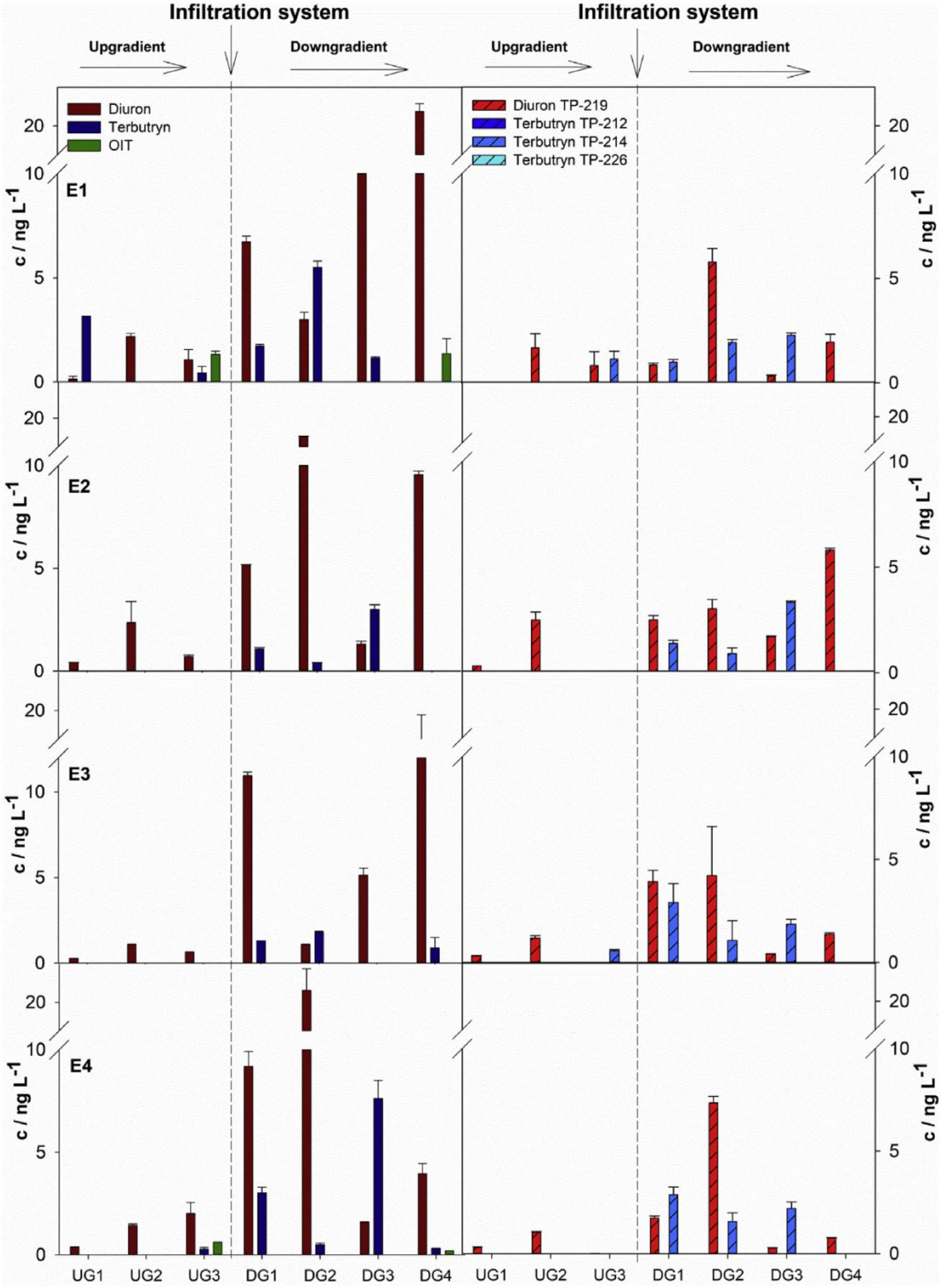


Fig. 4. Detected concentrations of biocides (left) and their related TPs (dashed bars, right) within four groundwater samplings. Wells are ordered in the flow direction of the groundwater with decreasing distance to the infiltration system (UG 1–3) and decreasing distance after its inflow (DG 1–4). Well locations are depicted in Fig. 1.

in pooling runoff with $c_{max} = 378.2$ ng L^{-1}. For Diuron, sprinkling runoff concentrations were similar to those in SW runoff. This was, however, not the case for TP-219. Burkhardt et al. (2012) found, conversely, a low recovery of TP-219 on an artificial wall under natural conditions and assumed further degradation processes. The duration of exposure and facade material, e.g. pigment color (Urbanczyk et al., 2017) seem to influence the transformation of Diuron to TP-219. Terbutryn leached from the facade in low

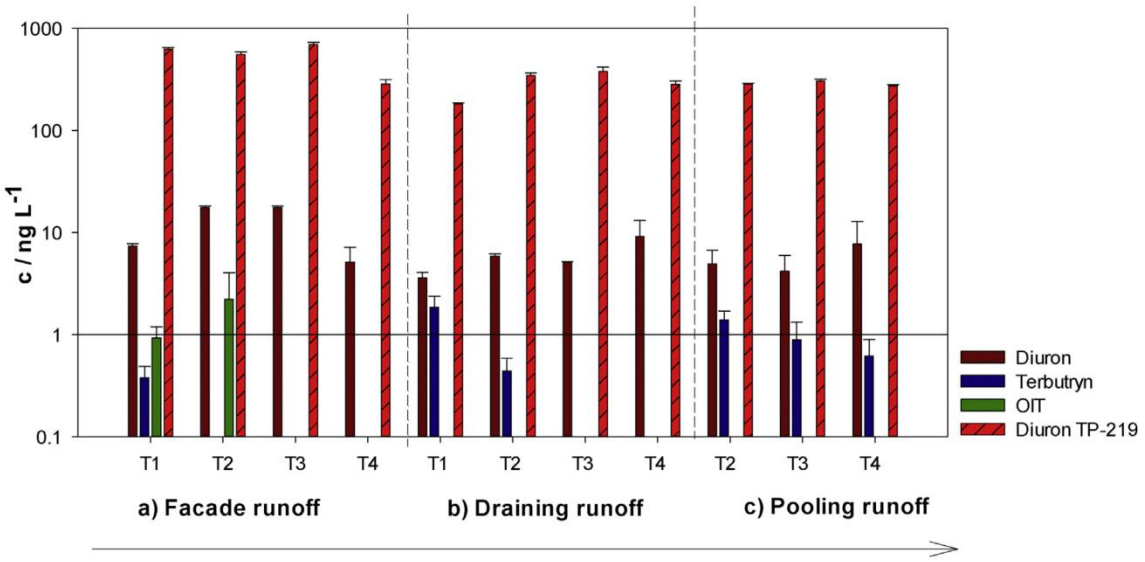


Fig. 5. Concentrations of biocides and the TP-219 of Diuron (in ng L^{-1}, logarithmic scale) in a facade sprinkling experiment at 4 different time points (T1-4). Samples were taken at three points: runoff water from facade (a), the draining runoff water (b), and the pooling runoff from the Swale 1 (c).

concentrations of 0.4 ng L^{-1} but was detected in higher concentrations in the S1 up to $c_{max} = 1.9$ ng L^{-1}. OIT was only detected in two samples of facade runoff but not in draining or pooling runoff samples. Not one of its TPs was detected.

Given these results, it is possible that Terbutryn and OIT (and their TPs) might have leached from other facades of the contributing drainage areas since both were detected in the swale during SW events. The results of the sprinkling experiment suggest that biocidal residues are released from facades and then transported to the swale-trench system. As discussed in the introduction, even a fourteen-year-old facade still releases biocidal residues. Higher concentrations of the parent compound can be expected in runoff from newer facades (Burkhardt et al., 2012). The in-situ development of TPs on facades described in other studies was confirmed here (Bollmann et al., 2016, 2017b).

4. Conclusion

This study analyzed the leaching of biocides and their TPs from facades during stormwater events into groundwater. We combined qualitative target screening with data provided by laboratory photolysis and confirmed the results using reference standards if they were available. Using this approach, we identified three new TPs of Terbutryn and overall nine different TPs in stormwater and groundwater samples.

This study extends the knowledge on the transport pathways of biocides and their TPs in urban stormwater and groundwater. Facades were confirmed as biocide sources releasing contaminants over their entire life span. The comparison of two subcatchments suggests that the mobilized contaminant concentrations strongly depend on study site characteristics such as different facade types and their connection to the stormwater drainage system. Since groundwater concentrations increased in downgradient groundwater, we hypothesize that biocidal groundwater contamination is caused by the investigated infiltration system.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.watres.2018.07.046

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Entry of biocides and their transformation products into groundwater via urban stormwater infiltration systems

- Supplementary material –

Birte Hensen, Jens Lange, Nicole Jackisch, Franziska Zieger, Oliver Olsson, Klaus Kümmerer

SM 1: Absorption spectra, absolute photon flux and calculation of quantum yield

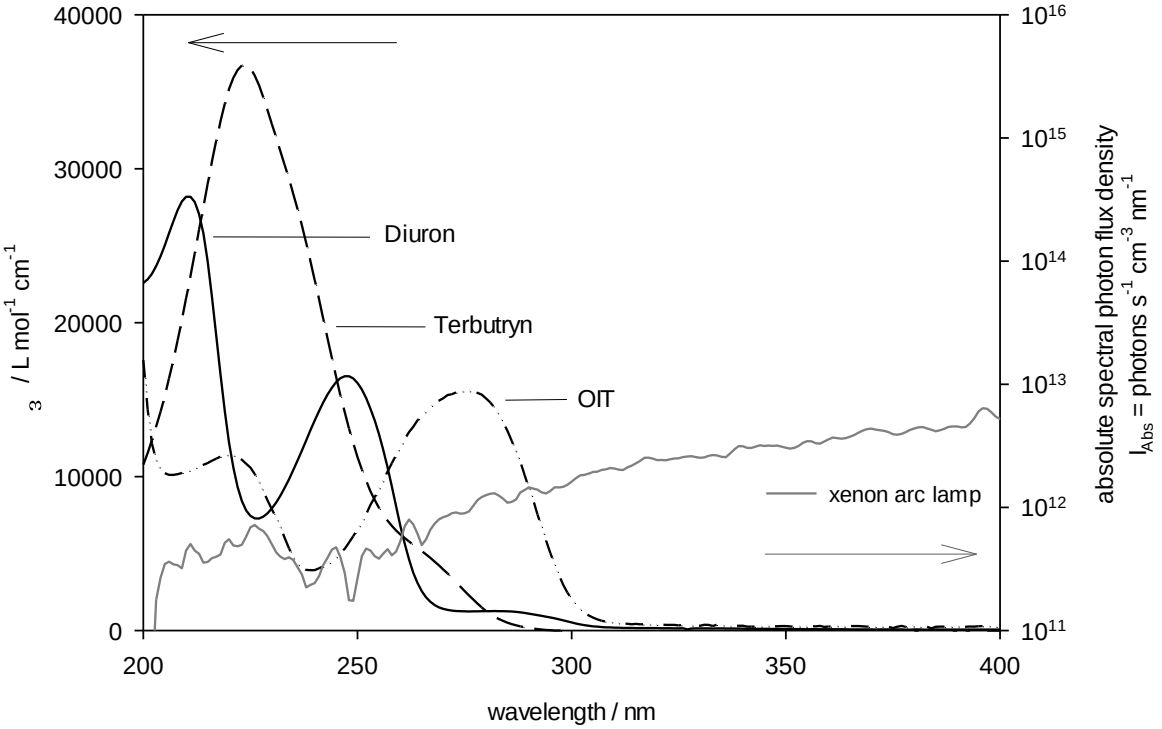
UV-Vis spectra (Lambda 45, Perkin Elmer, Massachusetts, USA) were measured in quartz cuvettes (d = 1 cm) with a resolution of 2 nm in 1 nm steps, baseline correction was done with ultrapure water. Actinometric measurements were implemented with Metamitron as an actinometer to determine the absolute photon flux $J_{\lambda,abs}$ of the used photolytic devices. Absolute photon fluxes were determined by measuring the relative emission spectra ($J_{\lambda,rel}$ in counts) by an UV-Vis Spectrometer (BLACK-Comet, StellarNET, Tempa, USA) within a range of λ = 190 to 850 nm (integration time = 10 ms) and calculating the factor between relative and absolute photon flux:

$$J_{\lambda,abs} = J_{\lambda,rel} \cdot F \quad (1)$$

$$\text{Factor } F = \frac{\frac{dc}{dt}}{\Phi_{Act} \cdot (\sum_{200nm}^{400nm} (J_{\lambda,rel} \cdot 2{,}303 \cdot \varepsilon_{\lambda} \cdot c \cdot l)} \quad (2)$$

with the degradation of the actinometer Metamitron at t_0 (dc/dt) in mol L^{-1} s^{-1}, the well known quantum yield ϕ of the actinometer the molar absorption coefficient ε in L mol^{-1} cm^{-1}, the concentration of the substance in mol L^{-1} and the penetration depth of the light through the solution in cm. Quantum yields Φ of each substance in each device to verify calculated absolute photon fluxes were determined by:

$$\Phi = \frac{k}{2.303 \cdot J_{\lambda,abs} \cdot \varepsilon_{\lambda}} \quad (3)$$



SM 2: Detailed description of the preparation of environmental samples

Preparation of environmental samples (approx. 1 liter; 2 liters for groundwater samples) was done by filtering with a folded filter (type 113 P Cellulose ø 240 mm). Supernatant was spiked with the internal standard Diuron-D6 (10 µl of 10 mg L^{-1}). Extraction procedure was a solid phase extraction (SPE). Cartridges (CHROMABOND® HR-X 6 mL/200 mg) were conditioned with 10 mL methanol and washed with 10 mL pure water. Environmental samples were enriched on the cartridges via teflon capillary and a vacuum extraction unit. After enrichment of the samples cartridges were washed with 5 mL pure water and air dried for about 5-10 minutes. Elution was done with a solvent mixture of methanol and chloroform (v/v; 1:1). The eluted phase was dried with nitrogen to dryness and 200 µl acetonitrile was added. 90 µL of the extract were spiked with 10 µl of Terbutryn-D5 as an internal standard. Each sample was a double determination. Measurements of environmental samples were conducted with a Triple Quadrupole (Agilent Technologies, 1200 Infinity LC-System and 6430 Triple Quad, Waldbronn, Germany).

Recovery was determined by spiking water samples with 100 µl of analytical standard (1 mg L^{-1}) and was found to be (97.7 % (Diuron), 88.5 % (Terbutryn) and 93.5 % (OIT), 85.0 % (Diuron TP-219), 66.2 % (Terbutryn TP-226), 50 % (Terbutryn TP-212) and 92 % (Terbutryn TP-214). The linearity between peak area and concentration of substances were obtained in a range of 0 - 5 µg L^{-1}. Hence limits of detection (LOD) and quantitation (LOQ) were calculated with DINTEST (2003) according to DIN 32645 and amounted to 0.11 and 0.39 ng L^{-1} (Diuron), 0.11 and 0.38 ng L^{-1} (Terbutryn), 0.21 and 0.77 ng L^{-1} (OIT), 0.25 and 0.93 ng L^{-1} (Diuron TP-219), 0.22 and 0.74 ng L^{-1} (Terbutryn TP-226),

0.19 and 0.67 ng L^{-1} (Terbutryn TP-214) and 0.22 and 0.77 ng L^{-1} (Terbutryn TP-212) considering an enrichment factor of 5000. As no analytical standards of TPs were available for the primal investigation of TPs, the following procedure was chosen to evaluate the results of the TP analysis for environmental samples. Elucidated masses of TPs of the photolytic mixtures were optimized and fragmented in the Agilent triple quadrupole. Thus, quantifier and qualifier transition could be preserved with related optimized fragmentation voltage (FV) and collision energy (CE). Qualifier and quantifier peak areas were detected in a defined ratio (relative response). To evaluate and validate the obtained results of environmental samples, quantifier-qualifier-ratios had to reach similarities with the photolytic mixture of ± 20 %. Equally, retention times of TPs in environmental samples had to conform with the photolytic mixture (RT ± 0.5 min.). Values for LOD and LOQ were assessed by evaluating an S/N-ration of 3 (LOD) and 10 (LOQ) referred to blank values.

SM 3: Photography's of the study site (2008)

Figure 1: Overview over the study site in Freiburg (aerial photograph 2008). Source: Stadt Freiburg, Luftbilder Vauban, http://www.freiburg.de/servlet/PB/menu/1236743_l1/index.html

Figure 2: *Left*: Swale S1 after a storm event (Sub-catchment 1 with arcade-like overhanging facades in the back; red facade = facade of sprinkling experiment). *Right:* Façade during sprinkling experiment (since façade overhangs a paved surface, façade wash-off is connected to the storm drainage and the capturing BR facility)

SM 4: Characteristics of the study site

Table 2: Land cover of the study site (as of 2011), modified after Jackisch & Weiler (2015).

Land cover type	**Total connected area (ha)**	**Total connected area (%)**	**Description**
Vegetated roofs	2.0 ha	13.1	Mostly extensive; incl. green balconies and roof terraces
Pebble roofs	0.2 ha	1.4	
Pervious pavements (concrete paver blocks; some gravel)	2.4 ha	15.9	On sidewalks, public places, public parking
Grass-covered areas	5.4 ha	35	Open spaces, back yards, swales of the BRS
Horticultural areas	1.5 ha	9.5	Front yards, gardens, tree pits
Impervious pavement	1.8 ha	11.8	Streets and bike lanes, ground-level terraces
Conventional roofs (tin, tile)	2.1 ha	13.4	Incl. non-greened balconies and roof terraces

Table 3: Characteristics of the study site (modified after Jackisch & Weiler (2015).

Parameter	Value	Remarks
Total connected area (A_{con})	15.34 ha	Area connected to the infiltration system
Runoff coefficient (RC)	0.47	Area-weighted average
Imperviousness	25.4%	Aerial Percentage of 100% impervious areas
Average height	278 m	a.s.l.
Average slope	1.2%	Terrain slope
Population density	145	inh./ha
Mean annual precipitation	955 mm	1961-90
Mean annual temperature	10.8 °C	1961-90
GW slope	1.4-1.5%	Source: Wibel, Leinenkugel und Partner (1994): „Hydrogeologische und geotechnische Untersuchungen auf dem „Vauban"-Gelände in Freiburg-Merzhausen", survey
hydraulic conductivity (kf) aquifer	1,00E-04 m/s	
effective porosity aquifer	0,15	
transport velocity aquifer	0,80 m/d	

SM 5: Sampling data

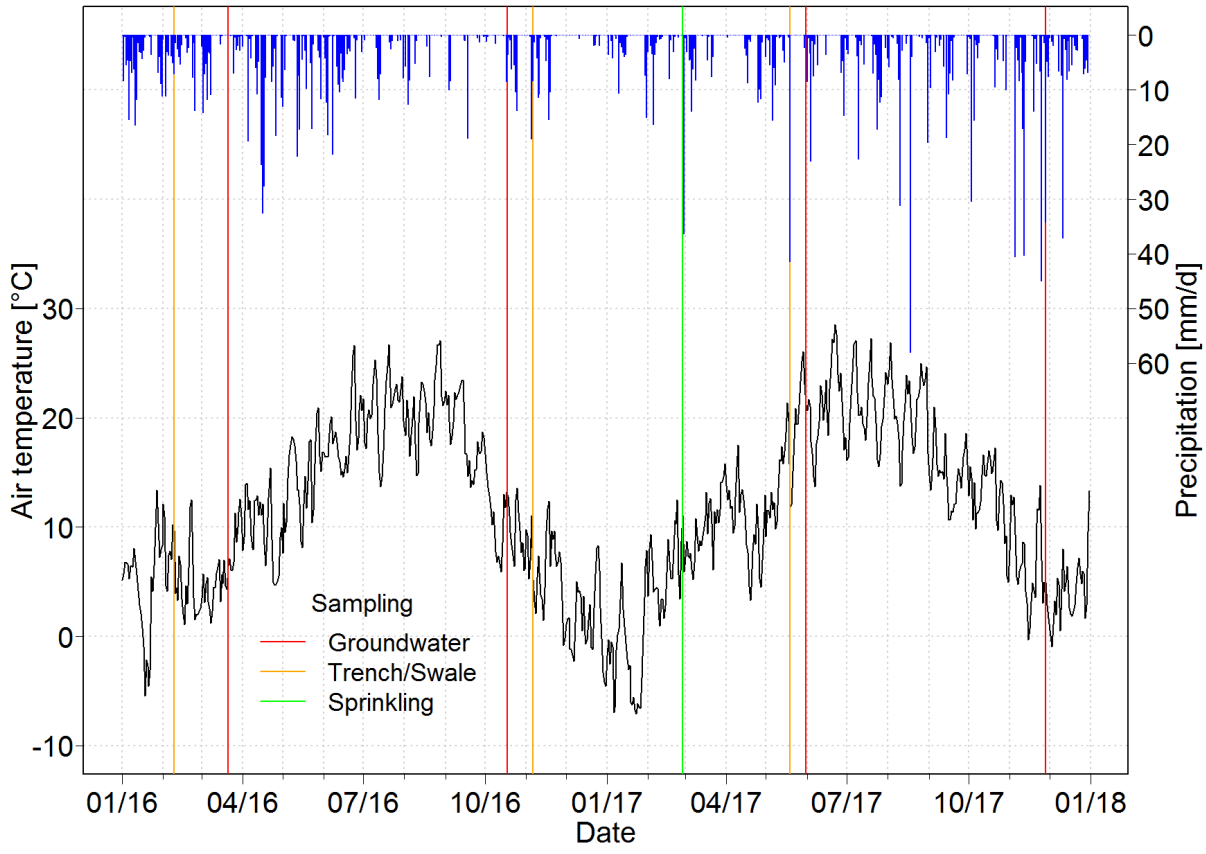


Figure 4: Time series of air temperature (daily average) and precipitation (daily total) for the whole period of samplings. Indicated are event samplings 1-3 (yellow lines; 9.2.2016, 6.11.2016, 19.5.2017), the three GW sampling campaigns (red lines; 21.03.2016, 18.10.2016, 31.05.2017, 05.12.2017) and the facade sprinkling experiment (green lines; 27.2.2017) (Source: WBI (State Viniculture Institute Freiburg) and LTZ (Agricultural Technology Centre Augustenberg).

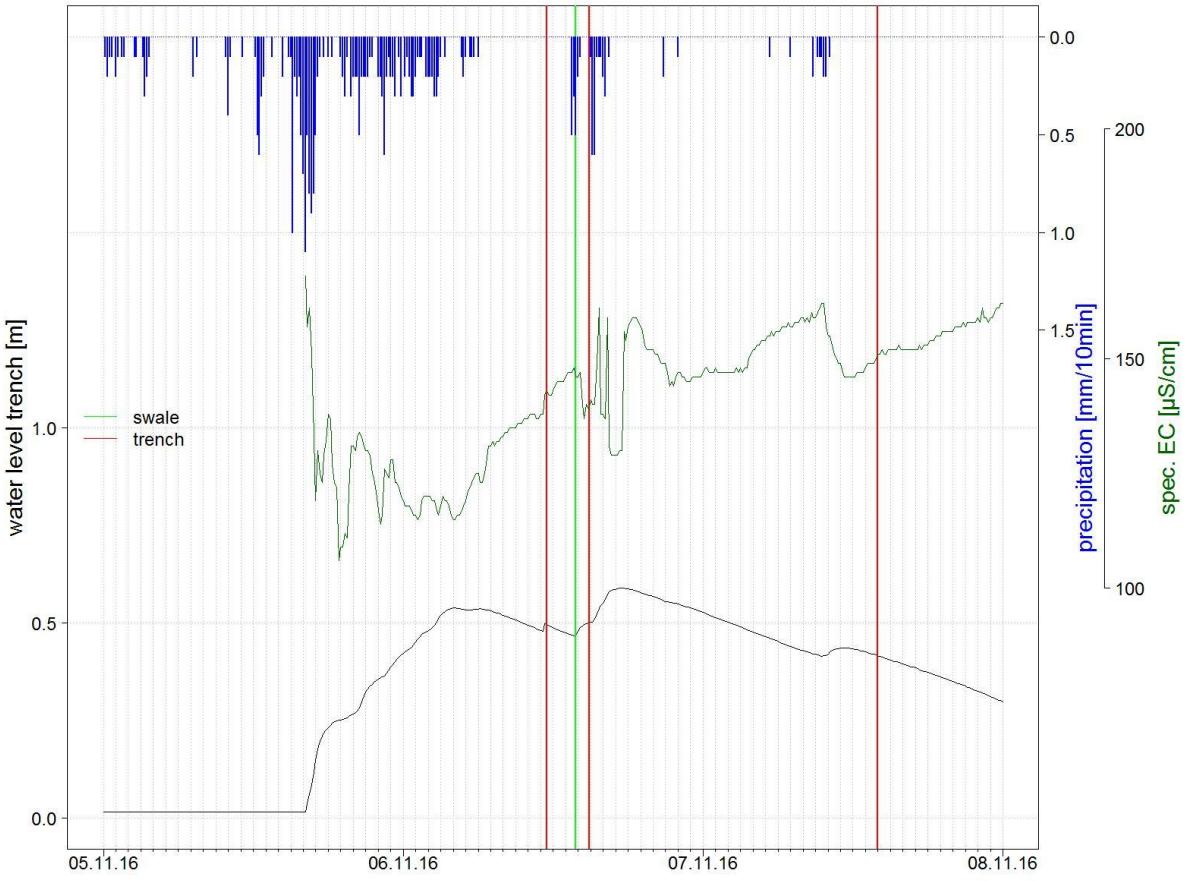


Figure 5: Time series of precipitation, water level and specific electrical conductivity in trench T1 during Event 3**.** Colored lines indicate precise sampling times for samples of subcatchment 1.

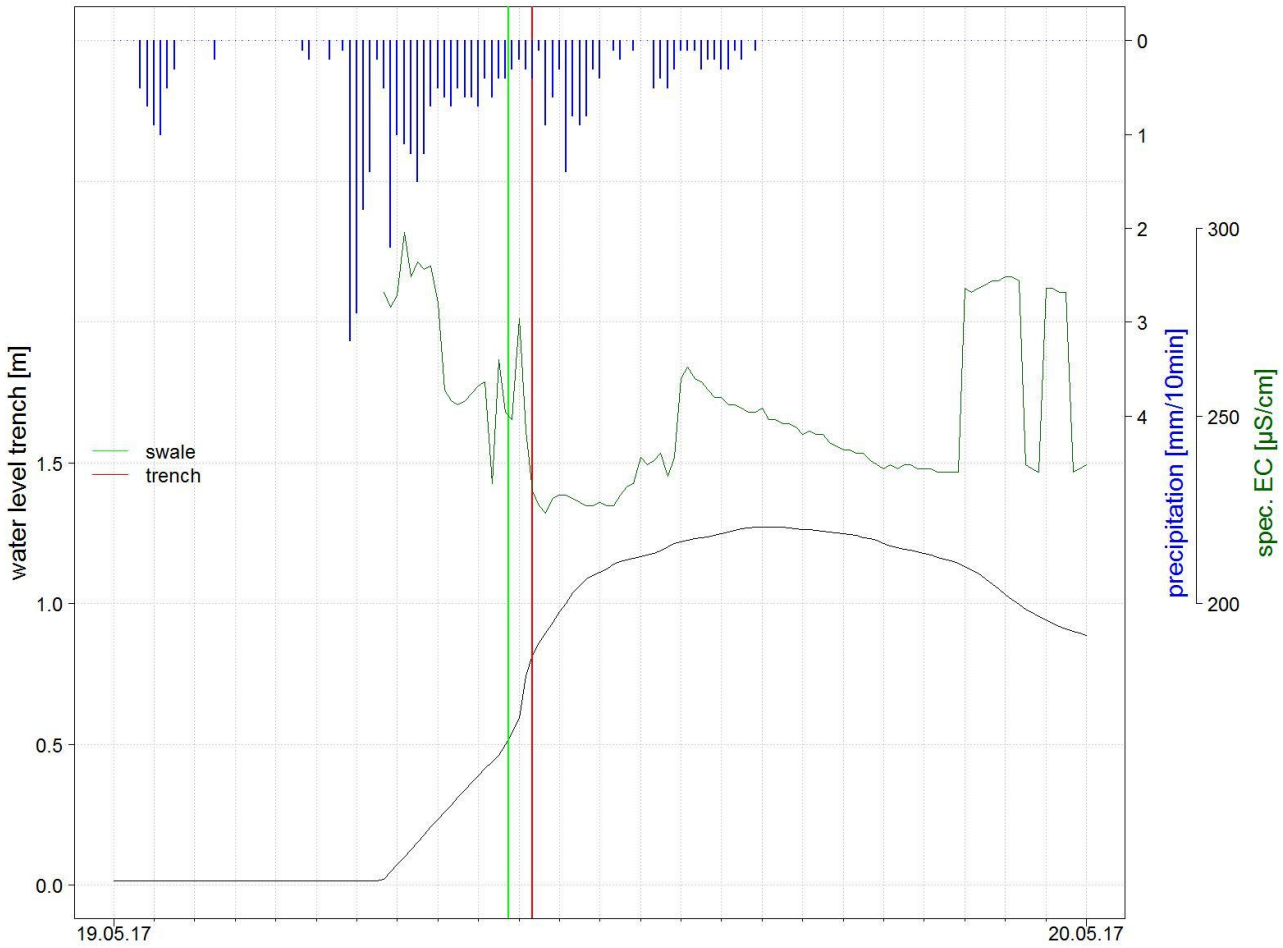


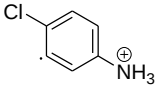
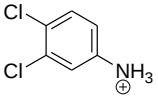
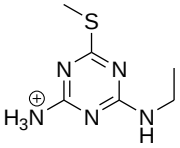
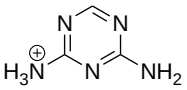
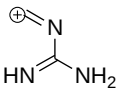
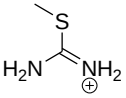
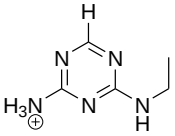
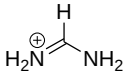
Figure 6: Time series of precipitation, water level and electrical conductivity in trench T1 during Event 4. Colored lines indicate precise sampling times for samples of sub-catchment 1.

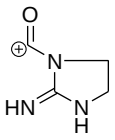
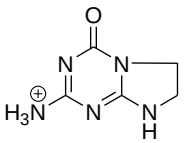
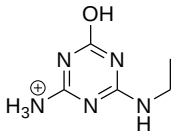
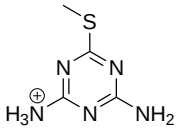
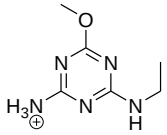
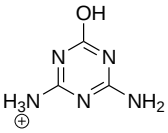
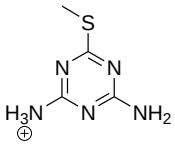
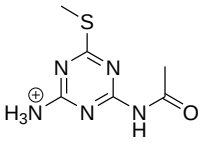
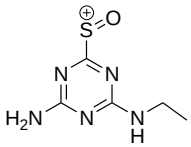
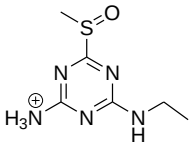
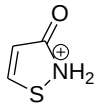
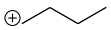
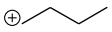
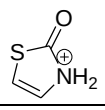
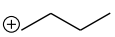
Table 4: Stormwater characteristics and meteorological conditions during event sampling.

	sampling time	**storm depth (mm)**	**storm period**	**solar influence on ponding SW**	**antecedent wetness**	**description**	**overflow S1 into S2**
Event 1	14:30	5.5	9.2.2016 5:00-9:00 and 12:30-19:00 (weak intensities)	weak	very wet (5 mm rain on 8.2.2016 and several rain events the 3 weeks before)	weak and small storm after a series of rain events	no
Event 2	13:50	26	5.-6.11.2016 16:00	weak	dry (no rain 2 days before onset)	main storm volume on 5.11. with moderate intensities after 15:00	no
Event 3	9:45	40	19.05.2017 2:00-6:00 and 7:00 - 12:00	weak	dry (no rain 4 days before onset)	main storm volume during 2nd storm period	yes

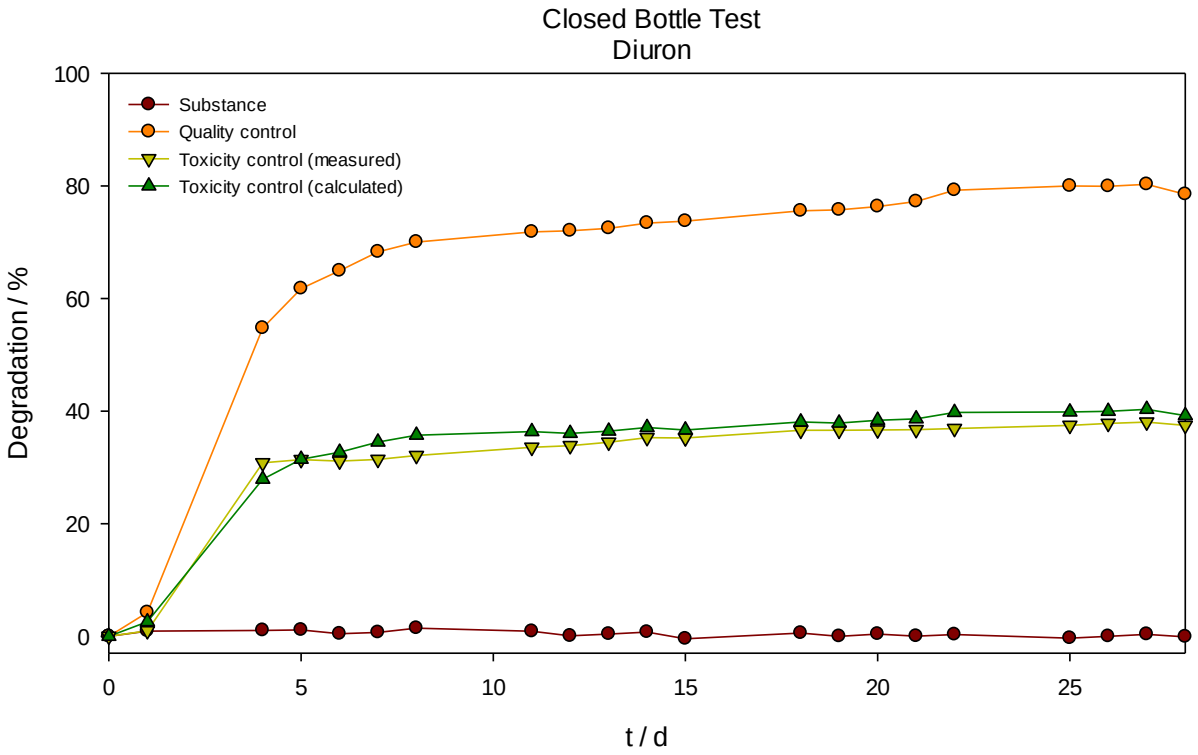
SM 6: Elucidated TPs

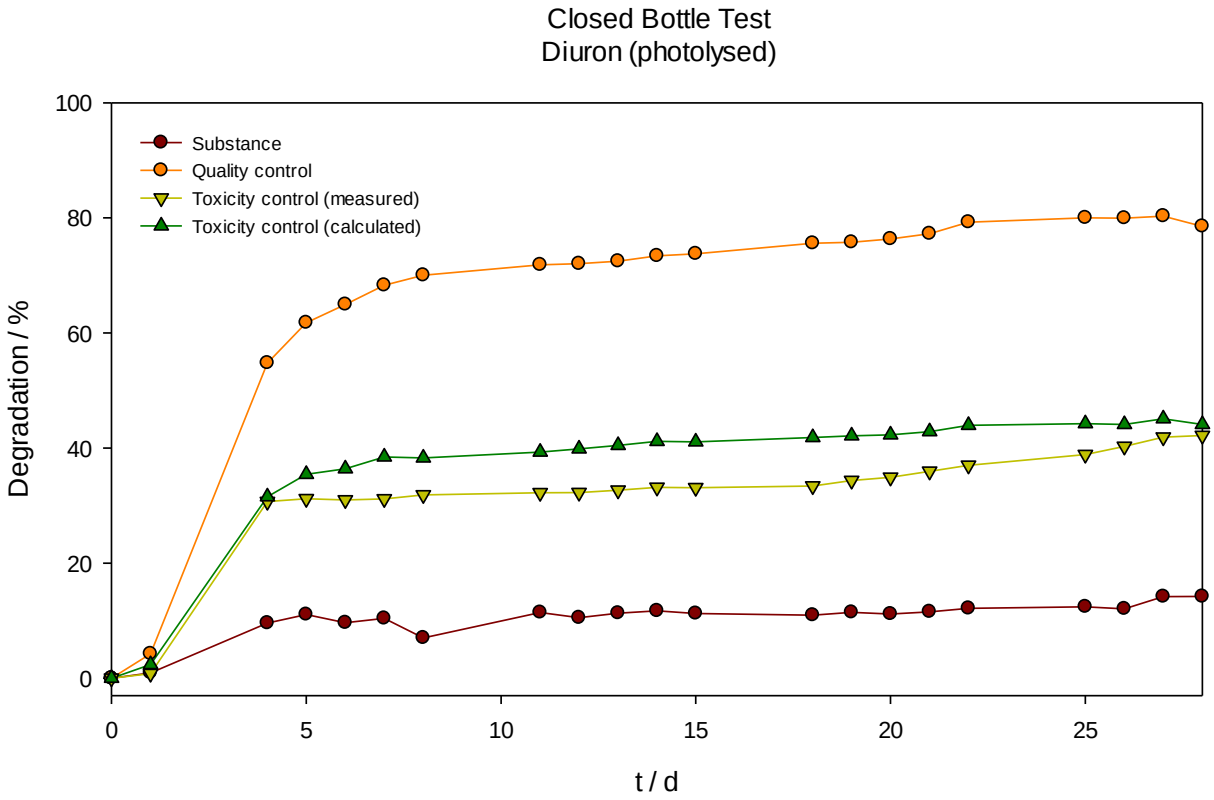
Table 5: Analytical parameters of elucidated TPs for the measurement of biocides and their Photo-TPs. Fragmentation voltage (FV) and Collision energy (CE).

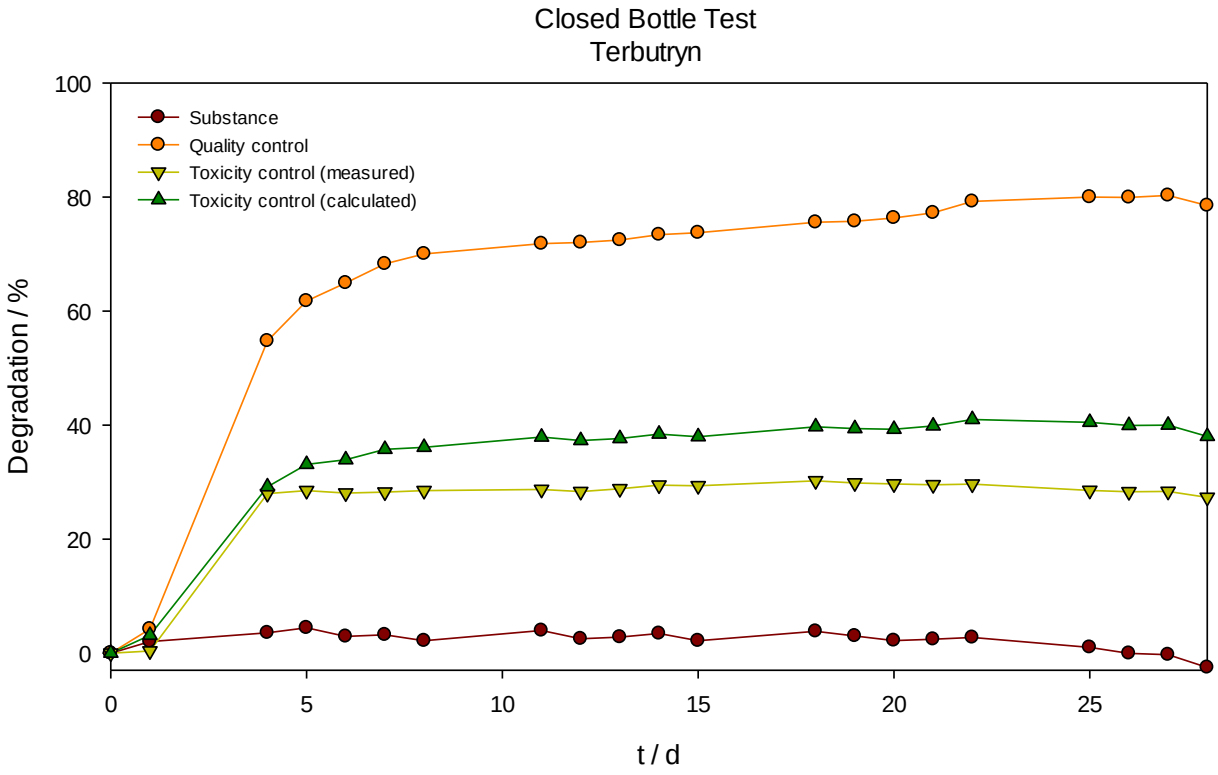
Exact mass MA (ppm)	Molecular formula	RT [min]	Transition 1 (FV/CE)	Transition 2 (FV/CE)
233.0275 (1.37)	C9H12ClN2O	8.5	233.03 ->72.1 (96/17) 	233.03 -> 56 (96/57) 
215.0607 (1.16)	C9H10ClN2O2	2.0	215.06 -> 72.1 (98/25) 	215.06 -> 46.1 (98/13) 
215.0607 (1.16)	C9H12ClN2O2	3.1	215.06 -> 72.1 (98/25) 	215.06 -> 46.1 (98/13) 
219.0112 (1.19)	C8H8Cl2N2O	7.9	219.01 -> 127 (98/33) 	219.01 -> 162 (98/13) 
242.1468 (1.40)	C10H19N5S	7.5	242.15 ->186,1 (91/13) 	242.15 -> 68 (91/45) 
168,1265 (0.71)	C7H13N5	1.0	168.13 -> 112 (98/13) 	168.13 -> 70 (98/29) 
186.0813 (0.00)	C6H11N5S	2.4	186,1 -> 68,0  $C_2H_2N_3^+$ Exact Mass: 68.024	186,1 -> 91,0  $C_2H_7N_2S^+$ Exact Mass: 91.032
196.1582 (1.02)	C9H17N5	1.5	196.16 -> 140.1 (88/13) 	196.16 -> 45 (88/25) 
210,1375 (0.95)	C9H15N5O	1.3	210.14 -> 112.1 (112/25)	210.14 -> 154 (112/9)

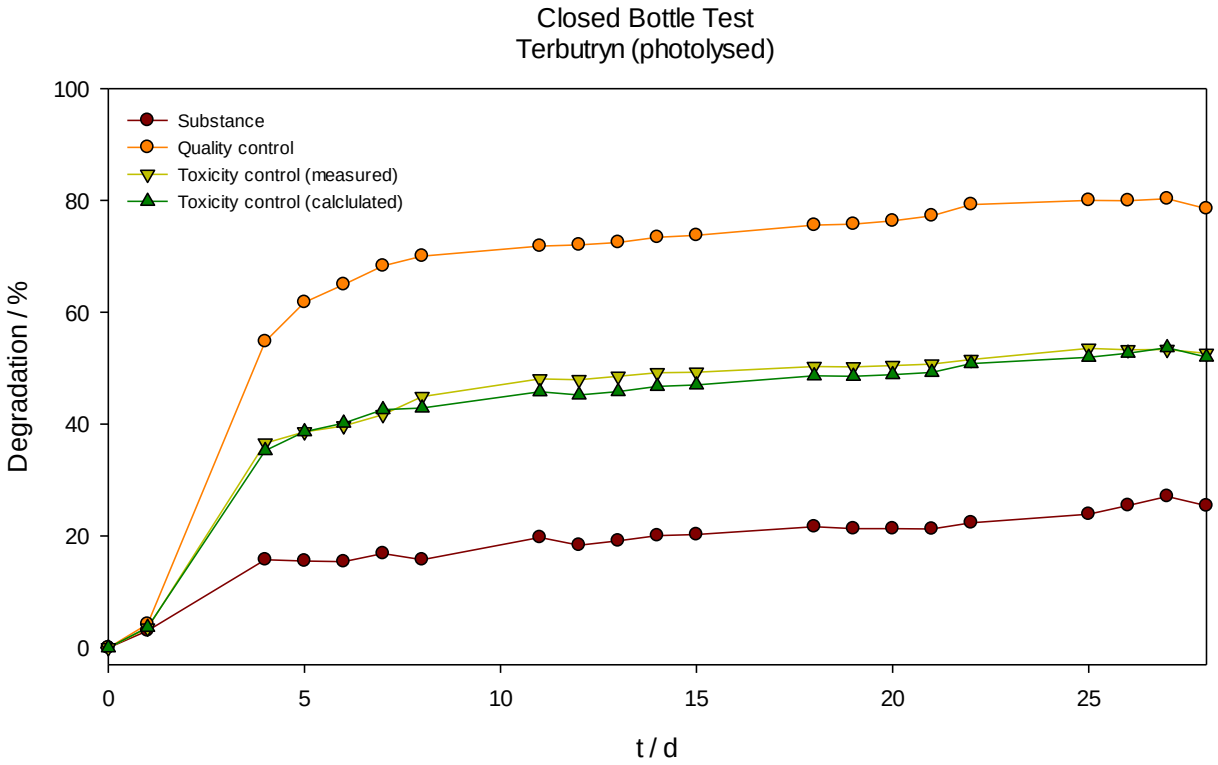
				
212.1533 (1.04)	C9H17N5O	1.2	212,2-> 156,0 	212,2 -> 69,0 
214.1149 (1.07)	C8H15N5S	3.8	214.11 -> 158 (93/13) 	214.11 -> 68 (93/45) 
226.1324 (14.94)	C9H19N5O	3.6	226,2-> 170,1 	226,2-> 128,1 
256.1258 (1.02)	C10H18N5SO	8.0	256.16 -> 158 (122/25) 	256.16 -> 200 (122/13) 
258.1418 (1.12)	C10H19N5OS	2.7	258,1-> 186,0  $C_5H_8N_5OS^+$ Exact Mass: 186.044	258,1-> 202,0  $C_6H_{12}N_5OS^+$ Exact Mass: 202.076
214.1285 (1.17)	C11H19NOS	10.8	214.13 -> 102 (86/9) 	214.13 -> 57.1 (86/13) 
130.1606 (1.23)	C8H19N	2.2	130.2 -> 57 (73/8) 	130.2 -> 43.1 (73/16) 
214.1286 (0.93)	C11H19NOS	12.3	214.13 -> 102 (86/9) 	214.13 -> 57.1 (86/13) 

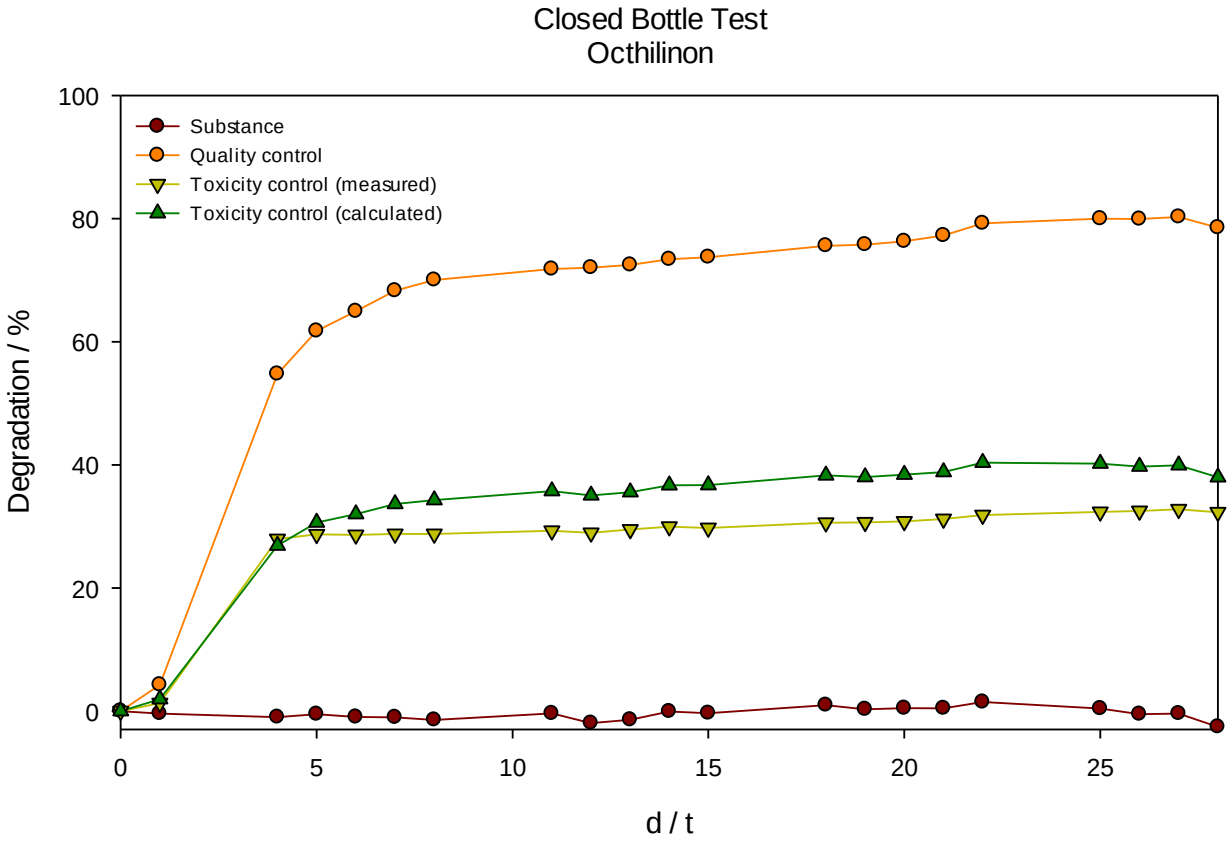
SM 7: Results of Closed Bottle Test (biodegradation test)

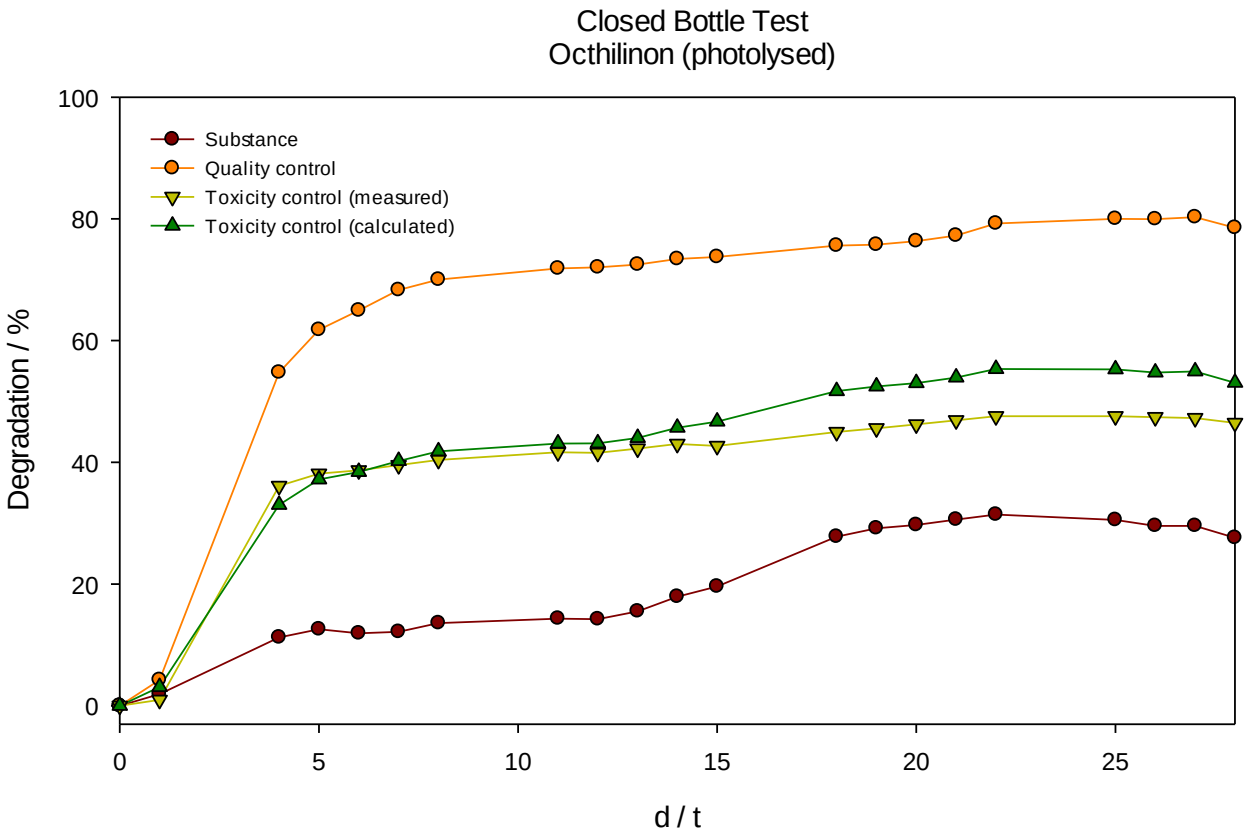





Closed Bottle Test
Terbutryn
Substance
Quality control
Toxicity control (measured)
Toxicity control (calculated)
Degradation / %
t / d
0
20
40
60
80
100
0
5
10
15
20
25


Closed Bottle Test
Terbutryn (photolysed)
Substance
Quality control
Toxicity control (measured)
Toxicity control (calclulated)
Degradation / %
t / d
0
20
40
60
80
100
0
5
10
15
20
25


Closed Bottle Test
Octhilinon
Substance
Quality control
Toxicity control (measured)
Toxicity control (calculated)
Degradation / %
d / t
0
20
40
60
80
100
0
5
10
15
20
25


Closed Bottle Test
Octhilinon (photolysed)
Substance
Quality control
Toxicity control (measured)
Toxicity control (calculated)
Degradation / %
d / t
0
20
40
60
80
100
0
5
10
15
20
25

Analysis of the recovery within CBT by comparing day 0 and day 28	
Substance/TP	**Recovery**
Diuron	86.0
TP-215(a)	6.1
TP-215(b)	14.8
TP-219	123.9
Terbutryn	100.4
TP-168	118.7
TP-196	107.7
TP-210	97.1
TP-212	104.9
TP-214	154.2
TP-226	89.5
TP-256	83.3
OIT	90.0
TP-130	0
TP-214	99.3

SM 8: Calculation of ThOD

$$\frac{\mathbf{16} \cdot (\mathbf{2} \cdot \boldsymbol{C}^{*} + \frac{\mathbf{1}}{\mathbf{2}} (\boldsymbol{H}^{*} - \boldsymbol{Cl}^{*} - \mathbf{3} \cdot \boldsymbol{N}^{*}) + \mathbf{3} \cdot \boldsymbol{S}^{*} + \frac{\mathbf{5}}{\mathbf{2}} \cdot \boldsymbol{P}^{*} + \frac{\mathbf{1}}{\mathbf{2}} \boldsymbol{Na}^{*} - \boldsymbol{O}^{*})}{\boldsymbol{Molar\ weight\ of\ substance}\ [\boldsymbol{in\ g\ mol^{-1}}]}$$

* Number of respective atoms in the molecule

Substance	ThOD of substances
Diuron	1.24
Terbutryn	1.66
OIT	2.40

To calculate the required concentration [mg L^{-1}] of each substance the ThoD of Substance [O_2 L^{-1} / mg $_{Substance}$] was multiplied with by the ThOD of test approach of 5 mg L^{-1}.

Artikel 3

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A strategy for an initial assessment of the ecotoxicological effects of transformation products of pesticides in aquatic systems following a tiered approach

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ABSTRACT

In order to conduct a fast and comprehensive toxicity screening of pesticide transformation products (TPs), this study used a tiered approach by a combination of *in silico* and experimental methods to determine the probability to be of relevance for risk assessment. The six pesticides Boscalid, Penconazole, Diuron, Terbutryn, Octhilinone (OIT), and Mecoprop were used as model compounds. Identification of corresponding environmental known and unknown TPs were done by literature analysis and photolysis experiments in combination. Aquatic solutions of the pesticides were photolysed to generate TPs which can be expected in the aquatic environment. The resulting mixtures were screened for TPs by high resolution LC-MS/MS. The herein developed approach was conducted at three different tiers: Literature review and *in silico* methods were used to predict exemplary the environmental bacterial toxicity and the genotoxicity of every single TP at tier I. In case of indications to be toxic, experiments at tier II were applied. Hereby, the photolytic mixtures containing parent compound and TPs were used for the consecutive toxicity test. Microtox assay for the parent compounds and the photolytic mixture was conducted to determine the acute and chronic toxicity and the growth inhibition of *V. fischeri*. Umu-tests were conducted to determine primary DNA damage. At tier III, single substance standards were used to conduct toxicity tests in case of toxic indication by previous tiers and availability of analytical standard. Identification of TPs revealed 45 known environmental TPs that originated from the six pesticides. The number of substances that need to be assessed was therefore more than sevenfold. By the tiered approach, it was possible to assess toxicological effects on environmental bacteria of 94% of the selected TPs. For 20% we found strong evidence to be toxic to environmental bacteria, as they were assessed at least at two tiers. For further 44% of the TPs we found slight evidence, as they could be assessed at one tier. Contrary, this approach turned out to be unsuitable to assess genotoxic effects of TPs neither by *in silico* tools nor by experiments. The number of substances that could probably pose a risk onto environment was quadrupled in comparison to the consideration of solely the parent compounds. Thus, this study demonstrates that the conducted screening approach allows for easy and fast identification of environmental relevant TPs. However, the study presented was a very first screening. Its applicability domain needs to be assessed further. For this purpose as a very next step the approach suggested here should be verified by applying additional endpoints and including additional parent compounds.

1. Introduction

TPs of organic pesticides that are formed by abiotic and biotic processes are increasingly identified in the environment (Burrows et al., 2002; Fenner et al., 2013). They increase the number of substances that need to be considered within risk assessment. However, TPs are still neglected even in current proposals of prospective assessments of pesticides risk (Schäfer et al., 2019). Within regulatory schemes only known and relevant TPs are considered and need to be assessed (European Union, 2009). Besides the exposed environmental concentration in the respective media, the risk of a substance is derived by its environmental properties. Studies that analyzed these properties showed that TPs are often more mobile and persistent in the aquatic environment than their parent compounds. Some of these studies also showed that TPs might pose similar or even higher toxic effects on different species (Belfroid et al., 1998; Bustos et al., 2019; Escher and Fenner, 2011; Gutowski et al., 2015c, 2015a; Sinclair and Boxall, 2009). Factors that could indicate a consistent or even increased

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toxicity of TPs compared to their parent compounds were described by Sinclair and Boxall (2009). As high mobility and persistency are well known for the majority of the known pesticide TPs, this study focused on the investigation of their toxic properties using the examples of environmental toxicity and genotoxicity.

In light of the increasing amount and diversity of chemicals and their TPs, it is questionable whether to deal with different required toxic endpoints - especially in case of the absence of an analytical standard (Kümmerer et al., 2019). To handle with that issue, there are different methods available to determine the toxicity of TPs (Escher and Fenner, 2011), e.g. by experimental effect-driven approaches. Such comparative analysis were conducted by toxicity testing of parent compounds and the corresponding reaction mixture containing TPs that were received by previous degradation experiments (Brack, 2003; Herrmann et al., 2016). Furthermore, computational methods such as QSAR/QSPR (quantitative structure-activity relationships/ quantitative structure-property relationship) were increasingly used for the assessment of environmental properties of pesticide TPs (Gutowski et al., 2015a, 2015b; Villaverde et al., 2017; Villaverde et al., 2018b). Finally, hybrid approaches were conducted in a combination of in vitro and *in silico* methods to evaluate the toxicity of pesticide TPs (Villaverde et al., 2018b). On this occasion, both the effect by a single TP and the effects of the mixture could be analyzed as it was successfully demonstrated for antibiotics (Menz et al., 2017).

By considering the high diversity of known and unknown TPs that need to be assessed, it became clear that there is a high demand for concepts that allow for a fast and comprehensive approach to identify relevant TPs (Escher et al., 2014; Menz et al., 2017). Both experimental and *in silico* methods have their specific limitations. However, to take advantage of both methodological strengths, both should be used in combination within an integrative approach. To go beyond the above-mentioned comparative methodology, this study made use of a tiered approach that allows for the gradual identification of the toxicity of pesticide TPs. At tier I, literature data and computational methods were used to identify TPs that might be toxic. In case of toxic indications an experimental approach were used to determine exemplary eco- and genotoxicity of a photolytic mixture including the parent compound and its TPs at tier II. In order to minimize the financial and temporal expense by synthesis of unknown or unavailable TPs, tier II provides a further pre-assessment of positive candidates at tier I. Finally, ecotoxicity of single TPs were only experimental examined at tier III if TPs showed toxic indication in tier II and an analytical standard was commercial available (Fig. 1). On the whole, this tiered approach provides a new strategy to assess the probability of TPs to be of relevance for risk assessment. This supports faster decision and priority setting to handle the great number of TPs which will become important in future especially in regulatory schemes.

This approach was explored by the TPs of Boscalid, Penconazole, Diuron, Terbutryn, OIT, and Mecoprop. These pesticides were chosen as model compounds as they including different substance classes and scopes of application, e.g. as plant protection agent and biocide. Additionally, they represent substances with different modes of action and levels of ecotoxicity. The parent compounds have different toxic EC_{50} values between 0.05 mg L^{-1} (OIT) and 59 mg L^{-1} (Diuron) on *V. fischeri* (Bollmann et al., 2017b; EPA, 2010; Hernando et al., 2007; Mottier et al., 2014; Rodríguez-Cabo et al., 2018; Strachan et al., 2001; Tixier et al., 2001), indicating specifically and non-specifically acting toxicants and a high variability in effect concentration. Furthermore, the substances Diuron and Terbutryn are currently used as biocides, although their former application as plant protection agent was prohibited due to their harmful effects on aquatic organisms (European Commission, 2019). As we want to assess environmental TPs, only TPs that were formed by biological, chemical, and/or physical processes in environmental compartments were considered.

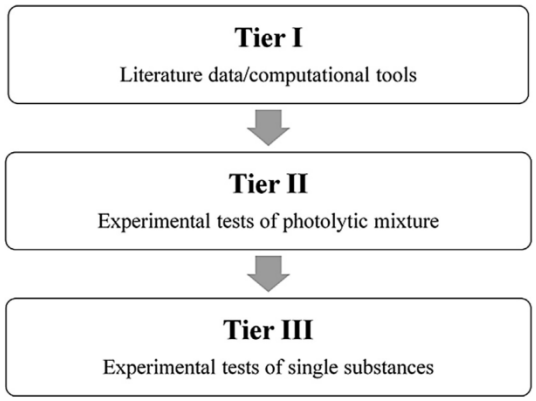

Fig. 1. Tiered pesticide TP assessment approach.

2. Methods

2.1. Workflow

A workflow was conducted for every test compound and their corresponding TPs in a tiered approach (Fig. 2). On this way already known and not yet known TPs could be identified. These TPs were used for subsequent assessment. In combination with the computer based *in silico* tool MultiCASE literature data was used to assess and evaluate the environmental toxicity and genotoxicity of TPs (Tier I). MultiCASE was used due to its inclusion of the required endpoints and its applicability for TPs of organic compounds as shown by previous studies (Gutowski et al., 2015c; Mahmoud et al., 2014; Menz et al., 2017; Rastogi et al., 2014). In case of toxic indications, photolysis experiments of the parent compounds were performed to produce a reaction mixture of TPs for further toxicity testing in a luminescent bacteria test (LBT) and a genotoxicity test (umu-test) (Tier II). Photolysis was chosen as transformation process due to its fast implementation unlike biodegradation experiments (OECD, 1992, 2008). This is even more underlined by the fact that TPs formed by photolysis and biodegradation are similar in many cases (Bollmann et al., 2016, 2017a, 2017b; Tixier et al., 2001). Moreover, it turned out, that TPs that are formed by photolysis experiments covers the majority of TPs that are known from literature. The respective parent compounds and the photolytic mixtures underwent a toxicity test for the comparative and initial assessment of TPs as it was done elsewhere (Escher and Fenner, 2011; Herrmann et al., 2015). In case of toxic indications by mixture analysis, single TPs were tested in our toxicity tests as long as an analytical standard was commercially available and they had not been tested in previous studies (Tier III).

Assessment of TPs was done as following (see Fig. 2): In case of toxic indication by neither the first nor the second tier, TPs were assessed to be probably not toxic. As the single toxicity test at tier III gave more evidence about their toxicity, TPs that were assessed to be most probably not toxic if test were negative. TPs that were tested positively at tier I but were not contained in the photolytic mixture were assessed to be probably toxic. If positive candidates of tier I were positively tested at tier II, TPs were assessed to be most probably tested as. Same applies for TPs that were positively tested in the single test at tier III.

2.2. Chemicals and reagents

Analytical standards were purchased from Sigma Aldrich, Neochema, and Dr. Ehrenstorfer and had a purity of $> 97.3\%$. Acetonitrile (VWR) was used as organic mobile phase and for the preparation of stock solutions. Aqueous mobile phase and solutions for the implementation of photodegradation experiments were prepared with ultrapure water (Membra Pure, Germany; Q1:16.6 MΩ and Q2: 18.2 MΩ).

The freeze-dried luminescent bacterium *V. fischeri* (NRRL-B-11177)

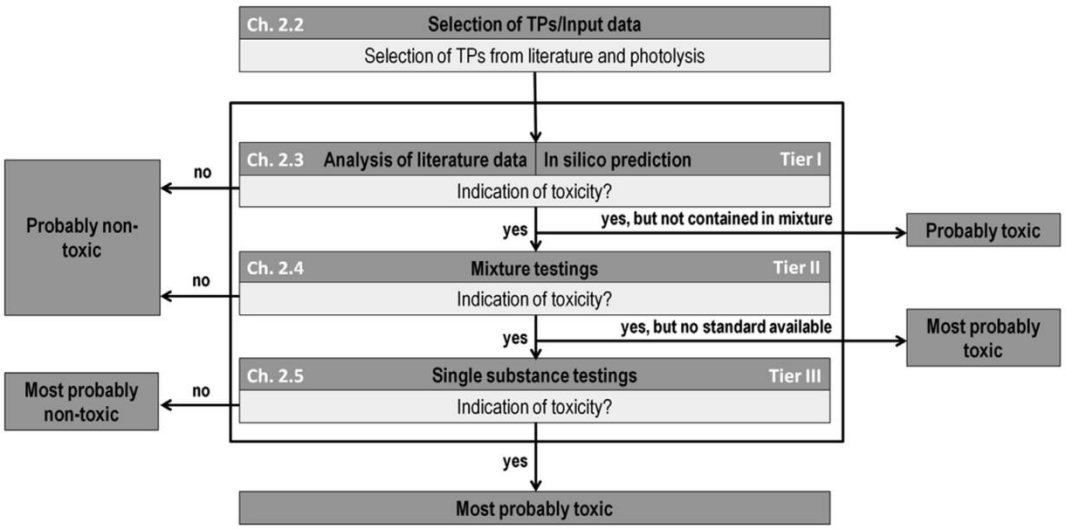

Fig. 2. Workflow and schedule of the tiered approach of this study. Toxicity assessment was conducted at different levels (I-III) that were described in detail in the corresponding chapters (2.2–2.5).

were purchased from Hach-Lange GmbH, Düsseldorf, and stored until usage at −20 °C. The gram-negative bacterium *Salmonella typhimurium* (TA1535pSK1002) was received from the German Collection of Micororganisms and Cell Cultures GmbH, Braunschweig, Germany.

2.3. Selection of environmental TPs (Input data)

Input data was generated by a comprehensive review of literature data and additional photolysis tests to receive a reaction mixture that contained TPs of every test compound.

2.3.1. Identification of TPs by literature and data research

As pesticide TPs are partly considered within regulatory schemes, analysis of available literature and reports on pesticides by the European Food Safety Authority (EFSA) and the Environmental Protection Agency of the United States of America (US-EPA) was evaluated. Data of pesticide reports were often fed by unpublished data which is why not only primary literature was used here. For each compound a comprehensive literature and database analysis was performed to identify known TPs. These TPs were selected due to their potential formation by environmental processes, e.g. known transformation pathways and processes such as photolysis, biodegradation, and hydrolysis in different compartments. TPs that were only formed by AOP or other technical processes were not considered. In addition, the state of knowledge on the aquatic environmental analysis and detection of the selected TPs were reviewed to get a more comprehensive understanding for their potential risk.

2.3.2. Generation of TPs and photolytic mixtures by photolysis

Each pesticide was diluted in pure water (pH 7) and approximately at their respective limit of solubility in water (S3). Photolysis was conducted with an initial volume of 800 mL using a xenon arc lamp (TXE 150, Peschl Ultraviolet, Mainz, Germany) which had an emission spectrum roughly matching the solar radiation (Yager and Yue, 1988; S1). However, cut-off wavelength is much beneath the natural border of 290 nm but was tested to be not relevant for the formation of transformation products (Hensen et al., submitted for publication). It was equipped with an ilmasil quartz immersion tube in a cylindrical batch reactor (T = 20 ± 2 °C). Photolysis experiments were performed over a time period of 8.0 h with hourly sampling (10 mL). Other test conditions and settings are listed in S2 and S3. The samples after 0, 1, 2, 3, 4, 5, 6 7, and 8 h were stored at −20 °C until one part of the samples was analyzed in mass spectrometer to identify formed TPs over the whole irradiation time. The other part was used for subsequent toxicity tests.

2.3.3. Analysis and elucidation of TPs

Analysis of primary elimination of parent compound and elucidation of TPs in photolytic mixture was done by LC-MS. Analysis was conducted using a RP-column (Nucleodur 100-3, 125/2, c18 ec; Macherey Nagel, Düren, Germany) as stationary phase while 0.01% Formic acid (A) and Acetonitrile (B) were used as mobile phase with a flow of 0.4 mL min^{-1} and a gradient as described in S2. Oven temperature was set to 30 °C. The structure of TPs were elucidated using an Iontrap (Dionex Ulitmate 3000 UHPLC system, Dionex, Idstein, Germany) and a LTQ Orbitrap-XL high-resolution mass spectrometer with ESI source (Thermo Scientific, Dreieich, Germany) in a full scan. Positive mode was used to analyze Boscalid, Penconazole, Diuron, Terbutryn, and OIT. Negative mode was used to analyze Mecoprop. Applied setting can be received from S2.

2.4. In silico prediction models (Tier I)

In silico analysis of the selected TPs employing QSARs was performed using different models provided by the CASE Ultra software package (v. 1.7.0.5, MultiCASE Inc.) expert system for the substructure based prediction of toxicity and bioactivity of chemicals. The model TOX_EB was used for the prediction of short-term bacterial luminescence inhibition in the Microtox assay. To analyze the bacterial mutagenicity, a statistical model (GT1_A7B) and an expert rule-based model (GT_EXPERT) were used in combination as recommended by the ICH M7 guidelines (EMA/CHMP/ICH/83812/2013). All *in silico* models used in this study have defined and validated applicability domains. More information about training sets, validity criteria, and predictive performance of the CASE Ultra software can be found elsewhere (Chakravarti et al., 2012).

Using this model, output variables were provided in a discrete categorical form that allow for the classification of chemicals. Therefore, substances could be assessed that were within a probability range of 35–55% of the model. All TPs that were found to be out of that range could only be inconclusively assessed (IN). All TPs that contain structural features that are not covered by the training set chemicals of the model were designated as “out of domain” (OD).

2.5. Cytotoxicity in bacteria: toxicity tests of the mixture (Tier II) and of single substances (Tier III)

A modified luminescent bacteria test (LBT) (Menz et al., 2013) was implemented to assess cytotoxic effects onto aquatic environmental bacteria (*Vibrio fischeri*) (NRRL_B_11177; Hach-Lange GmbH, Düsseldorf, Germany). In this test, three endpoints were analyzed: acute (30 min of exposure) and chronic toxicity (24 h) and growth inhibition (14 h). Due to the need of seawater conditions of luminescent bacteria, one half of initial solution was mixed with one half of a 2% (w/v) sodium chloride solution. In case of high toxic effects (about 100% luminescent inhibition) at the limit of solubility of substances, solutions were diluted until effects were reduced to 60–80% luminescence inhibition. Concentrations of parent compounds in the reaction mixture were dependent on the amount of elimination after eight hours and are depicted in S3. Concentration of TPs was not determined.

The umu-test and umu-test S9 were done to determine genotoxic effects on DNA of *Salmonella typhimurium* (TA1535 psk 1002; German Collection of Micororganisms and Cell Cultures GmbH, Braunschweig, Germany) (ISO/FDIS 13829 (1999)). The test based on measurement of induction ratio (IR) of the umuC gene. A substance is classified as genotoxic in case of IR $>$ 1.5. Test concentrations are also depicted in S3.

Single substances were diluted in water and stirred for half an hour. In some cases 1% DMSO was added to enable a better solubility of substances in water. Toxicity tests were conducted as described above.

Short-term luminescent inhibition of the conducted LBT was comparable to CASE Ultra model TOX_EB, whereas the umu-test was comparable with the models assessing bacterial mutagenicity (GT_A7B and GT-Expert) (Menz et al., 2017).

3. Results and discussion

3.1. Identification of TPs and their environmental occurrence

By data retrieved from literature and database research, and identification of TPs formed in photolysis experiments we compiled a list of 45 environmental TPs in total originating from the six pesticide parent compounds (Fig. 3). For more structural and analytical details of the TPs see S7 and S8. The TPs review demonstrated that the number of substances that need to be considered at environmental hazard and risk assessment was multiplied by a factor of 7.5 in comparison to the consideration of solely the parent compounds.

Seven TPs of Boscalid were found (Table 1). They are reported by the US-EPA (EPA, 2010) and in another study analyzing UV-treatment of Boscalid (Lassalle et al., 2014). This study was considered as an exception, since three of the TPs (TP-307(a), TP-307(b), and TP-325(a)) that were found in that study were also formed by our degradation experiments and the analysis of photolysis occurring under environmental conditions is still lacking for Boscalid. Out of the seven TPs, only the metabolites (TP-157, TP-158, TP-309, and TP-325(b)) are considered by approval reports so far. For Penconazole three studies were available that analyzed photodegradation (Hensen et al., submitted for publication; Rodríguez-Cabo et al., 2018; Schwack and Hartmann, 1994). Including three environmental relevant and already considered metabolites (TP-70, TP-130, and TP-286) from available reports, ten TPs of Penconazole were selected. Five TPs of Diuron that occurred from biodegradation and photodegradation were found. They are already known since 1982 (Ellis and Camper, 1982; Jirkovský et al., 1997; Tanaka et al., 1986). In total, twelve Terbutryn TPs were identified. Although this substance is used for decades, the elucidation of formed TPs has been done only recently (Bollmann et al., 2016, 2017a; Hensen et al., 2018). Seven OIT-TPs were found that were formed by biodegradation and photodegradation (Bollmann et al., 2017a). All four selected Mecoprop TPs are well known from various studies (Boule et al., 2002; Meunier and Boule, 2000), whereas two of them (TP-107 and TP-141) were already considered in the corresponding approval report (EFSA, 2017).

We found that 82% and 58% of the selected TPs were formed by photolytic processes and microbial degradation, respectively. 42% were formed by both processes. Under given analytical conditions (e.g. ionization and chromatography), LC-MS non-target screening indicated the presence of 27 TPs in the photolytic mixtures. In fact, 4 TPs of Boscalid, 7 TPs of Penconazole, 3 TPs of Diuron, 10 TPs of Terbutryn, 2 TPs of OIT, and 3 TPs of Mecoprop were detected in the respective photolytic mixture. Structures of all these TPs were already described in literature (Table 1). Some of the TPs that were described in literature, e.g. the majority of OIT-TPs, were not found in our photolysis experiments. One possible explanation is that other studies used different mass spectrometric devices and settings (i.e. mass spectrometer, ionization mode and source) or more likely the shorter duration time of our photolysis experiments.

Literature research (Table 1) demonstrated that one third of the identified TPs were already detected in different aquatic environments. In total, seven studies were found that detected at least one of the selected TPs. The studies were conducted from 1997 to 2018, whereby most of them were done in the past ten years. Out of the TPs that were analyzed in the aquatic environment 47% were detected in surface water and 40% in groundwater. Overall, 53% of the TPs could be detected in surface water and/or groundwater samples. However, 47% of the analyzed TPs could neither be detected nor quantified in any water sample. Hence, there is a lack of studies investigating the occurrence of TPs in the aquatic environment.

Most references were found for Diuron-TP-162, TP-205, and TP-219 and Terbutryn-TP-212 (Hydroxy-Terbutryn). Terbutryn-TP-212 (Hydroxy-Terbutryn) could also be formed by the degradation of Terbuthylazin as well. Thus, Terbutryn-TP-212 was referred to its other parent compound Terbuthylazin in most studies (Hernández et al., 2008; Reemtsma et al., 2013). None of the TPs of Boscalid and Penconazole was found in surface water or groundwater samples. The highest concentration in surface water (field runoff) was found for Diuron-TP-219 (cmax = 7.9 µg L^{-1}) (Field et al., 1997). In groundwater, highest concentration was found for Mecoprop-TP-141 of about c_{max} = 1.36 µg L^{-1} (McManus et al., 2014).

As it is well known that $logK_{OW}$ (logP) is negatively correlated to water solubility S_W (Isnard and Lambert, 1989) and this parameter shows generally the tendency of a substance to distribute into the aquatic environment, 82% of the analyzed TPs have a higher tendency to be more mobile in the aquatic environment than their corresponding parent compounds. This fact was received by the calculated logP values of TPs by QSAR (S5). Thus, there is in fact a high probability to detect them in the aquatic environment. The reason that most of the TPs were hitherto not analyzed could be explained to some extent that they were declared to be not relevant due to lacking awareness and assessment of TPs (Laabs et al., 2015). Hence, there is a great demand for the implementation of TPs in monitoring programs (Escher et al., 2014; German Federal Environmental Agency, 2019)

3.2. Genotoxicity of TPs

At tier I, it is shown by literature review that there were huge data gaps regarding the genotoxicity of TPs. Except a few non-specific TPs that were evaluated (Zeiger et al., 1992) none of these TPs were assessed by experiments. Prediction of TPs of Boscalid was done by the QSAR tool T.E.S.T. in a previous study and it was found that Bosclaid-TP-307(a), TP-307(b), and TP-325(b) were mutagenic in rat (Lassalle et al., 2014). This is supported by our *in silico* results, where we found that TP-307(a), TP-325(a), and TP-325(b) were genotoxic by employing the CASE Ultra statistical model GT-A7B on *S. typhimurium* (S5). No other TP was predicted to be genotoxic by QSAR. The absence of genotoxic potential could be confirmed for a variety of TPs, such as Boscalid-TP-157, Mecoprop-TP-107, Diuron-TP-162, and Mecoprop-TP-

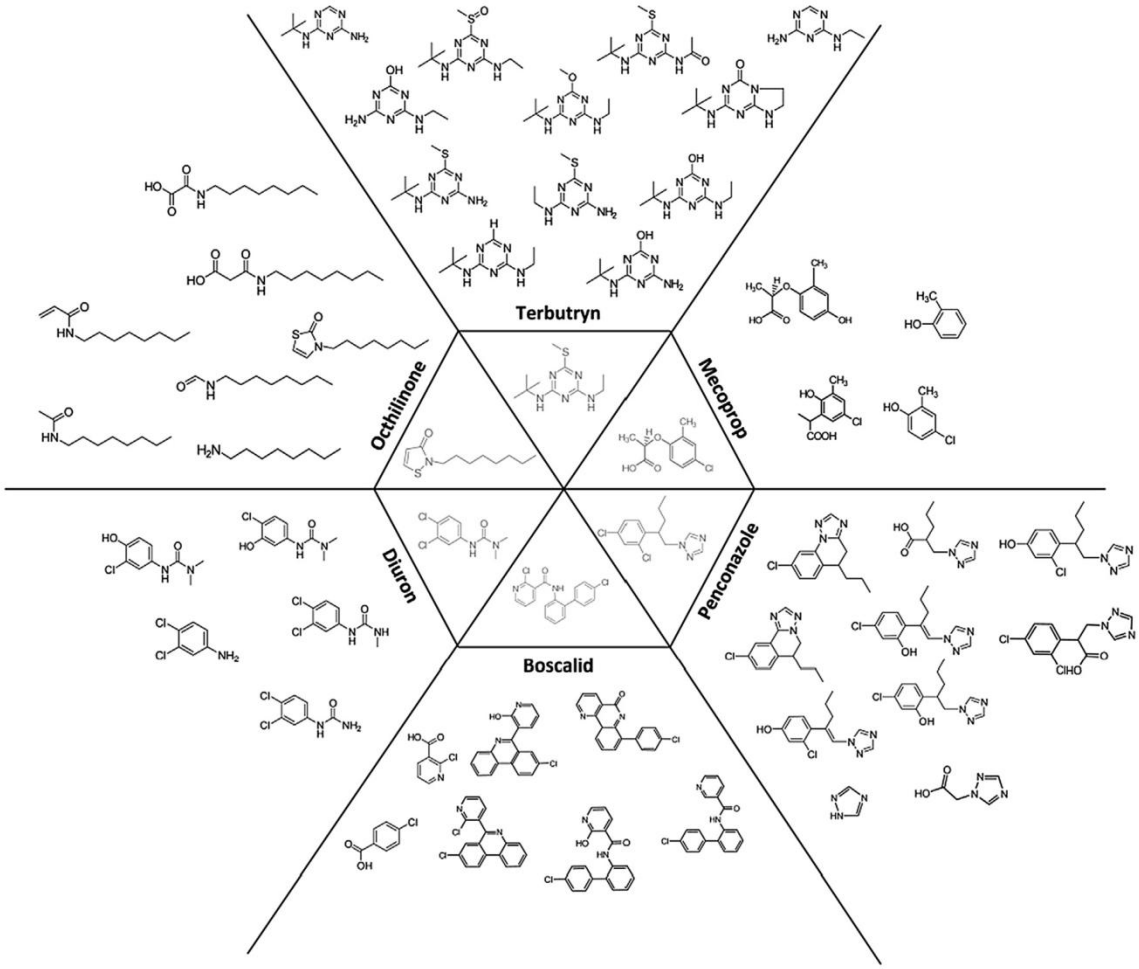

Fig. 3. 45 TPs originating from six pesticidal parent compounds – illustration of the multiplication of known substances that should be further investigates by an environmental risk assessment.

141 by a study that analyzed mutagenicity on *S. typhimurium* of 311 substances (Zeiger et al., 1992). At tier II, none of the TPs showed positive genotoxic effects in *S. typhimurium* in our experiments, neither the parent compounds nor the photolytic mixtures (S10). Hence, we concluded that the majority of TPs were not genotoxic to *S. typhimurium*. For this reason we did no further genotoxicity testing. Overall, it turned out that our approach was not useful to determine the genotoxicity of pesticide TPs in a photolytic mixture. This was also seen in other studies were the photolytic mixtures of different parent compounds had no genotoxic effect (Kotnik et al., 2016; Mahmoud et al., 2014; Menz et al., 2017; Toolaram et al., 2016). Thus, genotoxicity must be assessed in separate single tests in future approaches. Therefore, synthesis of single TPs that show genotoxic activity by QSAR seem to be unavoidable. In this particular case, it should be done for Boscalid-TP-307(a), TP-307(b), TP-325(a), and TP-325(b) as they were found to be probably genotoxic by *in silico* prediction.

3.3. Ecotoxicity of TPs

Ecotoxic properties of about 60% of the selected TPs were not known so far. No literature data was available for Boscalid-TPs. Contrary, every selected TP of Diuron was already assessed by previous studies (Ellis and Camper, 1982; Jirkovský et al., 1997; Tanaka et al., 1986). For the other TPs, ecotoxicity was done only occasionally. Overall, seven studies analyzed ecotoxicity of the selected TPs. In many studies luminescence inhibition of *V. fischeri* was used as ecotoxicological endpoint. In others test organisms such as *Pseudomonas putida* and aquatic organisms in general were used. Results of LBT are depicted in Table 2 and in S4.

3.3.1. Boscalid-TPs

At tier I there was no literature data available regarding the ecotoxicity (e.g. luminescence inhibition of *V. fischeri*) of Boscalid-TPs. By QSAR, we found positive results of Boscalid and its TPs TP-307(a), TP-307(b), TP-309, TP-325(a), and TP-325(b). It was shown that one common alert was found for all structures (S6) that caused the effect. Further testing of the photolysis mixture at tier II was not possible due to the low solubility of the substance. Hence, the required test concentration could not be reached due to interferences of solvents on photolysis and toxicity tests (Parvez et al., 2006). TPs that showed toxic activity (TP-325 and TP-309) could not be analyzed by a single toxicity test due to the non-availability of an analytical standard. Hence, tier II and III were not useful for Boscalid and data availability turned out to be really scarce as the toxic indications were only based on one tier. Hence, there is an urgent need to synthesize TPs with toxic indication to get stronger evidence for their ecotoxicity or its absence. This turned out to be especially important for TPs of parent compounds that have low water solubility. These difficulties in toxicity testing were already mentioned for low soluble parent compounds (Hernando et al., 2007; Tang et al., 2013), but were never the subject of discussion for mixture toxicity tests of TPs that originated from little soluble substances. The circumstance that TPs often have a higher water solubility compared to their parent compounds is of utmost importance due to the difficulties in toxicity tests described above and due to the possibly higher

Table 1
Literature review of the selected TPs. Known synonyms of the TPs, formation process by biodegradation/metabolism (M) or photodegradation (P) and the corresponding reference (REF) where they were elucidated. Environmental detection (ED) of TPs in surface water (SW) and groundwater (GW). ND = not detetcted, NQ = not quantified due to missing analytical standard.

Substance	Known Synonyms	Formation	ED SW [c in µg L^{-1}]	ED GW [c in µg L^{-1}]
Boscalid				
TP-157	M510F64, p-chlorobenzoic acid	M[1]	–	–
TP-158	M510F47, 2-chloronicotinic acid	M[1]	–	–
TP-307(a)*	–	P[2]	–	–
TP-307(b)*	–	P[2]	–	–
TP-309	M510F08	M[1]	–	–
TP-325(a)	–	P[2]	–	–
TP-325(b)*	M510F49	MP[1][2]	–	–
Penconazol				
TP-70	CGA 71019, 1,2,4-Triazole	M[3]	–	–
TP-130	CGA 142,856	M[3]	–	–
TP-184*	–	P[4]	–	–
TP-248(a)	–	P[5]	–	–
TP-248(b)*	–	P[6]	–	–
TP-264(a)*	–	P[4]	–	–
TP-264(b)*	–	P[4]	–	–
TP-266(a)*	–	P[4]	–	–
TP-266(b)*	–	P[4]	–	–
TP-286	CGA 179,944	M[3]	–	–
Diuron				
TP-162	DCA	M[7]	< 0.025 [15]; ND [15][16]	ND [15][16]
TP-205	DCPU	MP[8]	< 0.01 [14]*;0.9–3 [15]; ND [15]	< 0.01 [14]*;0.6 [15];ND [16]
TP-215(a)	–	P[9]	–	–
TP-215(b)	–	P[9]	–	–
TP-219	DCMPU	MP[9]	< 0.01 [14]*;1.3–7.9[15];ND [16]	< 0.01[11]; < 0.1[14]*;~1[15]; ND[16]
Terbutryn				
TP-140	Desthiomethyl-Desbutyl-T.	P[10]	–	–
TP-156	Desbutyl-2-Hydroxy-T.	P[10]	–	–
TP-168	Desthiomethyl-Desethyl-T.	MP[10]	NQ [11]	ND [11]
TP-184[c)]	Desethyl-2-Hydroxy-T.	MP[10]	0.2–1.2 [15]	1.2 [15]
TP-186	–	P[11]	ND [11]	ND [11]
TP-196	Desthiomethyl-T.	MP[10]	NQ [11]	ND [15]
TP-210	–	P[11]	NQ [11]	ND [11]
TP-212[c)]	2-Hydroxy-T.; MT13; GS 23,158	MP[10]	0.02 [11]; 0.023[14]*; ~0.1[16]; 0.8 [17]	0.023 [14]*ND [13]; 0.1 [16]
TP-214[d)]	Desethyl-T.	MP[10]	0.08 [11]	0.003 [11]
TP-226 [e)]	Terbumeton**	MP[10]	0.02 [11];0.006 [17]*	ND [11]
TP-256	–	P[11]	NQ [11]	ND [11]
TP-258	T.-Sulfoxid	MP[10]	ND [11]	ND [11]
OIT				
TP-130	Octylamin	MP[12]	–	–
TP-158	N-Octylformamide	MP[12]	–	–
TP-172	N-Octylamide	MP[12]	–	–
TP-184	N-Octylprop-2-enamide	MP[12]	–	–
TP-202	N-Octyloxamic acid	MP[12]	–	–
TP-214	2-Octylisothiazol-3(2H)-one	MP[12]	NQ [11]	ND [11]
TP-216	N-Octyl Malonamic acid	MP[12]	–	–
Mecoprop				
TP-107	o-Cresol, 2-Methylphenol	MP[13]	–	–
TP-141	2-MCP, 4-Chloro-o-cresol	MP[13]	NQ [18]*	0.005–1.36 [19]
TP-195	TP-195	P[13]	–	–
TP-213	TP-213	P[13]	–	–

References: [1]: EPA, 2010; [2]: Lassalle et al., 2014; [3]: EFSA, 2008; [4] Hensen et al., 2019; [5]: Schwack and Hartmann, 1994; [6]: Rodríguez-Cabo et al., 2018; [7]: Ellis and Camper, 1982; [8]: Jirkovský et al., 1997; [9]: Tanaka et al., 1986; [10]: (Bollmann et al., 2016) (Bollmannetal.,É; [11]: Hensen et al., 2018; [12]: Bollmann et al., 2017b [13]: Boule et al., 2002; [14]: Reemtsma et al., 2013; [15]: Field et al., 1997; [16]: Hernández et al., 2008; [17]: Benvenuto et al., 2010; [18]; Laganà et al., 2002; [19]: McManus et al., 2014.

* 50 percentile.

** Only studies relating to Terbumeton as TP and not as parent compound were considered,*** As a TP of MCPA.

probability to affect the aquatic environment.

3.3.2. Penconazole-TPs

EFSA report on Penconazole concludes that TP-70 and TP-286 are highly ecotoxic to aquatic organisms (EFSA, 2008). Precise ecotoxicological endpoints could not be obtained from this report. Our QSAR results revealed that seven out of ten assessed Penconazole-TPs showed toxic effects (Table 2). As many of them were with a probability of 50.7% out of the grey zone of 35 to 55% they could only be inconclusively assessed.

At tier II, the toxicity test of the photolytic mixture of Penconazole showed that chronic effects of Penconazole to luminescent bacteria and the photolytic mixture were similarly high. There were additionally moderate acute toxicity and growth inhibition effects of the mixture (> 20%). This was, however, not seen for the parent compound. As the mixture test results verify the results obtained at tier I, there were indications that TPs were probably more toxic than Penconazole.

At tier III, the toxicity of TP-70 was examined in a single LBT and turned out to be non-toxic to *V. fischeri* at a tested concentration of 30 mg L^{-1}. As studies revealed aquatic toxicity to other aquatic organisms than *V. fischeri* they might be affected by this TP. Regarding the toxicity of the photolytic mixture, TPs of phenyl ethyl azolic fungicides

Table 2
Predicted and experimental cytotoxicity in bacteria following the workflow at three different tiers: Tier I: Literature review and calculated by CASE Ultra model TOC_EB (MultiCASE Inc.; Model Version 1.5.2.0.899.500), Tier II: Toxicity test (luminescence bacteria test, LBT) of a photolytic mixture, and Tier III: LBT of single substance standards of TPs. Positive (+), negative (−), inconclusive (IN), and results that were out of domain (OD) of QSAR (MultiCASE). TPs that were identified in photolytic mixture (PM) and experimental results of LBT corresponding to three endpoints: acute toxicity (AT), chronic toxicity (CT) and growth inhibition (GI) as well as the results of the single LBT of some TPs if an analytical standard was available (Analyt.Std.).

Substance	Tier I		PM	Tier II			Analyt. Std.	Tier III		
	Literature	QSAR		LBT mixture				LBT single		
	mg L^{-1} (*V. fischeri*)	TOX_EB		AT	CT	GI		AT	CT	GI
Boscalid	EC_{50} = 5.33 [1]	+	+	–	–	–				
TP-157		–	–				+			
TP-158		–	–				+			
TP-307(a)		+	+				–			
TP-307(b)		+	+				–			
TP-309		+	–				–			
TP-325(a)		+	+				–			
TP-325(b)		+	+				–			
Penconazol	LC_{50} = 0.7 [2]*	+	+	–	+	–				
TP-70	toxic [2]*	OD	–				+	–	–	–
TP-130		OD	–				–			
TP-184		OD	+	+	+	+	–			
TP-248(a)	LC_{50} = 4.7 [2]*	IN (+)	+	+	+	+	–			
TP-248(b)	LC_{50} = 2.1[2]*	IN (+)	+	+	+	+	–			
TP-264(a)		IN (+)	+	+	+	+	–			
TP-264(b)		+	+	+	+	+	–			
TP-266(a)		IN(+)	+	+	+	+	–			
TP-266(b)		+	+	+	+	+	–			
TP-286	toxic [3]**	+	–				–			
Diuron	58 [4]	–	+	–	–	–				
TP-162	0.5 [4]	+	–				+			
TP-205	15 [4]	+	–				+			
TP-215(a)	71 [4]	IN (+)	+	+	+	+	–			
TP-215(b)	72 [4]	+	+	+	+	+	–			
TP-219	18 [4]	+	+	+	+	+	+			
Terbutryn	> 8.13 [5] [6]	OD	+		–	–	–			
TP-140	> 7.96 [5]	–	–				–			
TP-156		–	–				–			
TP-168	> 7.60 [5]	OD	+	+	–	–	–			
TP-184		OD	+	+	–	–	–			
TP-186		OD	+	+	–	–	–			
TP-196	> 6.54 [5]	OD	+	+	–	–	–			
TP-210		OD	+	+	–	–	–			
TP-212	Relevant [7]	OD	+	+	–	–	+	–	–	–
TP-214		OD	+	+	–	–	+[1]			
TP-226	89.4 [8]	OD	+	+	–	–	+	–	–	–
TP-256		OD	+	+	–	–	–			
TP-258		OD	+	+	–	–	–			
OIT	0.05 [5]	IN (+)	+	+	+	+				
TP-130		–	+	+	+	+	–			
TP-158		OD	–				–			
TP-172	> 8.36 [5]	–	–				–			
TP-184	4.51 [5]	–	–				–			
TP-202		–	–				–			
TP-214	1.09 [5]	IN (+)	+	+	+	+	–			
TP-216		–	–				–			
Mecoprop	91[9]*, 24 [10]***	+	+	–	+	–				
TP-107	27.1 mg L^{-1} [11]	–	–				–			
TP-141	0.29 [9]*	+	+	+	+	+	+	+	+	+
TP-195		+	+	+	+	+	–			
TP-213		+	+	+	+	+	–			

References: [1]: EPA, 2010; [2]: Rodríguez-Cabo et al., 2018; [3]: EFSA, 2008; [4]: Tixier et al., 2001; [5]: Bollmann et al., 2017a; [6]: Hernando et al., 2007; [7]: EFSA, 2011; [8]: Villa et al., 2012; [9]: Mottier et al., 2014; [10]: Strachan et al., 2001; [11]: Jenning et al., 2001

* Test organism: *Daphnia magna* (by QSAR);

** Test organism: aquatic organisms;

*** Test organism: *Pseudomonas putida;* [1] Solved in ACN that influences the luminescence of *V. fischeri* in the test conducted.

that were formed by cyclization (TP-248(a) and TP-248(b) might have similar or higher EC_{50} values than their parent compound (Rodríguez-Cabo et al., 2018). Derived from the *in silico* tool ECOSAR used by the authors, it was found that TP-248(a) and TP-248(b) of Penconazole were slightly less toxic than the parent compound to aquatic organisms (*Daphnia magna*, green algae, and fish). The increased effect of the photolytic mixture found in our study might be triggered by other TPs of the photolytic mixture than these both ones, e.g. TP-264(a), TP-264(b), TP-266(a) and/or TP-266(b) or could be provoked by synergistic effects of the photolytic mixture. However, our results showed for the first time that TPs of Penconazole could be ecotoxic on aquatic organisms and should receive more attention regarding their behavior

in the environment. This is especially important as TPs that were not formed by biological processes but by photolysis instead were generally not considered in studies. This can be explained by the fact that Penconazole is declared as not degradable by direct photolysis due to its absorption spectrum below the terrestrial sunlight (EFSA, 2008). As indirect photolysis could also be a relevant pathway (Remucal, 2014) and TPs of Penconazole formed by direct and indirect photolysis were found out to be similar in another study (Hensen et al., submitted for publication) these TPs need to be considered more closely in future research of risk assessment.

3.3.3. Diuron-TPs

The literature review (Tier I) revealed EC_{50} of 58 mg L^{-1}(Diuron), 71 mg L^{-1} (TP-215(a), 72 mg L^{-1} TP-215(b), 18 mg L^{-1} (TP-219), 15 mg L^{-1} (TP-205), and 0.5 mg L^{-1} (TP-162). Bacterial toxicity (tier I) were positively calculated for all TPs. In contradiction to the gathered literature data the results by QSAR calculation showed a difference between Diuron and the TP-215(a) and TP-215(b), and indicated a higher toxicity of these TPs (Table 2). The CASE Ultra model TOX_EB determined the dichlorophenyl group as the decisive toxic moiety, which is part of TP-162, TP-205, and TP-219 and explains their higher toxicity. The chloro-hydroxyphenyl group that is part of TP-215(a) and TP-215(b) was predicted with a lower probability to be toxic than the dichlorophenyl group. This confirmed the lower toxicity of TP-215(a) and TP-215(b) compared to the other TPs. Another study stated that the subsequent loss of the methylurea group of TP-219, TP-162, and TP-205 led to a decrease in toxicity in algae but to an increase in toxicity to daphnids (Neuwoehner et al., 2010). The results of the experimental LBT (Tier II) showed no inhibition of luminescence for the parent compound Diuron for all three endpoints. In contrast, the photolytic mixture showed high chronic toxicity and growth inhibition. The toxic effect of the photolytic mixture could therefore be caused by TP-219 as we could not indentify TP-205 and TP-162 in the photolytic mixture. Comprehensive data situation of Diuron TPs indicates that especially TP-219, TP-205, and TP-162 turned out to be more toxic than the parent compound. They are already considered in risk assessments. TP-205 and TP-219 were already declared to be relevant (EFSA, 2005). The experimental results received at tier II confirmed the results received at tier I and underline the accuracy of our approach.

3.3.4. Terbutryn-TPs

The results of the literature review (tier I) showed that Terbutryn and three TPs (TP-196, TP-168, and TP-140) did not inhibit luminescence of *V. fischeri* at tested concentration of about 6.5–8 mg L^{-1} (Bollmann et al., 2016). Villa et al. (2012) reported an EC_{50} value of 89.4 mg L^{-1} in an acute toxicity test (15 Min.) with *V. fischeri* of TP-226 (also known as Terbumeton). TP-212, which is known to be a TP of the herbicide Terbutylazin as well, is classified as relevant due to the fact that its parent compound Terbutylazin is classified as carcinogen category 3 although the risk of TP-212 onto aquatic organisms was assessed to be low (EFSA, 2011). Except two TPs that were predicted to have no ecotoxic effects, all other TPs were out of domain of the *in silico* prediction model, since fragments of these compounds were not present in any of the training sets of chemicals of the model used.

Toxicity tests of Terbutryn and its photolytic mixture (tier II) showed that there was an increase in acute toxicity by the photolytic mixture (Table 2), whereas no toxic effect could be measured for Terbutryn for all three endpoints. As chronic toxicity is generally the more sensitive parameter (Backhaus et al., 1997; Menz et al., 2013), it was surprising that the photolytic mixture of Terbutryn showed solely acute toxic effects. This might be a hint that TPs are acting more specifically in *V. fischeri* in this case. Derived from the results of the study by Bollmann et al. (2017a), we can assume that these effects can be caused by other TPs than TP-196, TP-168, and TP-140, as they were tested to have no effects. The conducted single LBT at tier III for available TP-212 turned out to be negative at the tested concentration of 2.5 mg L^{-1}. As no TP was found causing the toxic effect as it was seen in the photolytic mixture, other TPs of the mixture (TP-184, TP-186, TP-210, TP-214, TP-256, or TP-258) or mixture effects (Villa et al., 2012) by different TPs could have caused the luminescent inhibition. However, this study showed for the first time that some TPs of Terbutryn are more ecotoxic towards luminescent bacteria than their parent compound. This fact underlines the benefits of this multimethod approach as in silico results (applicability domain of the model) and single toxicity tests (availability of analytical standard) have reached their limits.

3.3.5. OIT-TPs

Literature stated that EC_{50} values of acute toxicity on *V. fischeri* of OIT, TP-184, and TP-214 were 0.05 mg L^{-1}, 4.51 mg L^{-1}, and 1.1 mg L^{-1}, respectively. EC_{50} values of other five TPs, such as TP-172, could not be assessed in the tested concentration range in that study (Bollmann et al., 2017b). QSAR results in our study at tier I underlined these results as OIT and OIT-TP-214 were the only substances that were (inconclusively) positively assessed with a probability of being positive of 46.2%.

At tier II, toxicity test of OIT showed high toxic effects for all three endpoints. The photolytic mixture showed also high acute toxicity but slightly lower effects for chronic toxicity and growth inhibition. Thus, TPs of the mixture are probably less specifically acting in *V. fischeri*. Calculated EC_{50} of chronic toxicity and growth inhibition values of OIT are depicted in S9. The results of tier II were in accordance with the results obtained at tier I. As no analytical standard of OIT-TP-214 was commercially available and as it was already tested previously with an synthesized standard by (Bollmann et al., 2017a) no LBT at tier III was conducted.

3.3.6. Mecoprop-TPs

Results of tier I revealed that no literature data of toxicity to*V. fischeri* were available for Mecoprop. Instead, an EC_{50} of Mecoprop for another test species (*Pseudomonas putida*) was found to be EC_{50} = 24 mg L^{-1} (Strachan et al., 2001). According to other studies, *P. putida* turned out to be generally more sensitive than *V. fischeri* which in turn means that EC_{50} for *V. fischeri* of Mecoprop is probably higher than 24 mg L^{-1} (Schmitz et al., 1998; de Zwart and Slooff, 1983). Regarding the toxicity to *V. fischeri* of TPs, EC_{50} of TP-107 was found to be 27.1 mg L^{-1} (Jenning et al., 2001) which indicates that the TP is probably more toxic than Mecoprop itself. TP-141 was found to be more toxic than Mecoprop to *D. magna* (Mottier et al., 2014). Toxicity of the photolytic mixture of MCPA, another chlorophenoxy herbicide, to *V. fischeri* increased after five minutes of UV irradiation (Zertal et al., 2001). Due to the fact that Mecoprop and MCPA only differ by different length of the carbon chain of the carboxyl group they have similar TPs such as TP-107 and TP-141. The toxic effect might therefore be triggered by these TPs. *In silico* prediction revealed toxic effects of the parent compound and TP-141, TP-195, and TP-213.

Due to the positive results received at tier I, we conducted toxicity test of the photolytic mixture at tier II. The results showed that no inhibition of luminescence was present for Mecoprop. In contrast to the parent compound the photolytic mixture showed clear effects of more than 60% luminescence inhibition for all three endpoints (Fig. 4A) indicating that the TPs are more toxic than Mecoprop on *V. fischeri*.

At tier III, we tested Mecoprop-TP-141 as this TP showed positive results at Tier I and II and an analytical standard was commercially available. To the authors best knowledge no toxicity test on *V. fischeri* was done previously for TP-141. It was seen found luminescence of *V. fischeri* was inhibited by 60% at a concentration of 12.5 mg L^{-1} for all three endpoints (Fig. 4B). EC_{50} values of 2.28 (acute toxicity), 7.63 (chronic toxicity), and 14.46 (growth inhibition) mg L^{-1} were calculated (Fig. 5).

It is thus most likely that the toxic effects observed in the photolytic mixture were caused by Mecoprop-TP-141. Nonetheless, other TPs such as TP-213 - the photoisomer of Mecoprop - might additionally cause the

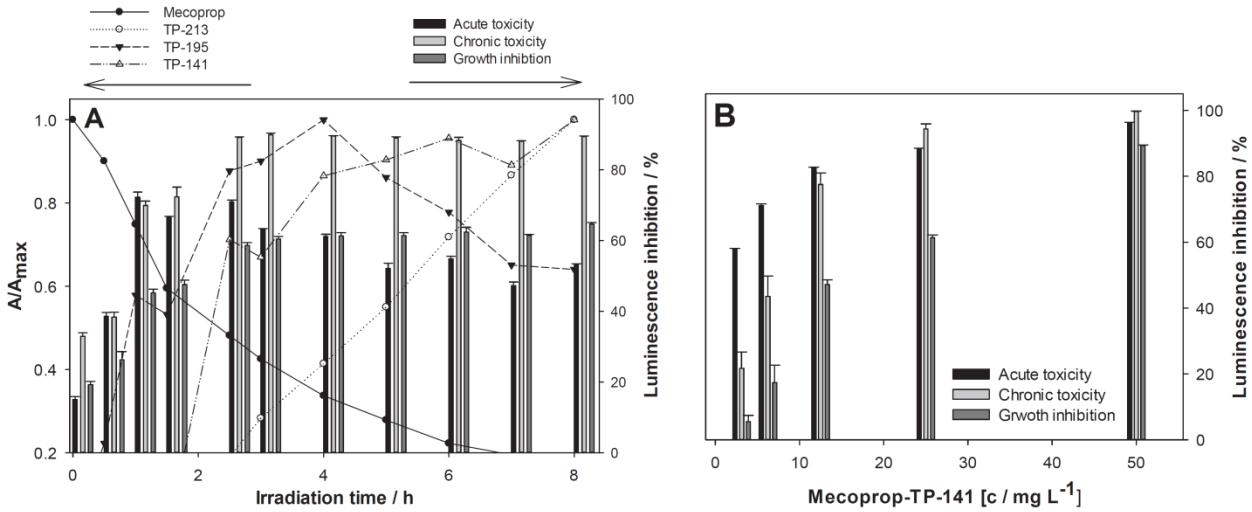

Fig. 4. A: Analysis of the toxicity of the photolytic mixture of Mecoprop over a time period of eight hours. Mean values of the Area of Mecoprop and its TPs (left ordinate) and luminescent inhibition of acute, chronic toxicity and growth inhibition (right ordinate). B: Single toxicity test of Mecoprop-TP-141 at a concentration range between 50 and 3.125 mg L^{-1}.

effect of the photolytic mixture. This was found for MCPA by (Zertal et al., 2001) as well. In that study the photoisomer of MCPA that was formed by photo-claisen-rearrangement as well as TP-213 here. It was found to be more toxic than MCPA itself. Hence, toxicity tests of the single substance of TP-213 need to be conducted in future. Besides, TP-195 should be taken into account as QSAR results showed positive results and it was present in the photolytic mixture as well.

3.4. Workflow and its classification of results

By the proposed workflow for the ecotoxicological assessment of TPs it was possible to initially assess 43 out of 45 TPs (96%). Here the benefits of the combined application arise. For example, in silico tests take effect for low soluble substances such as Boscalid that could not be tested by the conducted experiments. Or vice versa, experiments take effect for substances that do not fall under the applicability domain of the in silico tool such as Terbutryn and the majority of its TPs.

38% of the TPs (17 TPs) could be evaluated by more than one tier (Table 3), which resulted in a strong evidence to be toxic or non-toxic. For 58% of the TPs (26 TPs) slight evidence for their (non–) ecotoxicity was found based on the assessment at tier I. The knowledge on the potential environmental risk of TPs was therefore significantly enlarged by our approach, as the investigated ecotoxic properties were hitherto known for thirteen TPs only. Hence, the knowledge on the ecotoxic properties was more than tripled. Only 2 TPs (Penconazole-TP-130 and OIT-TP158) could not be evaluated by neither tier I nor tier II and III of the proposed workflow.

By starting at tier I (literature review and QSAR), it was possible to assess 80% of the TPs. Hence, the majority of TPs could be initially assessed here. By following the workflow at tier II (mixture toxicity testing) further 16% of TPs could be evaluated that were out of domain or not studied before at tier I. The number of TPs that could be assessed was not increased by conducting tier III (single toxicity test). But some TPs could be assessed more closely. Hence, for some TPs (Penconazole-TP-70, Terbutryn-TP-212, and Terbutryn-TP-226) single toxicity tests gave more information as their toxicities could be considered as negative in these tests. In case of Mecoprop-TP-141, the single LBT confirmed the results received by the toxicity test of the mixture at tier II as this TP was tested to be positive on *V. fischeri*. In total, 33% of the TPs could be assessed by both QSAR and mixture toxicity testings. Out of these TPs, 87% that could be assessed by QSAR could be confirmed by the toxicity testing of the reaction mixture. Hence, our procedure, which is much faster than an experimental assessment of each single TP and the high precision turned out to be beneficial.

By *in silico* prediction, 31% of the analyzed TPs were out of domain, implying that these TPs contain structural features that were not covered by the training set chemicals of the respective model. Most of such TPs were found for Terbutryn-TPs (83% of all Terbutryn-TPs). It

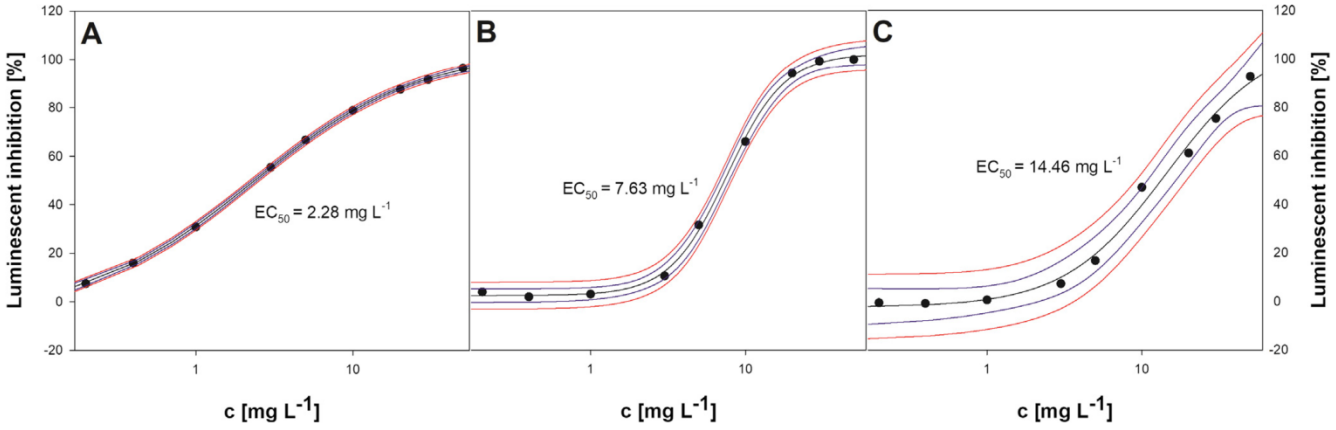

Fig. 5. Dose-Response-Curves of Mecoprop-TP-141. Luminescent inhibition is plotted against concentration between 60 and 0.2 mg L^{-1}. Luminescence inhibition was measured after 30 min (acute toxicity, A), 24 h (chronic toxicity, B), and 12 h (growth inhibition, C).

Table 3
Classification of TPs according to their probability to be toxic or non-toxic. No probability could be constituted if the TPs could neither be assessed by *in silico* nor experimental approaches. Categorization criteria could be received from the methodology approach depicted in Fig. 2.

Most probably toxic	Probably toxic	No probability	Probably non-toxic	Most probably non-toxic
Penconazole-TP-70	Boscalid- TP-307(a)	Penconazole-TP-130	Boscalid- TP-157	Terbutryn-TP-140
Penconazole-TP-286	Boscalid-TP-307(b)	OIT-TP-158	Boscalid- TP-158	Terbutryn-TP-168
Diuron-TP-162	Bosclaid-TP-325(a)		Boscalid- TP-309	Terbutryn-TP-196
Diuron-TP-205	Boscalid-TP-325(b)		Terbutryn-TP-156	Terbutryn-TP-212
Diuron-TP-219	Penconazole-TP-184		OIT-TP-202	Terbutryn-TP-226
OIT-TP-214	Penconazole-TP-248(a)		OIT-TP-216	OIT-TP-172
Mecoprop-TP-141	Penconazole-TP-248(b)			OIT-TP-130
Mecoprop-TP-195	Penconazole-TP-264(a)			Mecoprop-TP-107
Mecoprop-TP-213	Penconazole-TP-264(b)			
	Penconazole-TP-266(a)			
	Penconazole-TP-266(b)			
	Diuron-TP-215(a)			
	Diuron-TP-215(a)			
	Terbutryn-TP-184			
	Terbutryn-TP-186			
	Terbutryn-TP-210			
	Terbutryn-TP-214			
	Terbutryn-TP-256			
	Terbutryn-TP-258			
	OIT-TP-184			

became evident that the obtained results are strongly dependent on the availability of a sufficient large quantity of experimental results of test and training substances as it could be seen in case of Terbutryn-TPs.

By the additional photolysis experiments, it was possible to elucidate 62% of the selected and already known TPs. Most of them were found for Terbutryn and Penconazole. It can be assumed that some of the TPs described in literature were present in the photolytic mixture, although they could not be identified due to limitations in mass spectrometry or chromatography. This demonstrates the possibility to draw wrong conclusion regarding the toxicity of the photolytic mixture, as TPs could cause toxic effects of the mixture that were not detected.

For 9 TPs (20%) we found strong evidence to be toxic. Out of these TPs, TP-214 of OIT and TP-141, TP-195, and TP-213 of Mecoprop are not under consideration so far by any pesticide approval report. For 20 TPs (44%) there were slight indications to be ecotoxic. None of these TP was considered within approval procedure so far. Thus, the approach presented here can be considered as a first screening to guide further analysis and assessment with respect to the identification of possibly (eco-) toxicological relevant TPs.

The contribution of in silico models to the legal framework of pesticide risk assessment could be generally beneficial as time and costs are reduced compared to solely experimental approaches. This is, however, especially important when considering the huge amount of probably occurring TPs. Until now, the use of in silico methods is not generally recommended by the legislative framework of the EU since individual models have their own strengths and weaknesses (EFSA, 2010). However, according to the regulation (EC) No.1107/2009 the authorization shall be performed in light of current scientific and technical knowledge and, thus, in silico tools could support the risk assessment of pesticides (Villaverde et al., 2017) even of recent nanoformulations (Villaverde et al., 2018a). Hence, the herein introduced combination of experiment and *in silico* method in a tiered approach might be advantageous in terms of both applicability and validity considering the current legal requirements of risk assessment.

4. Conclusion

Our tiered approach for the preliminary assessment of pesticide TPs exemplified by selected endpoints demonstrates the extension of the body of the knowledge on the overall relevance and impact of TPs on human health and the environment. The combined use of published data, in vitro, and *in silico* toxicity assessment within the suggested tiered approach was demonstrated as a useful starting point to handle the increasing number of substances that need to be considered within hazard-oriented assessment of TPs. This approach supports faster decision and priority setting and depending on the issue being addressed the consideration of other endpoints (e.g. toxicity to fish and algae).

The comparison of parent compounds that show toxic effects to environmental bacteria to the number of probably toxic TPs suggests that the number of substances that pose a risk onto the aquatic environment increased by a factor of > 4. This is even more notable as about 33% of the TPs have already been detected in the surface- and groundwater. It may be necessary to implement this proactive assessment of TPs more consequently into the existing regulations to prevent the occurrence and effects of TPs in the water cycle. However, the study presented was a very first one. Its applicability domain needs to be assessed further. For this purpose as a very next step the approach suggested here should be verified by applying additional endpoints and including additional parent compounds.

CRediT authorship contribution statement

Birte Hensen: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft. **Oliver Olsson:** Conceptualization, Writing - review & editing, Project administration, Funding acquisition. **Klaus Kümmerer:** Resources, Writing - review & editing, Supervision.

Declaration of Competing Interest

We declare that we have no conflict of interest.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.105533.

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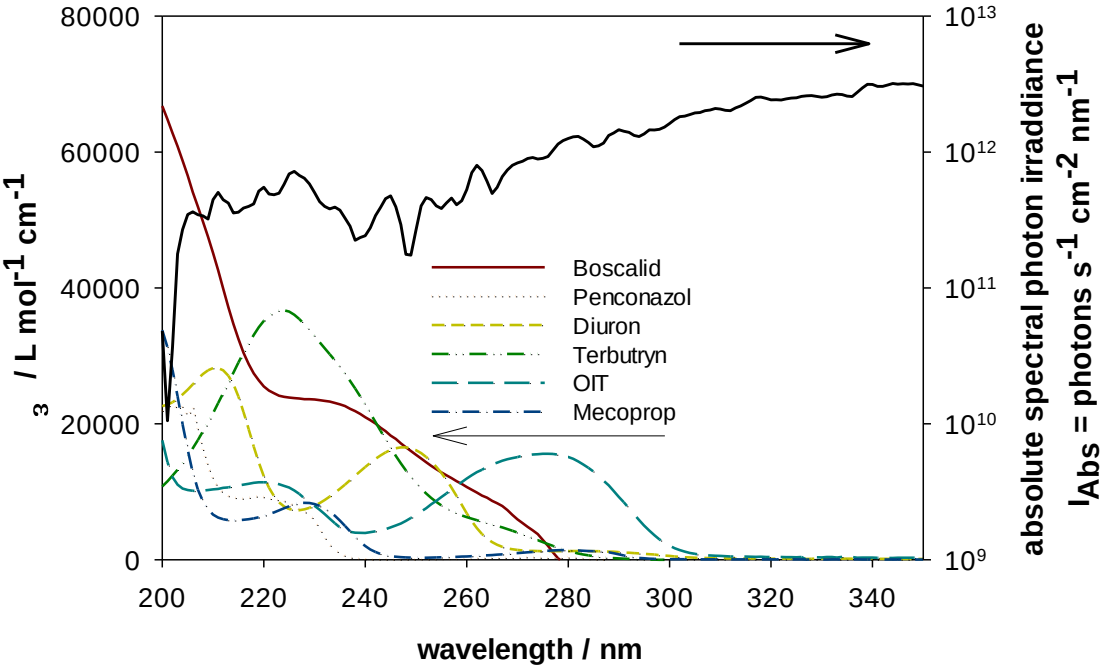
A strategy for an initial assessment of the ecotoxicological effects of transformation products of pesticides in aquatic systems following a tiered approach

Birte Hensen, Oliver Olsson, Klaus Kümmerer

- Supplementary information -

S1: Emission spectrum of xenon arc lamp and absorption spectra of substances



S2: Chromatographic and mass spectrometric settings

Time [min]	Mobile phase [% Acetonitrile]	Mobile phase [% Formic acid, 0.01 %]
0-1	10	90
1-11	10-50	90-50
11-18	50-85	50-15
18-21	85-90	15-10
21-24	90	10
24-26	90-10	10-90
26-30	10	90

MS setting	
Ionization mode	+ (Boscalid, Penconazole, Diuron, Terbutryn, and OIT)
	- (Mecoprop)
Ionization source	ESI
Scan mode	MS2 scan
Cut-off mass	100

S3: Test conditions of photolysis and toxicity tests

Substance	Photolysis		Toxicity	
	Test concentration [mg L^{-1}] of photolysis	**Elimination rate [%] after eight hours of irradiation**	**Test concentration [mg L^{-1}] of LBT**	**Test concentration [mg L^{-1}] of umu**
Boscalid	1	5	0.5	1
Penconazole	60	32	30	60
Diuron	10.8	37	5.4	10.8
Terbutryn	10	24	5	10
OIT	50.8*	25	0.6	50.8
Mecoprop	65*	100 (< detection limit)	16.3	65

***** We used concentrations below 100 mg L^{-1}. Concentration should be low enough to avoid inner filter effect (Douglas et al., 2013; Herrmann et al., 2015) but high enough to allow for a satisfactory TP elucidation.

S4: Results of the Luminescent Bacteria Test (LBT)

	Parent compound Growth inhibition [%]			Photolytic mixture (TPs) Growth inhibition [%]		
Substance	**Acute toxicity**	**Chronic toxicity**	**Growth inhibition**	**Acute toxicity**	**Chronic toxicity**	**Growth inhibition**
Boscalid	0	0	0	-	-	-
Penconazole	16	74	19	28	85	29
Diuron	10	26	23	14	26	30
Terbutryn	13	11	1	17	21	1
OIT	63	65	54	63	66	55
Mecoprop	16	33	22	86	100	94
Penconazole TP-70	0	1	6			
Terbutryn TP-212	0	9	0			
Terbutryn TP-226	1	21	0			
Mecoprop TP-141	96	99	93			

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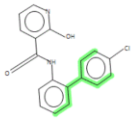
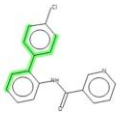
S5: Results of the literature analysis, experiments, and QSAR of genotoxicity

Substance	Rele-vance	Known Synonyms	logP	GT (Lit.)	GT (QSAR)		GT (experiments)	
					GT1_ A7B	GT_ Exp	IR (umu-S9)	IR (umu+S9)
Boscalid			**4.8**				0.8	
TP-157	NR	M510F64, p-chlorobenzoic acid	2.7	- [Zeiger]	--	--		
TP-158	NR	M510F47, 2-chloronicotinic acid	1.2		(-)	--		
TP-307(a)			5.1		+	+		
TP-307(b)			4.0		(-)	-		
TP-309	NR	M510F08	3.9		(-)	-		
TP-325(a)			3.8		+	+		
TP-325(b)	NR	M510F49	4.9		+	-		
Penconazol			**4.0**		--	--	1.1	1.1
TP-70	R	CGA 71019, 1,2,4-Triazole	-0.6		--	--		
TP-130	NR	CGA 142856, Penconazole Acetic acid	-0.8		--	--		
TP-184*			0.6		-	-	1.06	0.95
TP-248(a)			3.6		-	-	1.06	0.95
TP-248(b)*			2.1		-	-	1.06	0.95
TP-264(a)*			2.8		-	-	1.06	0.95
TP-264(b)*			2.8		-	-	1.06	0.95
TP-266(a)*			3.0		-	-	1.06	0.95
TP-266(b)*			3.0		-	-	1.06	0.95
TP-286	R	CGA 179944, Penconazole Propionic Acid	2.2		--	--		
Diuron			2.8		--	--	1.13	0.94
TP-162	NR	DCA; 3,4-Dichloranilin	2.7	- [Zeiger]	--	--		
TP-205	R	DCPU	2.7		-	-		
TP-215(a)			1.5		-	-	1.02	1.04
TP-215(b)			1.3		-	-	1.02	1.04
TP-219	R	DCPMU	2.9		-	-	1.02	1-04
Terbutryn			3.7		-	-	0-94	0.92
TP-140		Desthiomethyl-Desbutyl-Terb.	0.02		-	-		
TP-156		Desbutyl-2-Hydroxy-Terbutryn	0.2		--	--		
TP-168		Desthiomethyl-Desethyl-Terb.	0.8		-	-	0.91	0.92
TP-184		Desethyl-2-Hydroxy-Terbutryn	1.0		-	-	0.91	0.92
TP-186			1.0		-	-	0.91	0.92
TP-196		Desthiomethyl-Terbutryn	1.7		--	--	0.91	0.92
TP-210			1.2		--	--	0.91	0.92
TP-212	R	2-Hydroxy-Terbutryn; M13; GS23158	1.8		--	--	0.91	0.92
TP-214		Desethyl-Terbutryn	1.8		-	-	0.91	0.92
TP-226		Terbumeton	3.1		-	-	0.91	0.92
TP-256			1.9		+	-	0.91	0.92
TP-258		Terbutryn-Sulfoxid	0.8		-	-	0.91	0.92
OIT			2.5		--	--	1.08	0.98
TP-130		Octylamin	2.9		-	-	0.81	1.02
TP-158		N-Octylformamide	2.9		-	-		
TP-172		N-Octylamide	3.0		-	-		
TP-184		N-Octylprop-2-enamide	3.4		-	-		
TP-202		N-Octyloxamic acid	2.4		-	-		
TP-214		2-Octylisothiazol-3(2 H)-one	3.4		-	-	0.81	1.02
TP-216		N-Octyl Malonamic acid	2.4		-	-		
Mecoprop			3.1		--	--	1.0	1.2
TP-107	NR	o-Cresol, 2-Methylphenol	2.3	- [Zeiger]	--	--		
TP-141	NR	2-MCP, 4-Chloro-o-cresol	1.6	- [Zeiger]	--	--	0.74	0.92
TP-195		TP-195	2.0		-	-	0.74	0.92
TP-213		TP-213	2.6		-	-	0.74	0.92

R= Relevant metabolite; NR= not relevant metabolite;+=positive, - =negative;--= known negative;(-)inconclusive negative

References: Zeiger E, Anderson B, Haworth S, Lawlor T, Mortelmans K. Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals. Environ. Mol. Mutagen. 1992;19:2–41.

S6: QSAR - Positive alerts of Boscalid-TPs

TP-325	TP-309
	

Positive Alerts

One Positive Alert was found in the test chemical.

Alert ID 10: `c-c:cH:cH`

This alert occurs 3 times in the test chemical.

- 17 out of 23 (73.9%) molecules containing this alert are **positive** with an average activity of 3.97 mg/kg (-log10 transformed)
- 6 out of these 17 positive chemicals are supported by 5 other positive alerts of which none is present in this test chemical.
- 17 out of these 17 positive chemicals are supported by 2 positive modulators which are present in this test chemical.

Contribution in Binary Classification:

A local binary QSAR is available for this alert.

Positive modulators:

`c-c1:c:cH:cH:cH:cH1`, A/I = 5/0, Regression Coeff. = 0.929

`cH:cH:c(-c):cH:cH`, A/I = 14/3, Regression Coeff. = 0.959

Negative modulators:

No negative modulators of this alert were identified in this test chemical.

Regression Constant = 0.233

Sum of Factors = 0.929+0.959+0.233 = 2.122

Contribution of this alert to the binary global QSAR = $1/(1+e^{-1 * 2.122})$ = 0.893

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One Positive Alert was found in the test chemical.

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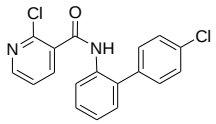
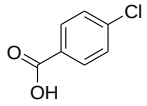
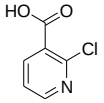
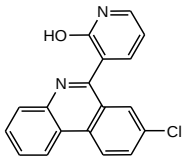
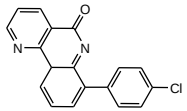
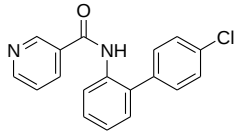
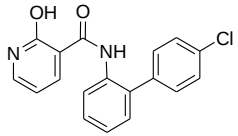
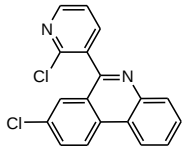
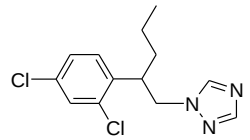
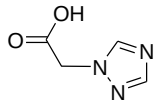
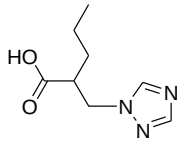
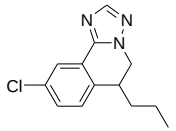
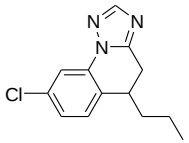
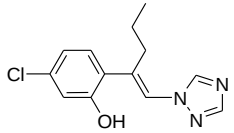
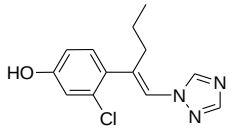
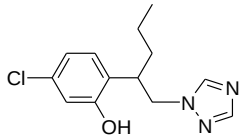
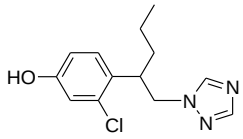
Sum of Factors = 0.929+0.959+0.233 = 2.122

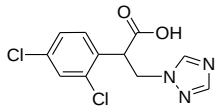
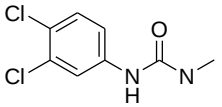
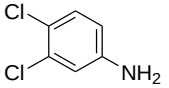
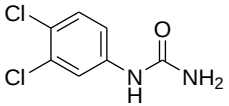
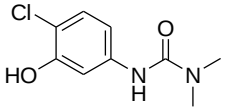
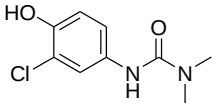
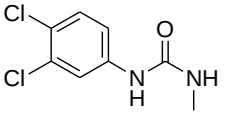
Contribution of this alert to the binary global QSAR = $1/(1+e^{-1 * 2.122})$ = 0.893

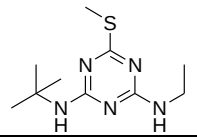
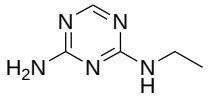
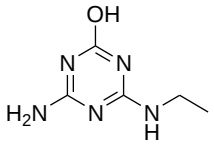
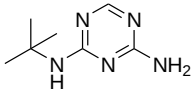
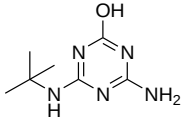
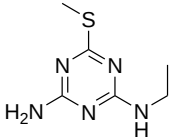
S7: Analytical details of TPs

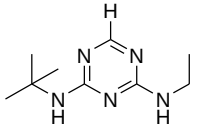
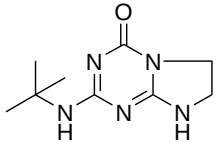
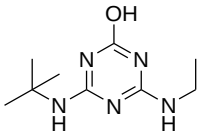
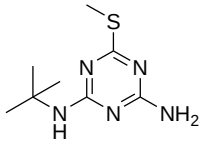
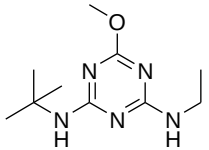
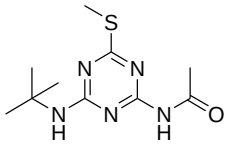
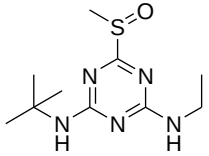
Substanz	RT [Min.]	Smiles Code	Exact Mass [Da]	Molecular formula	Structural formula	Transition 1	Transition 2
Boscalid	5.8	ClC1=NC=CC=C1C(NC2=CC=CC=C2C3=CC=C(Cl)C=C3)=O	342.033	$C_{18}H_{13}ClN_2O_2$		343.1 → 307.1	343.1 → 140
TP-157	-	ClC1=CC=C(C(O)=O)C=C1	155.998	$C_7H_5ClO_2$		-	-
TP-158	-	ClC1=NC=CC=C1C(O)=O	156.993	$C_6H_4ClNO_2$		-	-
TP-307(a)	2.2	ClC1=CC3=C(C2=CC=CC=C2N=C3C4=C(N=CC=C4)O)C=C1	306.056	$C_{18}H_{11}ClN_2O$		307.1→ 271.1	307.1→ 272.1
TP-307(b)	3.8	O=C(C4=C3N=CC=C4)N=C1C3C=CC=C1C2=CC=C(C=C2)Cl	306.056	$C_{18}H_{11}ClN_2O$		307.1→ 271.1	307.1→ 272.1

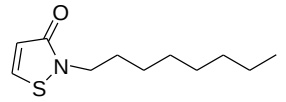
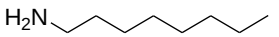
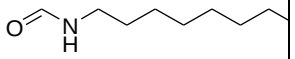
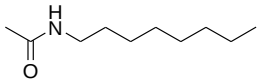
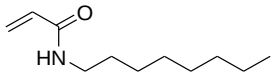
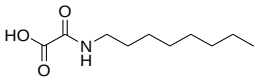
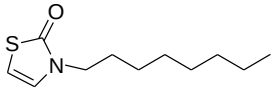
TP-309	-	ClC(C=C3)=CC=C3C1=CC=CC=C1NC(C2=CC=CN=C2)=O	398.072	$C_{18}H_{13}ClN_2O$		-	-
TP-325(a)	3.6	OC1=NC=CC=C1C(NC2=CC=CC=C2C3=CC=C(Cl)C=C3)=O	324.067	$C_{18}H_{13}ClN_2O_2$		325.1 → 307.1	325.1 → 271.1
TP-325(b)	11.8	ClC3=CC=C2C1=CC=CC=C1N=C(C4=CC=CN=C4Cl)C2=C3	324.067	$C_{18}H_{13}ClN_2O_2$			
Penconazol	16.2	c1(Cl)c(C(CCC)CN2C=NC=N2)ccc(Cl)c1	283.064	$C_{13}H_{15}Cl_2N_3$		284.1 → 70.1	284.1 → 159
TP-70	-	N1N=CN=C1	69.033	$C_2H_3N_3$		-	-
TP-130	-	O=C(O)CN1N=CN=C1	129.038	$C_4H_5N_3O_2$		-	-

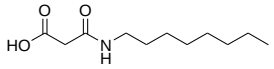
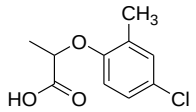
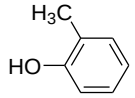
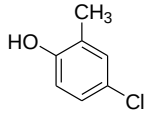
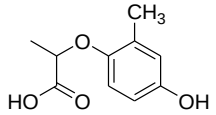
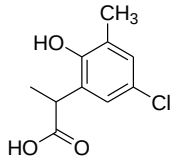
TP-184*	1.8	O=C(O)C(CN1N=CN=C1)CCC	183.101	$C_8H_{13}N_3O_2$		Keine Fragmente	
TP-248(a)	12.0	C12c3c(C(CCC)CN1N=CN=2)ccc(Cl)c3	247.088	$C_{13}H_{14}ClN_3$		248. → 204	248.1 → 192
TP-248(b)*	2.0	CCCC(C3)C1=CC=C(Cl)C=C1N2C3=NC=N2	247.132	$C_{13}H_{18}N_3O_2$		248.1 → 204	248.1 → 123
TP-264(a)*	3.0	OC1=C(/C(CCC)=C/N2C=NC=N2)C=CC(Cl)=C1	263.083	$C_{13}H_{17}N_3O_3$		264.1 → 220.1	264.1 → 246.1
TP-264(b)*	4.8	ClC1=C(/C(CCC)=C/N2C=NC=N2)C=CC(O)=C1	263.083	$C_{13}H_{14}ClN_3O$		264.1 → 220.1	264.1 → 246.1
TP-266(a)*	2.5	OC1=C(C(CN2C=NC=N2)CCC)C=CC(Cl)=C1	265.098	$C_{13}H_{16}ClN_3O$		266.1 → 141	266.1 → 69.9
TP-266(b)*	5.8	ClC1=C(C(CN2C=NC=N2)CCC)C=CC(O)=C1	265.098	C13H16ClN3O		266.1 → 141	266.1 → 69.9

TP-286	-	ClC1=CC=C(C(CN2N=CN=C2)C(O)=O)C(Cl)=C1	285.007	$C_{11}H_9Cl_2N_3O_2$		-	-
Diuron	13	ClC1=CC=C(NC(N(C)C)=O)C=C1Cl	232.017	$C_9H_{12}ClN_2O$		233.03 → 72.1	233.03 → 56
TP-162	-	ClC1=CC=C(N)C=C1Cl	160.090	$C_6H_5Cl_2N$		-	-
TP-205	-	ClC1=CC=C(NC(N)=O)C=C1Cl	203.986	$C_7H_6Cl_2N_2O$		-	-
TP-215(a)	9.5	ClC1=CC=C(NC(N(C)C)=O)C=C1O	215.051	$C_9H_{10}ClN_2O_2$		215.06 → 72.1	215.06 → 46.1
TP-215(b)	10.5	OC1=CC=C(NC(N(C)C)=O)C=C1Cl	215.051	$C_9H_{12}ClN_2O_2$		215.06 → 72.1	215.06 → 46.1
TP-219	12.5	ClC1=CC=C(NC(NC)=O)C=C1Cl	218.001	$C_8H_8Cl_2N_2O$		219.01 → 127	219.01 → 162

Terbutryn	19.9	CC(C)(C)NC1=NC(SC)=NC(NCC)=N1	242.147	$C_{10}H_{19}N_5S$		242.15 → 186,1	242.15 → 68
TP-140	-	NC1=NC=NC(NCC)=N1	139.086	$C_5H_9N_5$		-	-
TP-156	-	NC1=NC(O)=NC(NCC)=N1	155.081	$C_5H_9N_5O$		-	-
TP-168	11.8	NC1=NC(NC(C)(C)C)=NC=N1	167.117	$C_7H_{13}N_5$		168.13 → 112	168.13 → 70
TP-184	-	NC1=NC(NC(C)(C)C)=NC(O)=N1	183.112	$C_7H_{13}N_5O$		-	-
TP-186	19.5	NC1=NC(NCC)=NC(SC)=N1	185.074	$C_6H_{11}N_5S$		186,1 → 68,0	186,1 → 91,0

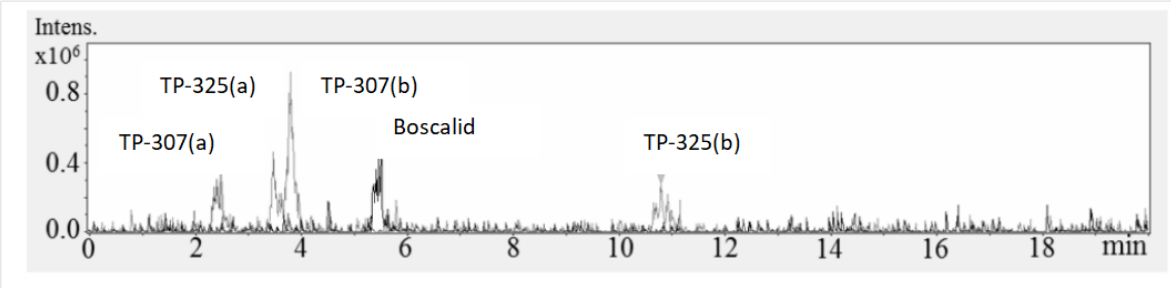
TP-196	13.8	CC(C)(C)NC1=NC([H])=NC(NCC)=N1	195.148	$C_9H1_7N_5$		196.16 → 140.1	196.16 → 45
TP-210	11	CC(C)(C)NC(N=C2N1CCCN2)=NC1=O	209.128	$C_9H_{15}N_5O$		210.14 → 112.1	210.14 → 154
TP-212	12.5	CC(C)(C)NC1=NC(O)=NC(NCC)=N1	211.143	$C_9H_{17}N_5O$		212,2 → 156,0	212,2 → 69,0
TP-214	15.9	NC1=NC(NC(C)(C)C)=NC(SC)=N1	213.105	$C_8H_{15}N_5S$		214.11 → 158	214.11 → 68
TP-226	10.2	CC(C)(C)NC1=NC(OC)=NC(NCC)=N1	225.159	$C_9H_{19}N_5O$		226,2 → 170,1	226,2 → 128,1
TP-256	20.2	CC(C)(C)NC1=NC(SC)=NC(NC(C)=O)=N1	255.115	$C_{10}H_{18}N_5SO$		256.16 → 158	256.16 → 200
TP-258	18.5	CC(C)(C)NC1=NC(S(C)=O)=NC(NCC)=N1	257.131	$C_{10}H_{19}N_5OS$		258,1→ 186,0	258,1 → 202,0

OIT	14.8	O=C1N(CCCCCCCC)SC=C1	213.119	$C_{11}H_{19}NOS$		214.13 → 102	214.13 → 57.1
TP-130	9.9	CCCCCCCCN	129.152	$C_8H_{19}N$		130.2 → 57	130.2 → 43.1
TP-158	-	CCCCCCCCNC=O	157.147	$C_9H_{19}NO$		-	-
TP-172	-	CCCCCCCCNC(C)=O	171.162	$C_{10}H_{21}NO$		-	-
TP-184	-	CCCCCCCCNC(C=C)=O	183.162	$C_{11}H_{21}NO$		-	-
TP-202	-	CCCCCCCCNC(C(O)=O)=O	201.136	$C_{10}H_{19}NO_3$		-	-
TP-214	15.5	O=C1N(CCCCCCCC)C=CS1	213.119	$C_{11}H_{19}NOS$		214.13 → 102	214.13 → 57.1

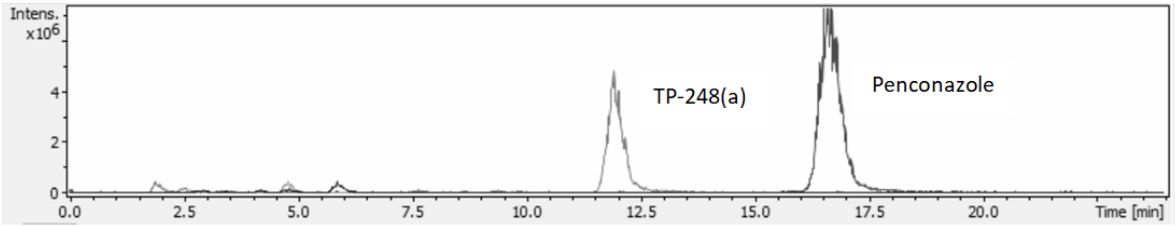
TP-216	-	CCCCCCCCNC(CC(O)=O)=O	215.152	$C_{11}H_{21}NO_3$		-	-
Mecoprop	14.5	ClC1=CC=C(OC(C(O)=O)C)C(C)=C1	214.040	$C_{10}H_{11}ClO_3$		213,0 → 141,0	213,0 → 70,9
TP-107	-	OC1=C(C)C=CC=C1	108.058	$C_{10}H_{11}ClO_3$		-	-
TP-141	8.5	OC1=CC=C(Cl)C=C1C	142.019	$C_{10}H_{12}O_4$		141.1 → 141.1 Keine Fragmente	
TP-195	8.9	OC1=CC=C(OC(C(O)=O)C)C(C)=C1	196.074	C_7H_7ClO		195,1 → 149,2	195,1 → 122,9
TP-213	10.8	ClC1=CC(C(C(O)=O)C)=C(O)C(C)=C1	214.040	C_7H_8O		155,0 → 141,0	-

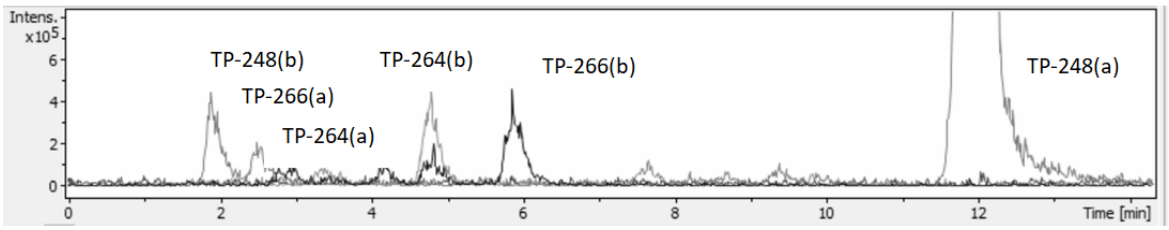
S8: Chromatograms of photolytic mixtures

Boscalid

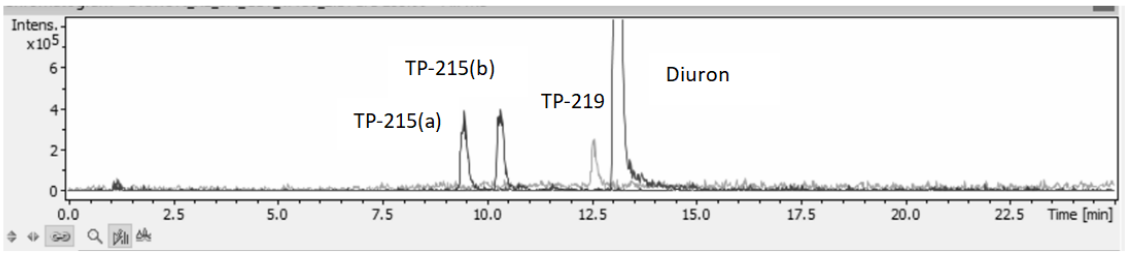


Penconazole

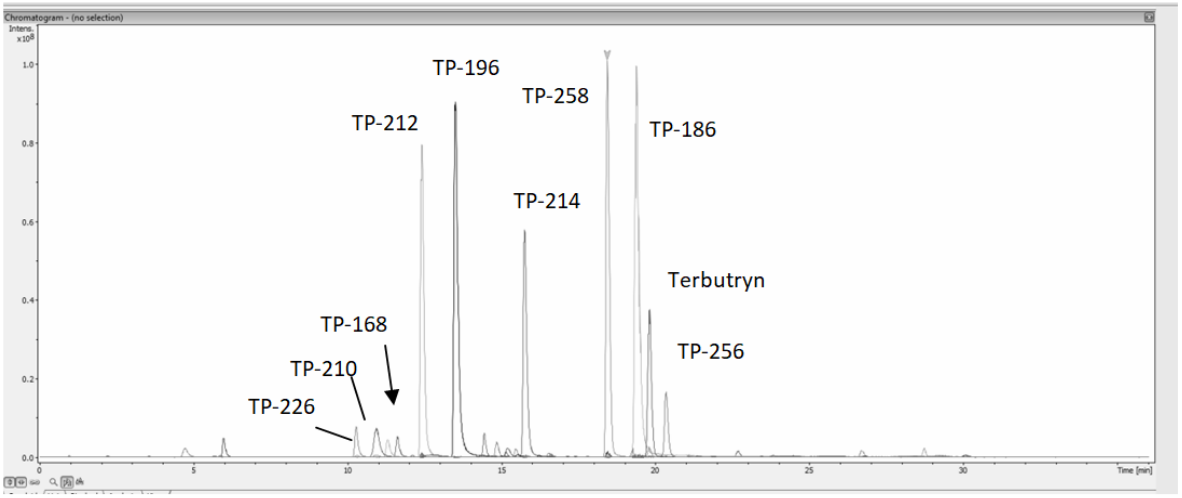




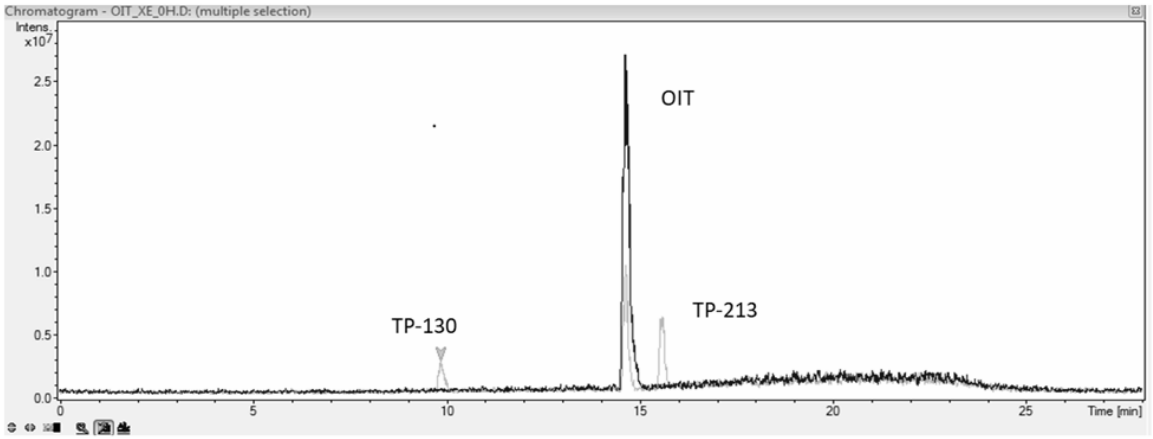
Diuron



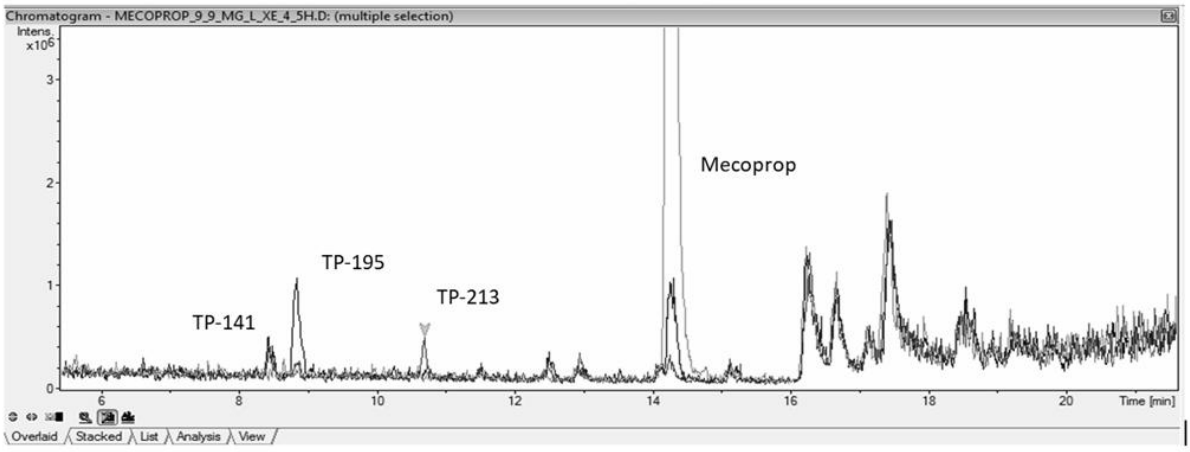
Terbutryn



OIT



Mecoprop



S9: Graphical results of the Luminescent Bacteria Test (LBT) of OIT

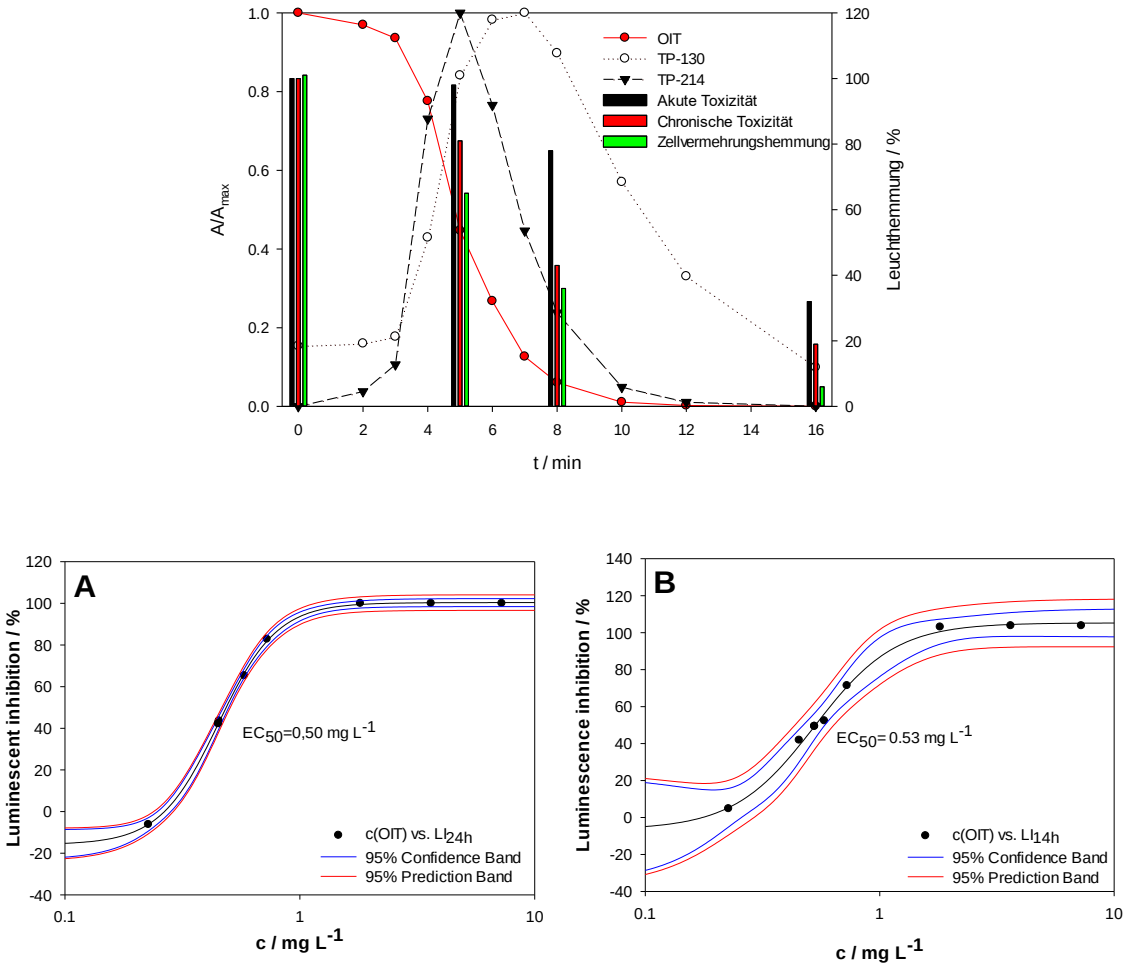


Figure: Dose-response curves of OIT for the chronic toxicity (A) and growth inhibition (B) in a modified luminescent bacteria test. Chronic toxicity test was conducted over a time period of 24 h, growth inhibition test was conducted over 14 h.

S10: Results of the umu-Test of pesticides and their photolytic mixtures

	Umu –S9	**Umu +S9**
Boscalid	**-**	**-**
Boscalid photo	**-**	**-**
Penconazole	**-**	**-**
Penconazole photo	**-**	**-**
Diuron	**-**	**-**
Diuron photo	**-**	**-**
Terbutryn	**-**	**-**
Terbutryn photo	**-**	**-**
OIT	**-**	**-**
OIT photo	**-**	**-**
Mecoprop	**-**	**-**
Mecoprop photo	**-**	**-**

References

(1) Zeiger, E.; Anderson, B.; Haworth, S.; Lawlor, T.; Mortelmans, K. Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals. *Environ. Mol. Mutagen.* **1992**, *19*, 2–41.